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Environment

Genetic diversity among black cumin (*Nigella sativa* L.) accessions based on quantitative and qualitative traitsSolomon Kassaye Ejigu^{1,2} | Wosene Gebreselassie Abtew² | Ermias Lulekal Molla³¹ Amhara Agricultural Research Institute, Sirinka Agricultural Research Center, Woldia, Ethiopia² Department of Horticulture and Plant Sciences, Jimma University College of Agriculture and Veterinary Medicine, Jimma, Ethiopia³ Department of Plant Biology and Biodiversity Management, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia

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Abstract

To investigate the genetic diversity in black cumin (*Nigella sativa* L.), a total of 64 genotypes were tested in an 8×8 simple lattice design at Sirinika Agricultural Research Center for two consecutive years during 2022/2023 and 2023/2024. Analysis of variance revealed the presence of significant difference among the genotypes for all quantitative traits except capsule length and diameter. The variation of the genotypes for seed yield was between 0.66 and 1.69 t ha⁻¹ with a mean of 1.02 t ha⁻¹. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for 16 traits are estimated in the range between 3.81% and 49.84% and 6.50% and 50.90%, respectively. H^2 and advance as percentage of mean (GAM) ranged from 19.18 to 98.25 and 4.67% and 100.70%, respectively. High to moderate estimates of GCV, PCV, H^2 , and GAM were observed for all parameters except days to bud formation, days to maturity, capsule length, and capsule diameter. Seed yield showed positive and significant correlation with almost all parameters. The first five PCAs accounted for 72% of the total variability, in which PCA1 contributed 32% and PCA2, PCA3, PCA4, and PCA5 exhibited 13%, 12%, 8%, and 7% contributions to the total variability, respectively. The qualitative data showed high variability except flower color, plant growth type, and stem hairiness. The cluster analysis for qualitative traits was classified into five groups. The research results clearly showed that there was sufficient diversity among the genotypes, which can be exploited for the future black cumin improvement program.

Plain Language Summary

Field trial was conducted to investigate the genetic diversity in black cumin (*Nigella sativa* L.) using 64 genotypes tested in an 8×8 simple lattice design at Sirinka Agricultural Research Center located in North Eastern Ethiopia for two consecutive

Abbreviations: GA, genetic advance; GAM, genetic advance as percentage of mean; GCV, genotypic coefficient of variation; PCA, principal component axis; PCV, phenotypic coefficient of variation; SARC, Sirinika Agricultural Research Center; SAS, statistical analysis system; UPGMA, unweighted paired grouping methods with arithmetic means.

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years during 2023 and 2024. Analysis of variance revealed the presence of significant difference among the genotypes for all quantitative traits except capsule length and diameter. The variation of the genotypes for seed yield was between 0.66 and 1.69 t ha⁻¹ with a mean of 1.02 t ha⁻¹. High to moderate estimates of genotypic coefficient of variation, phenotypic coefficient of variation, heritability, and advance as a percentage of mean were observed for all parameters. The first five principal component axes/PCAs/ accounted 72% of the total variability, in which PCA1 contributed 32% and PCA2, PCA3, and PCA4 exhibited 13%, 12%, 8%, and 7% contribution to the total variability, respectively. The qualitative data showed high variability in genotypes.

1 | INTRODUCTION

Medicinal plants have been in use by human beings for long time; one of these is black cumin (*Nigella sativa* L.) that has been used for healthcare since civilization. It is also known as black seeds, fennel flower, Roman coriander, and wild onion seed (Kulloli, 2016). It is a member of the Ranunculaceae (Weiss, 2002). Black cumin is resumed to originate in the Mediterranean region. It is widely cultivated in North Africa, South Europe, Middle East, China, Japan, Turkey, Iran, and Indian subcontinent (Bhutia et al., 2015; Rana et al., 2012).

The majority of the global commercial supply of these seeds is obtained from cultivation in India (Dessie et al., 2020). The estimated early world production is 600,000 tons, with an 86% share in India, followed by Iran (4%), Syria (3%), and Turkey (2%). The remaining 5% comes from different countries such as Egypt, Pakistan, and Afghanistan (CBI, 2023). In contrast, the top 10 countries that have the highest export values of black cumin in order are Malaysia, Indonesia, China, the Netherlands, Germany, Sweden, India, the United States, Russia, and Spain (Tridge, 2023).

In Ethiopia, black cumin is one of the most important spice crop and is mainly produced for flavoring foods, to prepare oil for perfumes and medicines, as a source of income, to diversify crop, and for export purposes (Desalegn & Wubshet, 2018; Wubshet & Desalegn, 2019). It is the second most important crop, which is exported to international markets next to ginger (Teshome & Anshiso, 2019). Ethiopia is a country with different and favorable agroecological zones for production of various spices, vegetables, and crops (Dessie, Koye, et al., 2019; Dessie, Abteu, et al., 2019). Many spice varieties, particularly black cumin, white cumin, pepper, turmeric, fenugreek, garlic, coriander, ginger, cardamom, and basil are grown in Ethiopia for consumption and commercial purposes (Tesfa et al., 2017).

Despite the country's favorable environmental condition for black cumin production and varieties namely, Silingo, Kena, Gemechis, Darbera, Soressaa, Keneni, Dershaye, and Eden were recommended, the national average productivity

is very low and was reported to be 0.64 t ha⁻¹, which is below the global average and some major producing countries, including India (2.20 t ha⁻¹) (Food and Agriculture Organization, 2023; Zigyalew, 2020). Lack of high-yielding variety, postharvest handling issues, and inadequate agricultural practices were the primary factors affecting its production and productivity (Getachew & Beriso, 2020).

Assessment of genetic diversity can be accomplished through the use of various data such as morphological and agronomic, pedigree, biochemical, and molecular data. Morphological traits are genetically controlled by number of genes and their expression depends on environmental factors in most cases (Abdi et al., 2002; Tuinstra et al., 1996; Van Beuningen & Busch, 1997).

There are both quantitative and qualitative morphological traits that play vital roles in classification of individuals. Qualitative morphological traits are subjected to comparatively less environmental influence and have mono- or oligogenic control (Lima et al., 2017). They do not have quantitative values and might be binary or multicategory. Utilization of qualitative morphological data is a fruitful strategy in genetic diversity studies not only because it is a practical, low-cost application, with no sophisticated equipment requirements, but also they are expected to provide additional information on hierarchical units.

Variation in highly heritable characteristics and qualitative traits provide an estimate of diversity, while quantitative traits are exploited to determine the magnitude of genetic variation within germplasm (Iqbal et al., 2010). Moreover, evaluation of available genetic stocks to assess the genetic variation for economically important characteristics is a prerequisite for combining desirable genes in a single accession (Palevitch, 2004).

Very few studies were carried out to determine the genetic diversity on quantitative traits in a specific location for 1 year in Ethiopia. According to Basazinew et al. (2023) with 64 accessions at Debre-Zeit and Kulumsa, it was found that there was sufficient diversity among genotypes, which can be exploited for future black cumin improvement programs.

In addition, Yewubdinber (2020) and Yewubdinber et al. (2022) with 25 accessions at Debre-Zeit reported the presence of variations among genotypes and suggested that further evaluation of black cumin germplasm in Ethiopia to seed yield. However, no diversity study was done on qualitative traits and no enough information was based on quantitative traits. Therefore, this research was conducted to investigate the diversity of Ethiopian black cumin genotypes based on quantitative and qualitative traits.

2 | MATERIALS AND METHODS

2.1 | Location of the experimental site

Field experiment was performed to evaluate the genetic diversity of black cumin accessions. The experiment was conducted at Sirinika Agricultural Research Center (SARC), Northeast Ethiopia. SARC is located at 11°45'00" N latitudes and 39°36'36" E longitudes with an altitude of 1850 m above sea level. The annual rainfall for the years 2022/2023 and 2023/2024 was 101.64 and 105.29 mm, respectively. In a similar fashion, the minimum-maximum temperature for the years 2022/2023 and 2023/2024 was 13.49/26.35 and 13.72/26.59 °C, respectively. The dominant soil type of the site was clay loam (SARC [Sirinika Agricultural Research Center], 2023).

2.2 | Plant materials and experimental design

Sixty-four black cumin accessions that were obtained from Ethiopian Biodiversity Institute (EBI) were used in this study (Table S1). I used the maximum number of genotypes that were collected from potential black cumin growing areas of Ethiopia by EBI. Materials were evaluated using an 8 × 8 simple lattice design in the main cropping season from July to January of the years 2022/2023 and 2023/2024. The plot size for each experimental unit was 1.2 × 2.1 m (four rows). The total area of a plot was 2.52 m². The spacing between plants, rows, plots, and blocks was 0.15, 0.30, 0.50, and 2.00 m, respectively. Sowing was done by hand drilling and covered slightly with the soil (Habtewold et al., 2017). Fertilizer at the rate of 64.90 kg ha⁻¹ N and 37.70 kg ha⁻¹ P₂O₅ was used to facilitate and increase root development and yield in black cumin (Amdie et al., 2024). Weeding, thinning, and plant protection operations were done after planting (Habtewold et al., 2017).

2.3 | Data collected

Qualitative data were collected on branch density, branching pattern, capsule hairiness, flower color, flowering rate, flow-

ering time, follicle size, growth power, leaf area, leaf color, main stem color, plant growth habit, plant growth type, root system, seed ripening, stem elasticity, stem hairiness, stem shape in cross-section, stem thickness, and capsule spiciness. Moreover, quantitative data were collected on days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, capsules per plant, seeds per capsule, diameter of capsule, length of capsule, plant height, primary branches per plant, secondary branches per plant, 1000 seed weight, seed yield per plant, seed yield per hectare, biomass yield per hectare, and harvest index. A descriptor list for characterizing black cumin is so far not available. Therefore, general plant descriptor using Bioversity International (2007) was used to record and analyze the qualitative characters (Table S2). The data of the quantitative characteristics was obtained on a plot basis and measured from 10 representative plants, and the average result was obtained.

2.4 | Data analysis

2.4.1 | Analysis of variance and mean comparison

All quantitative data generated were analyzed using Statistical Analysis System (SAS) software (SAS, 2019). Estimates of variance components were calculated by equating the observed mean squares from analysis of variance (ANOVA) with their expected mean squares. Prior to doing the combined ANOVA over years, each year's data were subjected to ANOVA and normality test using the Shapiro–Wilk W test. Bartlett's tests were used to determine the homogeneity of the error variances of the individual year experiments, and then the combined ANOVA over years was performed after confirming the homogeneity of the variances. The combined ANOVA was made using Generalized Linear Model (GLM) procedures for simple lattice design as follows:

$$X_{ijkl} = \mu + G_i + Y_j + GY_{ij} + R_k + B_{l(k)} + \epsilon_{ijkl}$$

where X_{ijkl} is the observed value of genotype i in block l , replication k of year j , μ is the grand mean, G_i is the effect of genotype i , Y_j is the effect of year j , GY_{ij} is the interaction effect of genotype i with year j , R_k is the effect of replication k , $B_{l(k)}$ is the effect of block l in replication k , and ϵ_{ijkl} is the error (residual) effect of genotype i in block l , replication k of year j .

2.4.2 | Phenotypic and genotypic variability

The phenotypic (σ^2_p) and genotypic (σ^2_g) variances and the corresponding phenotypic coefficient of variation (PCV) and

genotypic coefficient of variation (GCV) for each trait were calculated following the method described by Burton and De Vane (1953).

The genotypic variance was estimated as follows:

$$\sigma^2_g = \frac{(Msg - Mse)}{r}$$

where σ^2_g is the genotypic variance, r is the number of replications, Msg is the genotype mean square, and Mse is the error mean square.

The phenotypic variance was estimated following the estimated genotypic variance (Burton & De vane, 1953) as follows:

$$\sigma^2_p = \sigma^2_g + \sigma^2_e,$$

where σ^2_p is the phenotypic variance, σ^2_g is the genotypic variance, and σ^2_e is the environmental variance.

$$PVC = \frac{\sqrt{\sigma^2_p}}{\bar{x}} \times 100$$

$$GVC = \frac{\sqrt{\sigma^2_g}}{\bar{x}} \times 100$$

where PCV is the phenotypic coefficient of variation, GCV is the genotypic coefficient of variation, and $\bar{x}$ is the population mean of the trait evaluated.

The PCV and GCV values were categorized as low, moderate, and high values as indicated by Sivasubramaniam and Menon (1973) as follows: low = 0%–10%, moderate = 10%–20%, and high = >20%.

2.4.3 | Heritability and genetic advance (GA)

Broad-sense heritability values were estimated using the formula adopted by Falconer and Mackay (1996) as follows:

$$H^2 = \left(\frac{\sqrt{\sigma^2_g}}{\sqrt{\sigma^2_p}} \right) \times 100$$

where H^2 is the heritability in a broad sense, σ^2_p is the phenotypic variance, and σ^2_g is the genotypic variance

The heritability percentage was categorized as low, moderate, and high as suggested by Robinson et al. (1955) and Johnson et al. (1955): low = 0%–30%, moderate = 30%–60%, and high = >60%.

Expected GA under selection

GA in the absolute unit and percent of the mean (GAM), assuming selection of superior 5% of the genotypes, were esti-

mated in accordance with the methods illustrated by Johnson et al. (1955) as follows:

$$GA = K \times (\sqrt{\sigma^2_p}) * H^2$$

where GA is the genetic advance, K is the selection intensity at 5% ($K = 2.063$), $\sqrt{\sigma^2_p}$ is the phenotypic variance on mean basis, and H^2 heritability in the broad sense.

GA as percent of the mean was estimated as follows:

$$GAM (\%) = \left(\frac{GA}{\bar{x}} \right) \times 100$$

where GAM is the genetic advance as percent of mean, GA, and $\bar{x}$ is the populations mean for trait evaluated.

The GA as percent of mean was categorized as low, moderate, and high as suggested by Johnson et al. (1955) as follows: low = 0%–10%, moderate = 10–20, and high = >20.

2.4.4 | Principal component analysis

Principal component analysis (PCA) is one of the multivariate statistical techniques, which are a powerful tool for investigating and summarizing underlying trends in complex data structure (Legendre & Legendre, 1998). The data were standardized to mean zero and variance of one before computing principal component analysis. This analysis reflects the importance of the largest contributor to the total variation at each axis for differentiation (Sharma, 1998). Holland (2008) suggested that standard criteria permit ignore components whose variance explained is <1 when a correlation matrix is used. According to Brejda et al. (2000), data were considered in each component with an eigen value >1, which determined at least 10% of the variation. The higher eigen values were considered as best representative of system attributes in principal components.

2.4.5 | Correlation data analysis

Phenotypic correlations between two traits were estimated using the formula suggested by Johnson et al. (1955) and Singh and Chaudhury (1985).

$$r_{pxy} = \frac{cov_{pxy}}{\sqrt{\sigma^2_{px} \cdot \sigma^2_{py}}}$$

where r_{pxy} is the phenotypic correlation coefficient between character x and y ; cov_{pxy} is the phenotypic covariance between character x and y σ^2_{px} is the phenotypic variance for character x σ^2_{py} is the phenotypic variance for character y .

The coefficient of correlation at the phenotypic level was tested for significance by comparing the values of correlation

coefficient with tabulated t -value at $g-2$ degree of freedom, where “ g ” is number of genotypes. However, the coefficient of correlations at genotypic level was tested for the significance using the formula described by Robertson (1959).

2.4.6 | Qualitative data analysis and clustering

Morphological frequency distributions were estimated based on the characters observed in the accessions. To assess the phenotypic diversity of all accessions, the Shannon–Weaver diversity index (H') was computed using the phenotypic frequencies. The Shannon–Weaver was estimated using the following formula (Shannon, 1949):

$$H' = -\sum_{i=1}^n p_i \ln p_i$$

where p_i is the proportion of accessions in the i th class of the trait to the total number of accessions grouped in the trait, where “ n ” is the number of phenotypic classes in the trait. As adopted by Islam et al. (2019), H' is classified as low ($H' < 0.50$), medium ($H' = 0.50-0.75$), and high ($H' \geq 0.75$). Clustering analysis was performed based on unweighted paired grouping methods with arithmetic means (UPGMA). The numbers of clusters were determined using the cubic clustering criteria as described by Mohammadi and Prasanna (2003). The appropriate numbers of clusters were determined from the values of pseudo-F and pseudo-T2 using the SAS software (SAS, 2019).

3 | RESULTS

3.1 | ANOVA and mean performance

Since each year ANOVA showed the variance was normal and homogenous, combined analysis was done. Combined ANOVA over the 2 years revealed treatments showed a significant ($p \leq 0.01$) effect except for capsule diameter and capsule diameter. However, year $\times$ genotype interaction effects were non-significant in all the studied quantitative traits (Table 1). In addition, qualitative traits showed medium to high diversity difference among 64 genotypes of Ethiopian black cumin (Figure S1).

3.1.1 | Mean performance of phenological attributes

Based on the result presented in Table S3, there was a highly significant ($p \leq 0.01$) difference among genotypes for days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, plant height, primary branches per

TABLE 1 Combined analysis of variance for 16 characters of 64 black cumin accessions evaluated for 2 years.

SV	df	DBF	DCF	DF	DM	PH	NPB	NSB	TSW	NCP	CL	CD	NSC	SYPP	SYPH	BYPH	HI
Replication	1	26.32**	5.54*	2.30 ns	10.57**	3.26 ns	16.73**	13.89**	1.18 ns	14.58**	6.93**	0.08 ns	8.95**	40.32**	63.97**	206.75**	9.46**
Year	1	8.46**	73.35**	10.75**	90.55**	12.64**	8.17**	45.94**	54.44**	65.24**	54.06**	1.72 ns	4.60*	8.16**	3.29 ns	1.05*	1.44 ns
Block	7	0.82 ns	5.97**	2.16*	5.10**	0.53 ns	0.67 ns	1.27 ns	1.75 ns	2.36*	2.15*	1.25 ns	1.95 ns	0.95 ns	0.81 ns	3.46**	1.33 ns
Block(Replication)	7	1.56 ns	2.08 ns	2.76*	3.13**	0.87 ns	0.22 ns	1.11 ns	2.81**	3.29**	1.71 ns	1.98 ns	2.08 ns	2.13 ns	0.97 ns	1.66 ns	1.70 ns
Treatment	63	6.01**	41.13**	13.03**	5.86**	2.26**	2.08**	4.70**	7.30**	24.81**	0.79 ns	1.24 ns	3.63**	57.28**	10.27**	30.30**	24.38**
Year $\times$ treatment	63	1.2 ns	1.15 ns	1.44 ns	0.41 ns	0.97 ns	1.02 ns	1.36 ns	1.29 ns	0.74 ns	1.15 ns	1.2 ns	0.40 ns	0.99 ns	0.46 ns	0.40 ns	0.60 ns
Residual	113	14.63	6.23	9.31	22.18	30.54	1.24	4.54	0.05	7.29	0.02	0.01	106.52	0.12	0.02	0.34	6.78
CV		6.21	3.05	4.38	3.80	14.55	19.71	13.88	8.46	12.22	12.13	11.97	12.03	6.10	12.73	9.41	14.58

* and ** are significant at 0.05 and highly significant at 0.01. ns: non-significant at 0.05.

Abbreviations: BYPH, biomass yield per hectare (t ha^{-1}); CD, diameter of capsules (cm); CL, length of capsules (cm); DBF, days to bud formation; DCF, days to capsule formation; DF, days to 50% flowering; DM, days to maturity; HI, harvest index (%); NCP, capsules per plant; NPB, primary branches per plant; NSB, secondary branches per plant; NSC, seeds per capsule; PH, plant height (cm); SV, source of variation; SYPH, seed yield per hectare (t ha^{-1}); SYPP, seed yield per plant (g); TSW, 1000-seed weight (g).

plant, secondary branches per plant, and 1000 seed weight with wider ranges between minimum and maximum values. The days to bud formation performance ranged from 53.75 to 80.25 days, with a mean value of 61.59 days. The highest mean days to bud formation was obtained from genotypes 23,704, 23,705, and 23,706, whereas the least mean value was obtained equally from five genotypes, namely, 28,585, 28,592, 28,593, 30,785, and 19,935 (Table S3). This result indicates that the highest and least number of days imply the late and earliness of the genotype to reach bud formation, respectively. The days to capsule formation performance ranged from 69.25 to 107.75 days, with a mean value of 81.84 days. The highest mean days to capsule formation was obtained from genotype 23,704, 23,705, and 23,706, whereas the least mean value was obtained from genotypes 28,591, 28,588, 30,787, 28,585, and 28,595 (Table S3). This result indicates the highest and least number of days implies the late and earliness of the genotype to reach capsule formation, respectively. The days to 50% flowering performance ranged from 60.25 to 91.50 days, with a mean value of 69.59 days. The highest mean days to 50% flowering was obtained from genotype 23,706, whereas the least mean value was obtained equally from five genotypes, namely, 28,585, 30,788, 28,594, 28,593, and 30,790 (Table S3). This result indicates the highest and least number of days implies the late and earliness of the genotype to reach 50% flowering, respectively. The days to maturity performance ranged from 114.00 to 149.75 days, with a mean value of 123.96 days. The highest mean days to maturity was obtained from genotypes 23,704 and 23,706, whereas the least mean value was obtained equally from three genotypes, namely, 28,591, 19,935, and 30,790. This result indicates the highest and least number of days implies the late and earliness of the genotype to reach maturity, respectively. Accordingly, genotypes 23,704 and 23,706 were matured late, whereas genotypes 28,591, 19,935, and 30,790 matured earlier. The number of primary branches per plant performance ranged from 3.75 to 9.00, with a mean value of 5.66. The highest number of primary branches was obtained from genotypes 23,704 and 23,705, whereas the least mean value was obtained equally from three genotypes, namely, 20,798, 20,790, and 27,628. The number of secondary branches per plant performance ranged from 9.50 to 21.50, with a mean value of 15.35. The highest number of secondary branches was obtained from genotypes 31,106, 23,705, 9067, 19,926, 23,706, and 9068, whereas the least mean value was obtained equally from three genotypes, namely, 20,794, 31,112, and 20,793. The plant height performance ranged from 28.75 to 49.59 cm, with a mean value of 37.99 cm. The highest mean plant height was obtained from genotypes 20,941, 23,705, 23,704, and 23,706, followed by genotypes 19,926, 31,108, 9068, 31,109, 27,627, 31,114, 19,931, and 31,107, whereas the least mean value was obtained equally from five genotypes, namely, 28,595, 30,789, 30,791, 30,789, and 20,790 (Table S3). Thousand seed weight performance ranged from 2.18 to 4.06 g, with

a mean value of 2.64 g. The highest mean 1000 seed weight was obtained from genotype 31,106, followed by genotypes 23,705, 23,704, 30,790, and 19,935, whereas the least mean value was obtained equally from three genotypes, namely, 19,926, 27,626, and 19,932 (Table S3).

3.1.2 | Mean performance of growth, yield, and yield related attributes

There was a significant difference among genotypes for capsules per plant, seeds per capsule, seed yield per plant, seed yield per hectare, biomass yield per hectare, and harvest index with wider ranges between the minimum and the maximum values, but genotypes did not show any significant difference with capsule length and diameter (Table S4). The number of capsules per plant performance ranged from 11.75 to 43.25, with a mean value of 22.11. The highest mean number of capsules was obtained from genotypes 31,109, 31,114, 31,121, and 30,786, whereas the least mean value was obtained equally from five genotypes, namely, 30,793, 28,595, and 30,790 (Table S4). The number of seeds per capsule performance ranged from 63.50 to 122.75, with a mean value of 85.83. The highest mean number of seeds was obtained from genotypes 9068 and 19,926, whereas the least mean value was obtained equally from five genotypes, namely, 20,797, 20,790, and 28,588 (Table S4). The seed yield per plant performance ranged from 3.37 to 9.15 g, with a mean value of 5.60 g. The highest mean seed yield was obtained from genotype 31,121, whereas the least mean value was obtained equally from five genotypes, namely, 25,888, 30,793, and 27,629 (Table S4). The seed yield performance ranged from 0.66 to 1.69 t ha⁻¹, with the mean of 1.02 t ha⁻¹. The highest mean seed yield per hectare was obtained from genotypes 20,942, 31,114, and 9068, whereas the least mean value was obtained from 19,931, 27,629, and 30,792 (Table S4). The biomass yield per hectare performance ranged from 2.78 to 9.29 t ha⁻¹, with a mean value of 6.23 t ha⁻¹. The highest mean biomass yield per hectare was obtained from genotypes 31,121, 31,112, 31,109, 27,627, and 9068, whereas the least mean value was obtained equally from five genotypes, namely, 28,586, 27,626, and 20,792 (Table S4). The harvest index performance ranged from 10.43% to 43.09%, with a mean value of 17.85%. The highest mean harvest index was obtained from genotypes 27,626 and 20,792, whereas the least mean value was obtained equally from five genotypes, namely, 30,792, 31,106, 27,625, and 19,931 (Table S4).

3.2 | Estimates of phenotypic and GCV

Estimates of phenotypic (σ^2_p) and genotypic (σ^2_g) variances, and PCV and GCV are given in Table 2. The GCV and PCV ranged from 3.81% to 49.84% and 6.50% to 50.90%, respectively, for 16 traits of 64 black cumin genotypes. Seed

TABLE 2 Range, mean, variances, broad sense heritability, coefficient of variance, genetic advance, and genetic advance mean percent of 16 characters of 64 black cumin accessions.

Traits	Range	Mean $\pm$ SE	σ^2_g	σ^2_p	H^2	GCV	PCV	GA	GAM
DBF	53.75-80.25	61.59 $\pm$ 2.71	36.63	43.95	83.35	9.83	10.76	11.40	18.51
DCF	69.25-107.75	81.84 $\pm$ 1.76	124.97	128.08	97.57	13.66	13.83	22.78	27.84
DF	60.25-91.50	69.59 $\pm$ 2.16	55.95	60.60	92.32	10.75	11.19	14.83	21.31
DM	114.00-149.75	123.96 $\pm$ 3.33	53.91	65.00	82.94	5.92	6.50	13.79	11.13
PH	28.75-49.585	37.99 $\pm$ 3.91	19.19	34.45	55.68	11.53	15.45	6.74	17.75
NPB	3.75-9.00	5.66 $\pm$ 0.79	0.67	1.29	51.86	14.46	20.07	1.22	21.47
NSB	9.50-21.50	15.35 $\pm$ 1.51	8.40	10.67	78.74	18.88	21.28	5.31	34.57
TSW	2.18-4.06	2.64 $\pm$ 0.14	0.16	0.18	86.29	15.18	16.10	0.76	28.66
NCP	11.75-43.25	22.11 $\pm$ 1.91	86.82	90.46	95.97	42.15	43.03	18.83	85.18
CL	1.05-1.31	1.17 $\pm$ 0.10	0.00	0.01	26.60	3.81	8.51	0.05	4.67
CD	0.68-0.94	0.81 $\pm$ 0.0	0.00	0.01	19.18	3.89	12.31	0.04	4.87
NSC	63.50-122.75	85.83 $\pm$ 7.30	140.19	193.45	72.47	13.80	16.21	20.79	24.23
SYPP	3.37-9.15	5.6 $\pm$ 0.24	3.29	3.35	98.25	32.37	32.66	3.71	66.21
SYPH	0.66-1.69	1.02 $\pm$ 0.10	0.08	0.09	90.26	27.80	29.48	0.56	54.90
BYPH	2.78-9.29	6.23 $\pm$ 0.41	5.04	5.21	96.70	36.02	36.63	4.55	73.07
HI	10.43-43.09	17.85 $\pm$ 1.84	79.18	82.57	95.90	49.84	50.90	17.98	100.70

Abbreviations: BYPH, biomass yield per hectare (t ha^{-1}); CD, diameter of capsules (cm); CL, length of capsules (cm); DBF, days to bud formation; DCF, days to capsule formation; DF, days to 50% flowering; DM, days to maturity; GA, genetic advance; GAM, genetic advance as the percent of mean; GCV, genetic coefficient of variance; H^2 , broad sense heritability; HI, harvest index (%); NCP, capsules per plant; NPB, primary branches per plant; NSB, secondary branches per plant; NSC, seeds per capsule; PCV, phenotypic coefficient of variance; PH, plant height (cm); SYPH, seed yield per hectare (t ha^{-1}); SYPP, seed yield per plant (g); TSW, 1000 seed weight (g); σ^2_g , genotypic variance; σ^2_p , phenotypic variance.

yield per plant, seed yield per hectare, biomass yield per hectare, and harvest index had high GCV and PCV values, while days to capsule formation, days to 50% flowering, plant height, 1000 seed weight, and seeds per capsule had moderate GCV and PCV ranges from 10.75% to 16.21%. In addition, days to maturity and capsule length had low value of GCV and PCV. The estimate of PCV was moderate for days to bud formation (10.76%) and capsule diameter (12.31), while PCV was high for primary (20.07%) and secondary (21.28) branches. The estimates of GCV were <10% for days to bud formation and capsule diameter, while GCV was moderate for primary (14.46%) and secondary (18.88%) branches (Table 2).

3.3 | Estimates of heritability and GA

Broad sense heritability (H^2) estimated for 16 traits of 64 black cumin genotypes was found in the ranges from 19.18% (capsule diameter) to 98.25% (for seed yield per plant), while GA as percentage of mean ranged from 4.67% (for capsule diameter) to 100.70% (for harvest index) (Table 2). Accordingly, both H^2 and GAM estimates were high for days to bud formation, days to capsule formation, days to 50% flowering, number of secondary branches, 1000 seed weight, capsules per plant, seeds per capsule, seed yield per plant, seed yield

per hectare, biomass yield per hectare, and harvest index. High estimates of H^2 coupled with medium GAM values for days to maturity. Moderate estimates of H^2 coupled with high GAM values for plant height and primary branches, whereas low values were estimated both for H^2 and GAM for capsule length and capsule diameter.

3.4 | Principal component analysis

Principal component analysis results of 16 quantitative traits are presented in Table 3. The first four principal component axes (PCA1 to PCA5) accounted for a varied percentage of total variance that ranged from 7% to 32% and accounted for 72% of the total variation with eigen values ranged from 1.222 to 5.50. The PCA1 contributed 32% to the total variability among genotypes, while PCA2, PCA3, PCA4, and PCA5 exhibited 13%, 12%, 8%, and 7% contributions to the total variability, respectively. Days to capsule formation, plant height, days to bud formation, days to 50% flowering, and days to maturity had a higher contribution to PCA1. The harvest index, followed by biomass yield per hectare and 1000 seed weight, had a larger contribution to PCA2. Seed yield per plant had relatively more contribution to the total variance of PCA3, followed by capsule length and capsule diameter. Harvest index, 1000 seed weight, primary branches, capsule

TABLE 3 Eigen values, difference, proportion, cumulative variance, and component scores of the first five principal components for quantitative characters in 64 black cumin accessions.

Variables	PCI	PCII	PCIII	PCIV	PCV
Eigen value	5.5	2.2	1.96	1.33	1.22
Difference	3.3	0.24	0.63	0.11	0.26
Proportion (variability)	0.32	0.13	0.12	0.08	0.07
Cumulative	0.32	0.45	0.57	0.65	0.72
DBF	0.334	−0.054	0.145	−0.007	−0.105
DCF	0.366	−0.148	0.162	−0.167	−0.048
DF	0.32	−0.157	0.238	−0.259	0.069
DM	0.32	−0.166	0.036	−0.181	−0.073
PH	0.336	−0.013	0.25	0.106	−0.022
NPB	0.267	0.094	0.097	0.354	−0.329
NSB	0.232	0.097	−0.007	0.34	−0.365
TSW	0.127	0.328	−0.26	0.374	−0.178
NCP	0.194	−0.007	−0.199	0.029	0.258
CL	−0.013	0.254	0.379	0.355	0.296
CD	−0.158	0.299	0.347	0.189	0.302
NSC	0.13	0.153	0.189	−0.074	0.394
SYPP	0.253	0.166	−0.454	−0.012	0.266
SYPH	0.227	−0.258	0.029	0.234	0.321
BYPH	0.188	0.443	0.113	−0.289	0.015
HI	−0.015	−0.542	−0.073	0.417	0.241

Abbreviations: BYPH, biomass yield per hectare (t ha^{-1}); CD, diameter of capsules (cm); CL, length of capsules (cm); DBF, days to bud formation; DCF, days to capsule formation; DF, days to 50% flowering; DM, days to maturity; HI, harvest index (%); NCP, capsules per plant; NPB, primary branches per plant; NSB, secondary branches per plant; NSC, seeds per capsule; PCA1, first principle component axis; PCA2, second principle component axis; PCA3, third principle component axis; PCA4, fourth principle component axis; PCA5, fifth principle component axis, and number in parenthesis indicated the contribution of trait to the PCAs; PH, plant height (cm); SYPH, seed yield per hectare (t ha^{-1}); SYPP, seed yield per plant (g); TSW, 1000 seed weight (g).

length, and secondary branches had relatively more contribution to the total variance of PCA4. Finally, seeds per capsule, secondary branches, primary branches, seed yield per hectare, and capsule diameter had relatively more contribution to the total variance of PCA5.

3.5 | Analysis of correlation data

3.5.1 | Correlation coefficients of yield with other traits

The results of coefficients of correlations are presented in Table 4. Seed yield had positive and significant correlation coefficients with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, plant height (cm), primary branches per plant, and capsules per plant at phenotypic levels. Seed yield per hectare is highly and positively correlated with days to bud formation, days to capsule formation, days to 50% flowering, plant height, and capsules per plant. Seed yield per hectare also positively and significantly correlated with days to maturity and primary branches

per plant. It also had a positive correlation with length of capsules, seeds per capsule, seed yield per plant, biomass yield per hectare, and harvest index but a negative correlation with secondary branches per plant, 1000 seed weight, and diameter of capsules (Table 4).

3.5.2 | Correlation coefficients among other traits

Days to bud formation are highly and positively correlated with days to capsule formation, days to 50% flowering, days to maturity, plant height, primary branches per plant, capsules per plant, seeds per capsule, and seed yield per hectare but highly and negatively correlated with diameter of capsules. Days to bud formation also positively and significantly correlated with seed yield per plant and negatively and significantly correlated with harvest index. It also had a positive correlation with secondary branches per plant, 1000 seed weight, length of capsules, and biomass yield per hectare (Table 4). Days to capsule formation highly and positively correlated with days to bud formation, days to 50% flowering, days to maturity,

TABLE 4 Phenotypic correlation coefficients of quantitative traits among 64 accessions of black cumin.

Trait	DBF	DCF	DF	DM	PH	NPB	NSB	TSW	NCP	CL	CD	NSC	SYPP	SYPH	BYPH	HI
DBF																
DCF	0.96**															
DF	0.94**	0.94**														
DM	0.86**	0.83**	0.81**													
PH	0.78**	0.81**	0.79**	0.73**												
NPB	0.38**	0.40**	0.30*	0.37**	0.44**											
NSB	0.14	0.21	0.21	0.21	0.36**	0.54**										
TSW	0.02	0.01	-0.02	0.12	-0.02	0.12	0.01									
NCP	0.47**	0.49**	0.48**	0.44**	0.49**	0.21	0.11	0.12								
CL	0.17	0.18	0.12	0.04	0.38**	0.24	0.12	-0.04	-0.003							
CD	-0.35**	-0.42**	-0.38**	-0.46**	-0.31*	-0.16	-0.24	-0.13	-0.30*	0.42**						
NSC	0.38**	0.34**	0.42**	0.36**	0.44**	0.25*	0.07	0.07	0.29*	0.19	-0.01					
SYPP	0.29*	0.26*	0.27*	0.33**	0.28*	0.16	0.09	0.48**	0.48**	-0.03	-0.26*	0.28*				
SYPH	0.44**	0.38**	0.37**	0.31*	0.42**	0.28*	-0.001	-0.01	0.40**	0.01	-0.20	0.22	0.20			
BYPH	0.22	0.23	0.26*	0.23	0.31*	0.23	0.25*	0.26*	0.24	0.26*	0.10	0.36**	0.34**	0.19		
HI	-0.31*	-0.32**	-0.34**	-0.34**	-0.25*	-0.005	-0.074	-0.15	-0.21	-0.04	0.18	-0.19	-0.17	0.15	-0.42**	

* and ** are significant at 0.05, and highly significant 0.01 level of probability, respectively. Ns: non-significant at 0.05.

Abbreviations: BYPH, biomass yield per hectare (t ha^{-1}); CD, diameter of capsules (cm); CL, length of capsules (cm); DBF, days to bud formation; DCF, days to capsule formation; DF, days to 50% flowering; DM, days to maturity; HI, harvest index (%); NCP, capsules per plant; NPB, primary branches per plant; NSB, secondary branches per plant; NSC, seeds per capsule; PH, plant height (cm); SYPH, seed yield per hectare (t ha^{-1}); SYPP, seed yield per plant (g); TSW, 1000 seed weight (g).

plant height, primary branches per plant, capsules per plant, seeds per capsule, and seed yield per hectare but highly and negatively correlated with diameter of capsules and harvest index. Days to capsule formation also positively and significantly correlated with seed yield per plant and negatively and highly significantly correlated with diameter of capsules and harvest index. It also had a positive correlation with secondary branches per plant, 1000 seed weight, length of capsules, and biomass yield per hectare (Table 4). Days to 50% flowering were highly correlated with days to bud formation, days to capsule formation, days to maturity, plant height, capsules per plant, seeds per capsule, and seed yield per hectare. Days to 50% flowering, also positively and significantly correlated with primary branches per plant, seed yield per plant, and biomass yield per hectare negatively and highly significantly correlated with diameter of capsules and harvest index. It also had a positive correlation with secondary branches per plant and length of capsules but a negative correlation with 1000-seed weight (Table 4). Days to maturity highly correlated with days to bud formation, days to capsule formation, days to 50% flowering, plant height, primary branches per plant, capsules per plant, seeds per capsule, and seed yield per plant. Days to maturity also positively and significantly correlated with seed yield per hectare and negatively and highly significantly correlated with diameter of capsules and harvest index. It also had a positive correlation with secondary branches per plant, 1000 seed weight, length of capsules, and biomass yield per hectare (Table 4). Plant height was highly correlated with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, primary branches per plant, secondary branches per plant, capsules per plant, length of capsules, seeds per capsule, and seed yield per hectare. Plant height also positively and significantly correlated with seed yield per plant and biomass yield per hectare and negatively and significantly correlated with diameter of capsules and harvest index. It also had a negative correlation with 1000 seed weight (Table 4). Primary branches per plant were highly correlated with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, plant height, and secondary branches per plant. Primary branches per plant also positively and significantly correlated with seeds per capsule, seed yield per plant, and seed yield per hectare. It also had a positive correlation with 1000 seed weight, capsules per plant, length of capsules, seed yield per plant, and biomass yield per hectare but a negative correlation with diameter of capsules and harvest index (Table 4). Secondary branches per plant were highly correlated with plant height and primary branches per plant. Secondary branches per plant also positively and significantly correlated with biomass yield per hectare. It also had a positive correlation with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, 1000 seed weight, capsules per plant, length of capsules, seeds per capsule, and seed yield per plant but a negative cor-

relation with diameter of capsules, seed yield per hectare, and harvest index (Table 4). Thousand seed weight is highly correlated with only seed yield per plant. Thousand seed weight was also positively and significantly correlated with biomass yield per hectare. It also had a positive correlation with days to bud formation, days to capsule formation, days to maturity, primary branches per plant, secondary branches per plant, capsules per plant, and seeds per capsule but a negative correlation with days to 50% flowering, plant height, length of capsules, diameter of capsules, seed yield per hectare, and harvest index (Table 4). Capsules per plant were highly correlated with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, plant height, seed yield per plant, and seed yield per hectare. Capsules per plant also positively and significantly correlated with seeds per capsule and negatively and significantly correlated with diameter of capsules. It also had a positive correlation with primary branches per plant, secondary branches per plant, 1000 seed weight, and biomass yield per hectare but a negative correlation with length of capsules and harvest index (Table 4). Length of capsules highly and positively correlated with plant height and diameter of capsules. Length of capsules also positively and significantly correlated with biomass yield per hectare. It also had a positive correlation with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, primary branches per plant, secondary branches per plant, seeds per capsule, and seed yield per hectare but a negative correlation with 1000 seed weight, capsules per plant, seed yield per plant, and harvest index (Table 4). Diameter of capsules highly correlated positively with length of capsules but highly correlated negatively with days to bud formation, days to capsule formation, days to 50% flowering, and days to maturity. Diameter of capsules also negatively and significantly correlated with plant height, capsules per plant, and seed yield per plant. It also had a positive correlation with biomass yield per hectare and harvest index but a negative correlation with primary branches per plant, secondary branches per plant, 1000 seed weight, seeds per capsule, and seed yield per hectare (Table 4). Seeds per capsule were highly and positively correlated with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, plant height, and biomass yield per hectare. Seeds per capsule also positively and significantly correlated with primary branches per plant, capsules per plant, and seed yield per plant. It also had a positive correlation with secondary branches per plant, 1000 seed weight, length of capsules, and seed yield per hectare, but a negative correlation with diameter of capsules and harvest index (Table 4). Seed yield per plant was highly and positively correlated with days to maturity, 1000 seed weight, capsules per plant, and biomass yield per hectare. Seed yield per plant also positively and significantly correlated with days to bud formation, days to capsule formation, days to 50% flowering, plant height, and seeds

per capsule but negatively and significantly correlated with diameter of capsules. It also had a positive correlation with primary branches per plant, secondary branches per plant, and seed yield per hectare but a negative correlation with length of capsules and harvest index (Table 4). Biomass yield per hectare is highly and positively correlated with seeds per capsule and seed yield per plant but highly correlated negatively with harvest index. Biomass yield per hectare also positively and significantly correlated with days to 50% flowering, plant height, secondary branches per plant, 1000 seed weight, and length of capsules. It also had a positive correlation with days to bud formation, days to capsule formation, days to maturity, primary branches per plant, capsules per plant, diameter of capsules, and seed yield per hectare (Table 4). Harvest index highly and negatively correlated with days to capsule formation, days to 50% flowering, days to maturity, and biomass yield per hectare. Harvest index also negatively and significantly correlated with days to bud formation and plant height. It also had a positive correlation with diameter of capsules and seed yield per hectare but a negative correlation with primary branches per plant, secondary branches per plant, 1000 seed weight, capsules per plant, length of capsules, seeds per capsule, and seed yield per plant (Table 4).

3.6 | Analysis of qualitative data

3.6.1 | Distribution in characters

Accessions showed high to medium variations in the qualitative characters (Table S5). Branch Density: 39.06% of accessions were observed as low, 29.69% showed average, and 31.25% showed high (Table S5). Similarly, the distribution of branching pattern was observed to be non-branching (15%), basal branching (44.06%), and top branching (40.94%) among accessions. The capsule hairiness of the accessions varied more, with 39.06% having a medium hairiness, 26.56% having strong or profuse, 18.75% having weak or sparse hair, and 15.53% having a glabrous (hair absent) (Table S5). Some characters showed medium variability, such as flower color (62.50% white and 37.50% pale blue), plant growth type (60.94% determinate and 39.06% indeterminate), and stem shape in cross-section (54.69% round and 45.31% square). Furthermore, accessions displayed wide variation in the following leaf characteristics: the leaf color (48.44% green, 28.13% green with yellowish cast, 14.06% green with blue-gray cast, and 9.38% green with purple cast), the leaf area (51.56% broad width, 29.69% average width, and 18.75% narrow) (Table S5). The stem elasticity was 43.75% low, 40.63%, 17.81% moderate, and 7.81% high. In a similar way, the stem hairiness was observed to be weak or sparse (42.19%), glabrous (hair absent) (31.25%), and medium (26.56%), while the stem thickness was observed to

be very thick (48.44%), thick (20.31%), medium (20.31%), and narrow (10.94%). Flowering rate is as follows: 46.88% of accessions were observed as moderate, 28.13% were observed as low, and 25% were observed as high (Table S5). In a similar fashion, the flowering time was observed to be middle flowering (51.56%), early flowering (37.5%), and late flowering (10.94%) among accessions. The follicle size of the accessions varied more, with 40.63% having a moderate size, 26.56% having large, 23.44% having small, and 9.38% having very small (Table S5). The growth power was 26.56% very strong, 25% strong, 25% moderate, 14.06% weak, and 9.38% very weak. In a similar fashion, the main stem color is as follows: 43.75% purplish green, 21.88% purple, 18.75% yellow, and 15.63% green. Similarly, the plant growth habit was observed to be prostrate (43.75%), erect (hair absent) (31.25%), and semi-erect (25.00%), while the root system was observed to be deep thin taproot (46.88%), shallow fibrous (28.13%), and tuberous thick taproot (25.00%). Similarly, the distribution of seed ripening was observed to be early ripening (48.44%), medium ripening (37.50%), and late ripening (14.06%) among accessions (Table S5). Moreover, for capsule spiciness, 48.44%, 37.50%, and 14.06% of seeds tested mild, medium, and hot, respectively.

3.6.2 | The Shannon–Weaver diversity index (H')

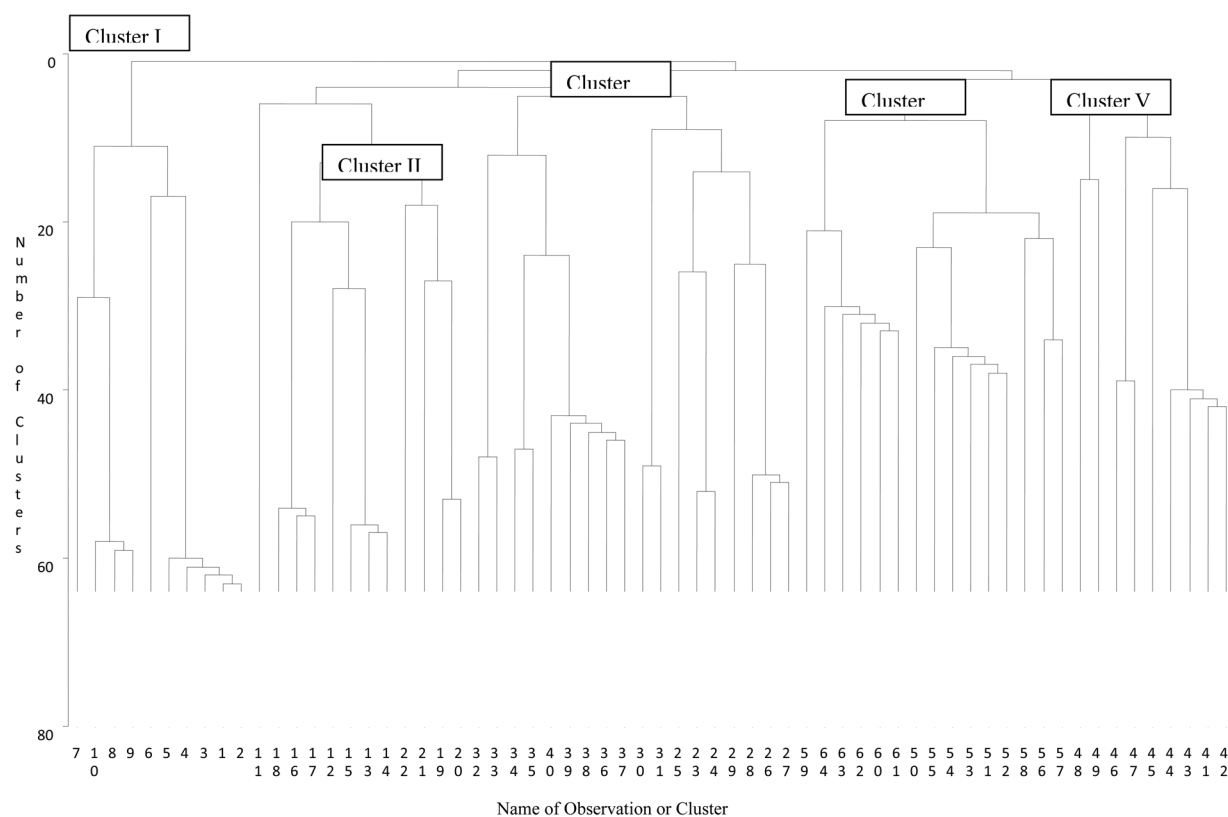
In this aspect, the H' value for most observed phenotypic characters exhibited a normal level of diversity among tested black cumin accessions, which ranged from 0.66 for flower color to 1.53 for growth power, with a mean value of 1.07 (Table S5). Based on this classification, three characters were categorized as medium diversity, such as flower color, plant growth type, and stem hairiness (Table S5). The rest of the 16 characters that were branch density, branching pattern, capsule hairiness, flowering rate, flowering time, follicle size, growth power, leaf area, leaf color, main stem color, plant growth habit, root system, seed ripening, stem thickness, stem hairiness, stem elasticity, and capsule spiciness had high genetic diversity.

3.6.3 | Cluster analysis

The Euclidean distance matrix of pair of genotypes estimated from quantitative traits was used to construct dendrograms based on the Unweighted Pair-group methods with Arithmetic Means (UPGMA). The 64 black cumin genotypes were grouped into five distinct clusters. Cluster III was the largest and consisted of 18 genotypes (28.13%) of the total genotypes. Cluster IV consisted of 15 (23.45%) and other three clusters (I, II, and V) consisted of 10 (15.61%), 12 (18.75%), and nine (14.06%), respectively (Table 5; Figure 1).

TABLE 5 Clusters of black cumin accessions based on qualitative characters.

Cluster	Number of accessions in each cluster	List of accessions within cluster	% of contribution
I	10	G1, G2, G3, G4, G5, G6, G7, G8, G9, & G10	15.61
II	12	G11, G12, G13, G14, G15, G16, G17, G18, G19, G20, G21, & G22	18.75
III	18	G23, G24, G25, G26, G27, G28, G29, G30, G31, G32, G33, G34, G35, G36, G37, G38, G39, & G40	28.13
IV	15	G50, G51, G52, G53, G54, G55, G56, G57, G58, G59, G60, G61, G62, G63, & G64	23.45
V	9	G41, G42, G43, G 44, G45, G46, G47, G48, & G49	14.06

**FIGURE 1** Dendrogram depicting dissimilarity of 64 black cumin genotypes by unweighted pair group method with arithmetic means (UPGMA) clustering method from Euclidean distances matrix estimated from 20 qualitative traits.

4 | DISCUSSION

4.1 | Mean performance of phenological, growth, yield, and yield related attributes

In the present study, the combined result showed that there were highly significant difference among genotypes for all traits except capsule length and diameter with wider ranges between minimum and maximum values. Supporting results were reported that 36 black cumin genotypes had 66–89 and 117–150 days to 50% flowering and days to maturity, respectively, as evaluated at Debre-Zeit (Tewodros et al., 2018).

Similarly, 20 black cumin genotypes were evaluated at three locations in Bale highlands (evaluated for 2 years) and were reported to have 142–150 days with a mean of 145 days to maturity (Fufa, 2016). The highest number of primary and secondary branches were registered for genotypes 30,763 (5.61) and 30,754 (5.12), respectively, while 30,772 had the lowest number of primary (3.86) and secondary branches (2.48) (Tewodros et al., 2018). Likewise, similar results were found in seed yield for tested 10 black cumin ecotypes by Golparvar et al. (2014). Gutiérrez-Prieto and Kirici (2021) report showed that under Mediterranean conditions, the life cycle of the plant lasts 225 days. The vegetative period lasted for 137

days and the reproductive period 88 days. Meanwhile, Haq et al. (2015) observed that the time required for 50% of the plants to reach the flower bud initiation was 146 days and registered the first capsule 78 days after sowing. In the study by Ürüsan (2016), the flowering stage started around 69.9 days after sowing and lasted roughly 43.9 days. The capsule setting stage for this study appeared 160 days after sowing, and 100% of the capsules had matured by day 225. Moreover, Asefa and Beriso (2020) reported that days to maturity and days to flowering ranged from 144.83 to 128.00 and 90.00 to 80.00, respectively. The highest means of total seed yield was 2.36 t ha^{-1} followed by 2.18 t ha^{-1} . This may be due to potential of the district for black cumin production than both sampled environment. Similar results were reported by Tewodros et al. (2018) on black cumin plant height, 1000 seed weight, seed yield per plant, and seed yield per plot. In addition, Ermias et al. (2015) reported plant height ranged from 45.88 to 52.20 with mean plant height of 49.43 cm at one location and 39.12–51.36 with mean 42.88 cm at the other location in Kaffa zone. Moreover, Fufa (2016) observed black cumin genotypes with shortest and tallest plant heights of 41.83 and 58.68 cm, respectively, and 20 genotypes had the overall mean plant height of 52.78 cm. This reflects the presence of variation among the tested genotypes. The variation in seed yield could be due to environmental, genetic, or interaction among these two factors (Rahimmalek et al., 2009; Shafie et al., 2009). The presence of variability among the black cumin genotypes was witnessed by the wide range of most of the traits studied. This showed that there is a possibility of improving these traits through selection. Fufa (2016) reported seed yields of 1.47, 0.92, and 0.79 t ha^{-1} at Sinana, Ginir, and Goro (all in Ethiopia), respectively. Ermias et al. (2015) reported mean seed yield of 612 kg ha^{-1} for three improved black cumin varieties cultivated by farmers at Kaya Kela experimental sites of Bonga Agricultural Research Center, Ethiopia. This indicated that black cumin germplasm in Ethiopia had a wide range of variation for seed yield. Emran et al. (2017) research result on thirty-seven accessions showed significant differences for all the characters, including seed yield per plant, during 2014. During 2015, the accessions did differ significantly for most characters except plant height and seeds per capsule. During the year 2014. During 2015, the accessions did differ significantly for most characters except plant height and seeds per capsule. The highest yielding accession gave 3.35 g per plant, while least yielding accession gave 1.44 g during 2014. In addition, Asefa and Beriso (2020) reported that the mean total seed yield of genotypes across environment ranged from 2.45 to 1.61 t ha^{-1} . The means for capsule number per plant, biomass, and primary branch ranged from 10.72 to 6.96, 63.74 to 39.79, and 4.67 to 3.33, respectively. Yewubdinber (2020) did research on 25 black cumin genotypes at Debre-Zeit Agriculture Research Center in 2018/2019. The results from ANOVA revealed the variation of the genotypes for seed

yield was between 1.86 and 2.87 t ha^{-1} with a mean of 2.29 kg ha^{-1} . Moreover, Basazinew et al. (2023) reported that combined ANOVA over the two locations revealed a significant effect for genotypes in all quantitative traits except number of primary branches per plant. The variability of the genotypes for days to maturity creates a great opportunity for developing early maturing varieties by improving the traits that are associate to days to maturity. This result would help the breeders to develop improved and suitable varieties for different agroecologies of the country. Saeed et al. (2016) in Iran indicated the significant difference between ecotypes for plant height, number of branches, number of capsules, days to beginning of blooming, days to full blooming, 1000 seed weight, and grain filling rate. The range in days to flowering and maturity will help to select early maturing lines for future use. The lowest genotype took 23 days for flowering. Highest harvest index ranged from 8.54% to 35.85% (Iqbal et al., 2019). The significant variation between the black cumin materials tested for the measured agro-morphological traits indicated the presence of high degree of phenotypic variability, and it implies the availability of great potential for future improvement of black cumin.

4.2 | Estimates of phenotypic and GCV

The GCV and PCV ranged from 3.81% to 49.84% and 6.50% to 50.90%, respectively, for 16 traits of 64 black cumin genotypes. Satesh et al. (2010), Bharathiveeraman et al. (2012), Nwangburuka et al. (2012), and Swati et al. (2014) concluded that the PCV and GCV are indications of the lower influence of environmental factors in the expression of traits and the higher chance to improve the traits through breeding. Similarly, Gholizade et al. (2019) reported high GCV and PCV for number of capsules and moderate PCV for number of primary branches. Preeti et al. (2019) also reported high GCV and PCV for the number of capsules. Tewodros et al. (2018) also reported low PCV and GCV estimates for days to 50% flowering, days to maturity, plant height, and 1000 seeds weight. The lower GCV than PCV values for the traits reflect the apparent variation is not only due to genotypic effect but also the involvement of environmental effects (Gholizade et al., 2019; Misra et al., 2013; Sarkar et al., 2015). Analysis of variability carried out for 10 characters in 16 diverse genotypes of black cumin revealed high GCV and PCV for secondary branches per plant, number of grains per fruit, number of fruits per plant, length of fruit (cm), and width of fruit (cm) (Singh & Singh, 2018). Moreover, Yewubdinber (2020) reported the GCV and PCV for 10 traits estimated in the range between 3.91% and 48.36% and 6.90% and 48.94%, respectively. High to moderate estimates of GCV and PCV were observed for number of capsules, number of secondary branches, and number of capsules. Basazinew et al. (2023)

indicated that the degree of variability for a given trait is mandatory for the improvement program of any crops, including spices. The research result showed that the estimated values of GCV ranged from 0.66% to 18.41% for days to maturity and seed yield per plot, respectively. Likewise, the values of PCV ranged from 1.37% to 22.96% for days to maturity and seed yield per plot, respectively.

4.3 | Estimates of heritability and GA

In the present study, broad sense heritability (H^2) estimated for 16 traits of 64 black cumin genotypes was from 19.18% (capsule diameter) to 98.25% (for seed yield per plant), while GA as percentage of mean ranged from 4.67% (for capsule diameter) to 100.70% (for harvest index). Similarly, PhaniKrishna et al. (2015) suggested that selection based on phenotypic performance of genotypes would be effective to improve the traits for which high GA as percent of mean coupled with high heritability estimates. Preeti et al. (2019) reported moderate heritability for day to 50% flowering and day to maturity. Moreover, estimates of broad sense heritability ranged from 30.17% for seed yield per plant to 90.31% for days to 50% flowering (Tewodros et al., 2018). In addition, the same author showed that high heritability values for days to 50% flowering (90.31%) and days to first flowering (87.34%). Moderate estimates for flower duration (71.82%), 1000 seeds weight (55.85), and plant height (47.48%). On the other hand, low heritability was recorded for biomass per plot (34.75%), days to maturity (31.75%), and seed yield per plant (30.17%) (Tewodros et al., 2018). Tamoor et al. (2018) investigated the interrelationship between quantitative characters in 13 black seed accessions under rainfed conditions in Pakistan and reported that heritability values were greater for primary branches per plant, plant height, number of capsules per plant, number of seeds per capsule, 1000 seed weight, and seed yield. Similarly, Singh and Singh (2018) reported that heritability estimates were high for number of seeds per capsule (0.86), number of capsule per plant (0.78), length of capsule (0.64), days to maturity (0.59), number of secondary branches per plant (0.56), and yield per plant (0.47). Iqbal et al. (2019) reported that heritability estimates in broad sense ranged from 0.28 for number of branches to 0.98 for days to maturity. High heritability (<50%) was observed for all the traits except number of branches, which indicated the scope of selection from this material. Parents diverse in nature could be selected to start hybridization as well as breeding program. Preeti et al. (2019) recorded high heritability estimates, and high estimates of GA as a percent of mean were observed for seed yield per plant, biological yield per plant, harvest index, and capsule per plant, indicating predominance of additive gene effects for these traits. The highest heritability estimates were found for biological yield, seed yield per

plant, number of capsules per plant, harvest index, days to flowering, test weight, and plant height. The characters like days to maturity, number of primary and secondary branches, and seeds per plant showed moderate estimates of heritability. This implies that selection can be exercised for these traits as they are less influenced by the environment. The GA was highest for seeds per plant, followed by harvest index, plant height, and days to 50% flowering. Genetic gain was highest for seed yield and biological yield and lowest for days to maturity and days to 50% flowering. Yewubdinber (2020) showed that broad sense heritability (H^2) and GA as percentage of mean (GAM) ranged from 32.16 to 99.74 and 4.58 to 98.61%, respectively. High to moderate estimates of H^2 and GAM were observed for number of capsules, number of secondary branches, and number of capsules. Basazinew et al. (2023) indicated that broad sense heritability for the studied quantitative traits ranged from the least 8.16% for number of seeds per capsule to the highest 89.47% for 1000 seed weight. Accordingly, high heritability values were obtained on 1000 seed weight (89.47%), plant height (78.3%), days to flowering (76.65%), days to full blooming (74.71%), number of capsules per plant (66.86%), seed yield per plot (64.33%), and days to 50% flowering (62.56%). Supporting results were reported by Tewodros et al. (2018) on black cumin days to maturity, days to flowering, and days to 50% flowering. Moderate heritability in broad sense was recorded on number of seeds per plant, seed yield per hectare, number of primary branches per plant, and seed yield per plant. However, low broad sense heritability values were showed by days to maturity and number of seeds per capsule. The high H^2 would be a close correspondence between the genotypic and phenotypic variations due to relatively small contribution of the environment to the phenotype expression of the trait (Singh et al., 1990).

4.4 | Principal component analysis

Similar result was reported at Debre-Zeit that the first four principal components accounted for about 76.05% in 33 black cumin genotype (Tewodros et al., 2018). In addition, Emran et al. (2017) reported that the PCA could extract only 84.18% and 84.12% of the variability among the accessions for all the traits. The first three PCAs could extract 55.79% during 2014 and 53.78 during 2015. During the years 2014 and 2015, the first PCA extracted 21.41% and 21.77%, the second PCA extracted 18.66% and 17.22%, and the third PCA 15.72% and 14.79%, respectively. The 37 accessions separated widely during both years, suggesting that the collections from India and Afghanistan are clearly distinct accessions with less genetic overlap among them. The Indian and Afghanistan accessions showed a clear separation in the PCA space. Yewubdinber et al. (2022) reported that PCA results of 12 quantitative traits. PCA1 to PCA4 accounted for a varied percentage of total

variance that ranged from 9.98% to 28.43% and accounted for 70.62% of the total variation. The PCA1 contributed 28.43% to the total variability among genotypes, while PCA2, PCA3, and PCA4 exhibited 18.91%, 13.30%, and 9.98% contribution to the total variability, respectively. Days to first flowering (24.44%), days to 50% flowering (23.50%), days to 90% maturity (21.64%), and plant height (9.02%) had a higher contribution to PCA1. The number of secondary branches (29.72%), followed by number of primary branches, number of capsules, and number of seed per capsule with 23.54%, 21.52%, and 10.99%, respectively, had larger contribution to PCA2. Seed yield (16.71%), number of seeds per capsule (13.34%), and 1000 seed weights (39.33%) had relatively more contribution to the total variance of PCA4. In addition, Iqbal et al. (2013) indicated that the greater part of genetic variation was accounted for by days to maturity, number of branches per plant, number of capsules per plant, biomass, and seed yield in PCA for quantitative traits. The accessions possessing the genes of interest for traits of economic importance were identified and hence are recommended to be utilized directly or included in hybridization program for varietal development. During 2009, six principal components were formed that contributed 87.85% of variability, while during 2010, three PCs were formed and contributed 79.75% of variability. For 2011, five PCs were formed and contributed 79.42% of variability. The PCs showed variable contribution of traits in each year; this might be attributed to the non-uniform climatic conditions over the years. High fluctuation of the seasonal rain, temperature, evaporation rate, and humidity influenced the seed and oil yield in the reported years. Moreover, Basazinew et al. (2023) reported that the first four principal components explained 81.7% of the variation that existed among the studied genotypes. Days to 50% flowering, days to maturity, days to full blooming, days to flowering, number of seeds per plant, seed yield per plant, and number of capsules per plant were the main contributor traits for the variation in the second PC, which contributed 24.6% from the total variations. The main contributor traits for the third PC were variations mainly in the seed yield per plot, seed yield per hectare, number of capsules per plant, number of seeds per plant, and seed yield per plant. Moreover, 1000 seed weight, plant height, and number of seeds per capsule were the main contributor traits for the variations in the fourth PC. The proportion of the total phenotypic variance of the genotypes accounted for by the third and fourth PCs was about 16.7% and 8.5%, respectively. Hence, the PCA confirmed that the collected Ethiopian black cumin genotypes have high diversity, and most of the traits considered seem to have high contributions toward the total phenotypic variability. Alboukadel (2017) described that traits such as days to flowering, days to full blooming, seed yield per plant, days to maturity, number of seeds per plant, days to 50% flowering, and plant height were the main contributor to the variation in the first PC,

whereas days to 50% flowering, days to maturity, days to full blooming, days to flowering, number of seeds per plant, seed yield per plant, and number of capsules per plant were the main contributor to the variation in PCA2. The traits that had more contribution to the total variance of each PCA suggested these traits were responsible for the differentiation of the black cumin genotypes into different groups.

4.5 | Analysis of correlation coefficients

Significant correlation of characters suggested that there is much scope for direct and indirect selection for further improvement. Significant correlation was observed among seed yield and other traits studied, but highly significant positive correlation with highly and positively correlated with days to bud formation, days to capsule formation, days to 50% flowering, plant height, and capsules per plant. This indicated that these traits are the major contributing traits for seed yield improvement in black cumin genotypes. Therefore, these characters should be considered while making selection for yield improvement in nigella. Similar results were reported by Fufa (2016), Tewodros et al. (2018), Iqbal et al. (2019), Yewubdinber (2020), Zigayalew (2020), and Basazinew et al. (2023). Days to bud formation were highly and positively correlated with days to capsule formation, days to 50% flowering, days to maturity, plant height, primary branches per plant, capsules per plant, seeds per capsule, and seed yield per hectare. Days to capsule formation highly and positively correlated with days to bud formation, days to 50% flowering, days to maturity, plant height, primary branches per plant, capsules per plant, seeds per capsule, and seed yield per hectare. Days to 50% flowering were highly correlated with days to bud formation, days to capsule formation, days to maturity, plant height, capsules per plant, seeds per capsule, and seed yield per hectare. Days to maturity were highly correlated with days to bud formation, days to capsule formation, days to 50% flowering, plant height, primary branches per plant, capsules per plant, seeds per capsule, and seed yield per plant. Plant height was highly correlated with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, primary branches per plant, secondary branches per plant, capsules per plant, length of capsules, seeds per capsule, and seed yield per hectare. Primary branches per plant were highly correlated with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, plant height, and secondary branches per plant. Secondary branches per plant were highly correlated with plant height and primary branches per plant. Thousand seed weight is highly correlated with only seed yield per plant. Capsules per plant were highly correlated with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, plant height, seed yield per plant,

and seed yield per hectare. Length of capsules highly and positively correlated with plant height and diameter of capsules. Diameter of capsules highly correlated positively with length of capsules but highly correlated negatively with days to bud formation, days to capsule formation, days to 50% flowering, and days to maturity. Seeds per capsule were highly and positively correlated with days to bud formation, days to capsule formation, days to 50% flowering, days to maturity, plant height, and biomass yield per hectare. Seed yield per plant is highly and positively correlated with days to maturity, 1000 seed weight, capsules per plant, and biomass yield per hectare. Biomass yield per hectare is highly and positively correlated with seeds per capsule and seed yield per plant. Harvest index highly and negatively correlated with days to capsule formation, days to 50% flowering, days to maturity, and biomass yield per hectare. Similar results were reported by Fufa (2018), Tewodros et al. (2018), Preeti et al. (2019), Iqbal et al. (2019), Yewubdinber (2020), Zigyalaw (2020), and Basazinew et al. (2023).

4.6 | Analysis of qualitative data

Similarly, an experiment was recorded for the experiment done to assess the diversity of 24 black cumin genotypes considering 10 qualitative parameters that were stem elasticity, flower color, seed ripening, follicle size, flowering rate, flowering time, leaf area, branch density, growth power, and stem thickness. Based on Shannon–Weaver diversity index (H'), all traits were categorized as high except flower color (Mehri et al., 2022). In this aspect, the H' value for most observed phenotypic characters exhibited a normal level of diversity among tested black cumin accessions, which ranged from 0.47 for flower color to 1.58 for growth power, with a mean value of 1.02 (Mehri et al., 2022). The result suggested that the genotypes grouped under same cluster had similarity for many traits but dissimilarity to other genotypes in other clusters with one or more traits. Cluster analysis sequesters genotypes into clusters, which exhibit high homogeneity within a cluster and high heterogeneity between clusters (Jaynes et al., 2003).

5 | CONCLUSION

The result of this study showed the existence of significant variation for all of the studied traits among black cumin genotypes collected from different regions of Ethiopia. The mean performance of the genotypes revealed wider ranges between the minimum and maximum values of all quantitative traits except capsule length and diameter; this showed the presence of significant variation among black cumin genotypes

included in this study. High to moderate estimates of GCV, PCV, H^2 , and GAM were observed for all parameters except days to bud formation, days to maturity, capsule length, and capsule diameter. About 76.05% of the variation that occurred among the studied genotypes was explained by the first five principal components. The PCA confirmed the presence of high phenotypic variability among the black cumin genotypes of Ethiopia. Regarding to the qualitative data, the evaluated accessions showed high diversity except for flower color, plant growth type, and stem hairiness. In the cluster analysis for qualitative traits for the 64 accessions are classified into five groups. The observed diversity among black cumin genotypes is an opportunity to improve the productivity of the crop either through selection or hybridization to combine desirable traits from different genotypes into the segregating or hybrid progenies. Moreover, a number of the key economical traits of black cumin, such as seed yield and yield-related traits, can be improved through selection. Nevertheless, the experiment has to be repeated at more locations to confirm the findings and to get consolidated information that is essential in breeding program of black cumin.

AUTHOR CONTRIBUTIONS

Solomon Kassaye Ejigu: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; writing—original draft; writing—review and editing. **Wosene Gebreselassie Abteu:** Investigation; project administration; software; supervision; writing—review and editing. **Ermias Lulekal Molla:** Data curation; investigation; project administration; supervision; validation; visualization; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data are available from the corresponding author upon request. Supplementary data to this article can be found online at <https://doi.org/10.5061/dryad.2fqz61316>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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