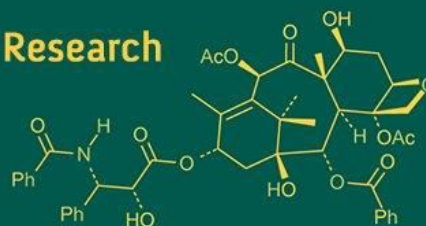
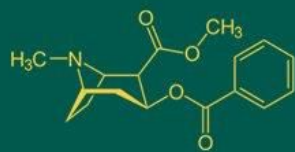


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(1): 629-634
www.biochemjournal.com
Received: 01-11-2025
Accepted: 04-12-2025

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Variability and genetic gain for yield and biofortification traits in lentil (*Lens culinaris* L.)

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i1Sh.7048>

Abstract

The present investigation was conducted to evaluate the extent of genetic variability and to understand the inheritance pattern of yield, yield-related traits, and micronutrient content in lentil (*Lens culinaris* L.). A set of diverse lentil genotypes was assessed for twelve quantitative characters comprising phenological traits, morphological attributes, yield components, and nutritional parameters such as iron (Fe) and zinc (Zn) content. Analysis of variance revealed highly significant differences among the genotypes for all the traits studied, indicating the presence of substantial genetic diversity within the experimental material. The estimates of phenotypic coefficient of variation (PCV) were consistently higher than the corresponding genotypic coefficient of variation (GCV) for all characters, suggesting varying degrees of environmental influence on trait expression. High GCV and PCV were observed for the total number of pods per plant, 1000-seed weight, iron content, zinc content, and biological yield, reflecting greater scope for selection. Heritability estimates ranged from low to high among the traits, with high heritability recorded for zinc content, days to maturity, grain yield, 1000-seed weight, and iron content. Importantly, high heritability coupled with high genetic advance as a percentage of mean was observed for 1000-seed weight, total number of pods per plant, iron content, and zinc content, indicating the predominance of additive gene action and the effectiveness of direct phenotypic selection. Moderate heritability and genetic advance were recorded for plant height, secondary branches per plant, biological yield, and grain yield, suggesting that improvement of these traits may require selection over multiple environments or generations. In contrast, days to 50% flowering, primary branches per plant, and number of seeds per pod exhibited low genetic advance, indicating limited response to direct selection. The study identified key yield and micronutrient traits with high genetic gain potential, which can be effectively exploited in lentil breeding programs aimed at improving productivity and enhancing nutritional quality through biofortification.

Keywords: Genetic advance, genetic variability, heritability, yield components

Introduction

Lentil (*Lens culinaris* L.), a member of the Leguminosae family, is a diploid ($2n = 2x = 14$), self-pollinated, and annual cool-season grain legume with a genome size of 4,063 Mbp is an important food component in most parts of the world (Faris *et al.*, 2013) ^[10]. The cultivated lentil, known scientifically as *Lens culinaris*, belongs to the genus *Lens*. Early researchers used morphological comparisons and "crossability" to classify different individuals within this genus. A comprehensive review of changes in *Lens* classification is extensive, but a summary reveals that the number of recognised species fluctuated over the years (Darai *et al.*, 2020) ^[8]. Lentil is widely consumed because of their rich nutritional profile, providing high levels of protein, essential vitamins, and important micronutrients that contribute significantly to human nutrition. In addition, its vegetative biomass is commonly utilized as fodder for livestock (Pandey and Bhatore, 2018) ^[19]. Lentil seeds contain high-quality protein, typically ranging from 22% to 35%, and are composed of a balanced array of amino acids, including lysine and isoleucine. Owing to these attributes, lentil serves as an economical and valuable protein source for both human consumption and animal feed in many parts of the world (Gaad *et al.*, 2018) ^[11]. Germplasm provides traits necessary for developing input-responsive genotypes with resistance to various biotic and abiotic stress. Therefore, evaluating germplasm under local environmental conditions is crucial to identify the potential of donor parents for breeding specific traits.

The processes of collecting, evaluating, and conserving germplasm are critical not only for current breeding efforts but also for upcoming crop improvement programs. The selection of genetically varied parents is based on the premise that crosses between genetically distant lines have a higher likelihood of producing superior segregants in the progeny. Several researchers have highlighted the importance of utilizing.

Genetic parameters such as variability, heritability, correlation, and path coefficients serve as essential tools for breeders in identifying superior parental lines and formulating effective selection strategies to enhance yield-related traits (Mohammed *et al.*, 2019) ^[17]. These parameters are important to the selection process in breeding programs aiming to improve seed yield. Identifying parameters that have positive correlations with yield can enhance selection efficiency, while traits with negative correlations, often due to developmental constraints, should be minimized (Noor *et al.*, 2017) ^[18]. Similar studies have found moderate heritability for traits, i.e., days to maturity and seed weight, whereas traits such as biological yield and seed yield have shown low heritability estimates.

Numerous studies worldwide have investigated genetic diversity in lentils and other legume crops using phenotypic and morpho-agronomic evaluations (Ates, 2019) ^[3]. Germplasm repositories represent invaluable resources, as they conserve a vast array of beneficial genes that can be exploited for crop improvement. However, their effective utilisation depends on a clear understanding of the magnitude and structure of genetic diversity present within the collections (Noor *et al.*, 2017) ^[18]. Genetic diversity can be assessed using both qualitative and quantitative traits, and a wide range of morphometric and statistical approaches have been applied to characterize and classify genotypes in

several crops, including mustard, pea, mungbean, alfalfa, cowpea, soybean, and lentil (Roy *et al.*, 2013) ^[22].

Materials and Methods

Experimental Site and Climatic Conditions

The present study of genetic parameters and phenotypic diversity for yield and micronutrient (iron and zinc) component traits in lentil was carried out during the *Rabi* season of 2023-24 at the P.G. Research Farm, College of Agriculture, Banda University of Agriculture and Technology, Banda, Uttar Pradesh, India. The experiment was conducted to evaluate 25 lentil genotypes, including two check varieties, under irrigated conditions and normal soil using a randomized block design (RBD). Each genotype was sown in a plot consisting of three rows, with each row measuring 2 meters in length and spaced 30 cm apart, covering a total plot area of 1.2 m². This site is located between 24053' and 25055'N latitude and 80007' and 81034'E longitude in the Bundelkhand region of Uttar Pradesh. The climatic conditions of this region are characterized by a hot and humid climate. Temperature varies from 4-50 °C during the colder days of the winter months to 45-50 °C in the hot summer. Apart from extreme temperatures, this area also experiences strong heat waves and dry, sandy summer breezes across its terrain.

Experimental Materials

The plant materials were comprised of fifty advanced breeding lines, including two check varieties, viz., PL 04 & PSL 9, for this experiment. The lentil varieties used as checks in the experiment are adopted varieties of the area. The list of genotypes used in the present study is given in Table 1.

Table 1: List of 25 genotypes and 2 checks with sources.

S. No.	Genotype Name	Source
1	X20125-109-513	ICARDA, AMLA, Sehore (MP)
2	IPL-406	BUAT, Banda
3	IPL-316	BUAT, Banda
4	10072/1712/4-1	ICARDA, AMLA, Sehore (MP)
5	PDL-1	BUAT, Banda
6	6002/7716/4-4-S2	ICARDA, AMLA, Sehore (MP)
7	ILL-753/ILL-8461	ICARDA, AMLA, Sehore (MP)
8	L-4076	IARI, New Delhi
9	ILL-6778 × ILL-5480-S3-S4	ICARDA, AMLA, Sehore (MP)
10	6002/LiRL-21-50-1-1/17-S3-S1	ICARDA, AMLA, Sehore (MP)
11	6002/LiRL-21-50-1-1-1/24-6	ICARDA, AMLA, Sehore (MP)
12	590/8461/2-4-S1	ICARDA, AMLA, Sehore (MP)
13	4605/4380/2SPS	ICARDA, AMLA, Sehore (MP)
14	ILL-7537/ILL-800-S4	ICARDA, AMLA, Sehore (MP)
15	X20125-146-S1	ILL 4605 × ILL 6002
16	X20125-171-17-S1	ICARDA, AMLA, Sehore (MP)
17	X20115-89-23-S4	ICARDA, AMLA, Sehore (MP)
18	L-4147	IARI, New Delhi
19	ILL-10657	BUAT, Banda
20	PL-04	BUAT, Banda
21	L-4729	IARI, New Delhi
22	PSL-9	BUAT, Banda
23	KM-1	BUAT, Banda
24	IPL-220	BUAT, Banda
25	Black Lentil	BUAT, Banda

Morphological Traits

Data were recorded from five randomly selected plants per genotype for the traits under study. Observations on the

following morphological traits were recorded on randomly selected plants in each plot for the entry of lentil. Days to 50% flowering (days), number of primary branches per

plant, number of secondary branches per plant, plant height (cm), number of pods per plant, total number of seed per pod, days to maturity(days), biological yield per plot (kg), 1000 grain weight (g), iron (mg/kg), zinc (mg/kg), Grain yield per plot (kg)

The estimation of iron and zinc content was carried out using the Agilent ICP-MS 7850 instrument, which is housed in the Central Laboratory Facility at Banda University of Agriculture and Technology (BUAT), Banda. This advanced inductively coupled plasma mass spectrometry (ICP-MS) system provides high sensitivity and precision for trace element analysis.

Statistical analysis

Statistical analyses were conducted using the R Studio software package R 4.3.0, including analysis of variance (ANOVA), and genetic variability was assessed to assess genetic variability and relationships among the genotypes.

Estimation of variability parameters

The genotypic (σ^2_g) and phenotypic (σ^2_p) variances were calculated according to the following formula:

A. Mean: It was determined using the following formula:

$$\text{Mean (X)} = \sum X/N$$

Where,

$\sum X$ = Summation of N variables.

N = Represents the number of observations.

B. Range: It was taken at the lowest and highest value for each character:

$$\text{Range} = X_1 \text{ to } X_n$$

Where,

X_1 = Lower limit

X_n = Upper limit

C. Coefficient of variation (CV in %): The coefficient of variance was calculated using the following formula:

$$C.V = \frac{\text{Standard deviation(s)}}{\text{Sample Mean}} \times 100$$

D. Genotypic coefficient of variation (GCV): The extent of genotypic variation that exists in a character was calculated by the formula suggested by Burton and de Vane (1953)^[6]:

$$GCV\% = \text{Genotypic standard/Mean Deviation} \times 1000$$

E. Phenotypic coefficient of variation (PCV): The extent of phenotypic variation that exists in a character was calculated by the formula suggested by Burton and de Vane (1953)^[6]:

$$GCV\% = \text{Phenotypic standard/Mean Deviation} \times 1000$$

F. Heritability (in broad sense): It is the heritable proportion of total variability/phenotypic variability.

Heritability in the broad sense (h^2) (Falconer, 1996)^[9]:
 $h^2 = \sigma^2_g / \sigma^2_p$

where,

σ^2_g -genotypic variance.

σ^2_p -phenotypic variance.

Genotypic (σ^2_g) and Phenotypic variances (σ^2_p) were acquired from the analysis of the variance table that is according to Comstock and Robinson (1952)^[13].

G. Genetic advance (GA): Genetic advance (GA) was calculated with the method suggested by Allard (1960)^[2]

$$GA = K.h^2$$

Where,

K = constant = 2.06 at 5% selection intensity, h^2 = heritability in a broad sense.

H. Genetic advance as percent of the mean (Genetic gain): It was calculated by using the following formula suggested by Johnson *et al.* (1955)^[13].

$$\text{Genetic gain} = \frac{\text{Genetic advance}}{\bar{X}}$$

Where,

$\bar{X}$ = Grand mean

Results

Analysis of Variance

The analysis of variance (ANOVA) revealed statistically significant differences among the pigeon pea genotypes for nearly all the traits examined (table 2), suggesting the presence of substantial inherent genetic variability within the population. This variability offers ample opportunity to identify genotypes with favorable traits for yield improvement, particularly when appropriate selection pressure is applied. Significant genotypic differences were observed for key agronomic and nutritional traits, including days to 50% flowering (DF), number of primary branches (PB), number of secondary branches (SB), plant height (PH), number of pods per plant (PP), seeds per pod (SP), days to 50% maturity (DM), biological yield per plot (BY), 1000-grain weight (TGW), iron content (Fe, mg/kg), zinc content (Zn, mg/kg), and grain yield per plot (GY) (table 2).

Table 2: An analysis of variance (ANOVA) was conducted for twelve morphological and yield-related traits across lentil genotypes.

Source	Replication	Genotype	Error
df	2	24	48
Days to 50% flowering	10.253	23.27*	10.587
Primary branches per plant	0.2125	0.5476**	0.09677
Secondary branches per plant	3.5897	7.0756***	0.8514
Plant height (cm)	49.204	38.44*	9.217
Total no. of pods per plant	1089.5	29950.8***	333.1
Total no. of seed per pod	0.011111	0.042133*	0.013244
Days to maturity	18.8333	0.2133	1.13
Biological yield (kg)	0.03824	0.68931***	0.01451
1000 seed wt. (gm)	103.455	43.944**	7.312
Iron (mg/kg)	774.52	262.25*	71.05
zinc (mg/kg)	93.545	33.739***	0.61
grain yield (gm/plot)	0.003072	0.150492***	0.001786

***significance level at (0.001), **significance level at (0.01), and *significance level at (0.05)

Genetic parameters

The mean, range, genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), heritability, genetic advance, and genetic advance as per cent of the mean were presented in Table 3.

Genotypic coefficient of variation (GCV) ranged from 1.39% to 21.74%. Higher magnitude of GCV was observed for 1000 seed weight (21.74%), followed by total no. of pods per plant (18.86%), iron (16.69%), biological yield (14.69%), secondary branches per plant (12.63%), zinc (10.95%), grain yield per plot (10.69%), plant height (8.91%), primary branches per plant (5.43%), days to 50% flowering (3.53%), days to maturity (2.26%) and total seed per pod (1.91%) displayed lowest GCV values. The phenotypic coefficient of variation (PCV) was varied from 2.47% to 28.73% in Table 3. High magnitude of phenotypic coefficient of variation (PCV) was observed for total no. of pod per plant (28.73%) followed grain yield per plot (25.08%), biological yield (24.19%), 1000 seed weight (24.10%), iron (19.05%), secondary branches per plant (17.56%), plant height (11.59%), zinc (11.06%), primary branches per plant (10.16%), days to 50% flowering (6.61%), total seed per pod (5.86%), and days to maturity (2.47%) exhibited lowest PCV value (table 3). The heritability (%) in a broad sense for twelve characters was estimated, which ranged from 98.07% to 28.51%. Maximum percentage of heritability was recorded for zinc (98.07%),

days to maturity (83.93%), grain yield per plot (81.81%), 1000 seed weight (81.42%), iron (76.75%), plant height (59.12%), secondary branches per plant (51.74%), total seed per pod (45.60%), total no. of pods per plant (43.08%), biological yield per plot (35.27%), days to 50% flowering (28.54%), and primary branches per plant (28.51%), while displayed low heritability (table 3). The genetic advance (GA) and genetic advance as percent of mean (GA%) were estimated for twelve characters and showed considerable variation among the traits. The GA values ranged from 0.01 to 27.63. Maximum GA was observed for iron content (27.63), followed by total number of pods per plant (21.47), 1000-seed weight (10.52), zinc content (11.35), plant height (5.78), days to maturity (4.58), secondary branches per plant (1.42), days to 50% flowering (2.26), biological yield per plot (0.11), primary branches per plant (0.22), grain yield per plot (0.02), and total number of seeds per pod (0.01) (table 3). Similarly, genetic advance as percent of mean ranged from 0.68% to 40.42%. The highest GA% was recorded for 1000-seed weight (40.42%), followed by iron content (30.12%), total number of pods per plant (25.50%), zinc content (22.34%), secondary branches per plant (18.72%), biological yield per plot (17.57%), plant height (14.11%), grain yield per plot (9.41%), primary branches per plant (5.97%), days to maturity (4.27%), days to 50% flowering (3.89%), and total number of seeds per pod (0.68%) ((table 3).

Table 3: Genetic parameters for yield and yield components in Lentil genotypes during Rabi 2024-25.

Characters	Range		Mean	GCV (%)	PCV (%)	Heritability %	GA	GA % mean
	Maximum	Minimum						
Days to 50% flowering	66.00	52.00	58.23	3.53	6.61	28.54	2.26	3.89
Primary branches per plant	5.00	2.80	3.62	5.43	10.16	28.51	0.22	5.97
Secondary branches per plant	11.20	4.60	7.56	12.63	17.56	51.74	1.42	18.72
Plant height (cm)	52.20	28.60	40.97	8.91	11.59	59.12	5.78	14.11
Total no. of pods per plant	190.60	33.80	84.20	18.86	28.73	43.08	21.47	25.50
Total no. of seed per pod	2.20	1.60	1.91	1.39	5.86	45.60	0.01	0.68
Days to maturity	113.00	101.00	107.33	2.26	2.47	83.93	4.58	4.27
Biological yield (kg)	1.09	0.30	0.62	14.37	24.19	35.27	0.11	17.57
1000 seed wt. (gm)	40.03	15.19	26.04	21.74	24.10	81.42	10.52	40.42
Iron (mg/kg)	171.80	67.81	91.74	16.69	19.05	76.75	27.63	30.12
Zn (mg/kg)	62.30	41.60	50.82	10.95	11.06	98.07	11.35	22.34
Grain yield (gm)	0.36	0.03	0.19	10.69	25.08	81.81	0.02	9.41

Discussion

The magnitude of genetic variability, heritability, genetic advance (GA), and genetic advance as percent of mean (GA%) provides a reliable basis for assessing the effectiveness of selection and understanding the nature of gene action controlling different traits (Burton, 1952; Johnson *et al.*, 1955) [5, 13]. In the present study, all twelve characters exhibited varying degrees of genetic variability, indicating the presence of exploitable diversity among the lentil genotypes.

Genetic diversity and effective selection approaches are fundamental to the success of any breeding program, as they enable the utilization of available genetic variability (Cristobal *et al.*, 2014) [7]. These findings are consistent with previous reports by (Alemayo *et al.* 2021 Kumar *et al.* 2022; Ghimire *et al.* 2024) [1, 16, 12]. In general, phenotypic coefficient of variation (PCV) values were higher than the corresponding genotypic coefficient of variation (GCV) for all traits, suggesting that environmental factors influenced trait expression to some extent. Similar observations have been reported earlier in lentil and other legumes (Yadav *et*

al., 2017; Singh *et al.*, 2019) [26, 23]. However, traits such as 1000-seed weight, iron content, zinc content, and days to maturity showed relatively narrow differences between PCV and GCV, indicating a stronger genetic control and greater reliability of phenotypic selection. High heritability coupled with high genetic advance as percent of mean was observed for 1000-seed weight, iron content, zinc content, and total number of pods per plant. This combination suggests the predominance of additive gene action and indicates that these traits can be effectively improved through direct selection (Johnson *et al.*, 1955; Falconer & Mackay, 1996) [13, 9]. Similar results have been reported for seed weight and micronutrient traits in lentil, highlighting their importance as stable selection criteria (Kumar *et al.*, 2018; Singh *et al.*, 2021) [15, 24]. Moderate heritability along with moderate GA% was recorded for secondary branches per plant, plant height, biological yield, and grain yield. These traits appear to be governed by both additive and non-additive gene effects and are moderately influenced by environmental conditions. Therefore, improvement in these characters may

require selection across multiple environments or the use of advanced breeding strategies (Panse & Sukhatme, 1967; Tsegaye *et al.*, 2020) ^[20, 25]. In contrast, days to 50% flowering, days to maturity, primary branches per plant, and number of seeds per pod exhibited low GA and GA% despite moderate to high heritability in some cases. This indicates the predominance of non-additive gene action or epistatic effects, limiting the response to simple selection (Allard, 1960) ^[2]. Similar findings have been documented in lentil and related pulse crops, where phenological traits showed lower genetic gain despite moderate heritability (Reddy *et al.*, 2016; Younis *et al.*, 2022) ^[21, 27]. Notably, micronutrient traits such as iron and zinc content exhibited high heritability along with high GA and GA%, indicating substantial potential for genetic improvement. This is particularly significant in the context of biofortification, as lentil is an important dietary source of micronutrients in many developing countries (Khazaei *et al.*, 2019; Bouis & Saltzman, 2017) ^[14, 4].

Overall, the present findings suggest that traits showing high heritability in combination with high genetic advance—namely 1000-seed weight, total number of pods per plant, iron content, and zinc content—should be given priority in lentil breeding programs aimed at improving yield and nutritional quality. Traits with low genetic advance may require alternative breeding approaches such as hybridization and recurrent selection to achieve meaningful improvement.

Conclusion

The present investigation revealed substantial genetic variability among the lentil genotypes for yield, yield components, phenological traits, and micronutrient content, indicating ample scope for genetic improvement through selection. The observed differences between phenotypic and genotypic coefficients of variation for most traits suggested varying degrees of environmental influence; however, several characters exhibited strong genetic control. High heritability estimates coupled with high genetic advance as a percentage of mean were recorded for 1000-seed weight, total number of pods per plant, iron content, and zinc content, indicating the predominance of additive gene action and the effectiveness of direct phenotypic selection for these traits. Moderate heritability and genetic advance were observed for plant height, secondary branches per plant, biological yield, and grain yield, suggesting that improvement of these traits may be achieved through selection over multiple environments or generations. In contrast, low genetic advance for days to 50% flowering, days to maturity, primary branches per plant, and number of seeds per pod indicated limited scope for improvement through simple selection, possibly due to non-additive gene action or greater environmental influence. Notably, the high heritability and genetic advance associated with iron and zinc content highlight the potential of lentil as a candidate crop for biofortification programs aimed at addressing micronutrient malnutrition. Overall, the study identified 1000-seed weight, total number of pods per plant, iron content, and zinc content as reliable selection criteria for developing high-yielding and nutritionally enriched lentil varieties. The findings provide valuable information for lentil breeding programs and can be effectively utilized to enhance productivity, nutritional quality, and food security through the development of improved cultivars.

Acknowledgements

The authors are grateful to acknowledge the Department of Genetics & Plant Breeding, Banda University of Agriculture and Technology, Banda, India.

Competing Interests

Authors have declared that no competing interests exist.

Author Contributions

This work was carried out in collaboration among all authors. Authors NK, KD, HJ and AJ contributed to conceptualisation and methodology, Author NK performed data collection and data curation, and VS did statistical analysis. NK prepared the initial draft of the manuscript. Authors HJ, VS, NPS, and KD reviewed and edited the manuscript. K provide resources to carry out experiments. All authors read and approved the final manuscript.

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