



Genetic diversity of morphological, biochemical and mineral traits in Indian onion (*Allium cepa*) genotypes

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ABSTRACT

The present study was carried out during 2021 and 2022 at ICAR-Indian Agricultural Research Institute, New Delhi to evaluate the diversity among 83 onion (*Allium cepa* L.) genotypes utilizing morphological, biochemical, and mineral profiling. Substantial genetic variances were observed across all the investigated traits. Traits such as bulb phenol content, bulb pyruvic acid content, neck thickness, average bulb weight, iron, zinc, and sulphur recorded high genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV) values, whereas plant height, total soluble solids, marketable yield, dry matter, and calcium had moderate GCV as well as PCV values. High heritability was observed for all traits except for iron content, which ranged from 98.32% (bulb phenol content) to 37.93% (Iron). Principle Component Analysis (PCA) extracted 5 principal components (PC1–PC5), accounting for a cumulative variance of 59.88%. The primary contributors to PC1 were average bulb weight, marketable yield, and equatorial diameter, while PC2 was primarily influenced by iron content, bulb pyruvic acid content, and neck thickness. On the basis of Euclidean distance and Ward's D² analysis, all the genotypes were grouped into three clusters. Cluster 1 showed the highest values for dry matter, iron and zinc content. Cluster 2 consisted of genotypes with higher values for plant height, polar diameter, average bulb weight, calcium, potassium, and sulphur content, whereas it showed lower values for neck thickness. Cluster 3 exhibited higher values for equatorial diameter, total soluble solids and marketable yield. Greater genetic diversity offers breeders enhanced opportunities to identify promising genotypes for selection or utilization as parents in hybrid breeding programmes.

Keywords: Biochemical, Heritability, Minerals, Onion, PCA, Variability

Onion (*Allium cepa* L.) is considered as a vital vegetable crop because of its immense culinary, nutritional, and medicinal value. Onions are also highly valued as raw material in the processing industry for producing dehydrated powder, rings, shreds, and pickled onions in vinegar or brine. Onions possess diverse medicinal properties, encompassing antioxidant, anti-inflammatory, and antimicrobial effects. Globally, onions are cultivated across an area of 5.77 million hectares, yielding a production of 106.59 million metric tonnes. India leads in production, contributing 26.64 million metric tonnes from an area of 1.62 million hectares (FAOSTAT 2023). India, a significant onion producer, lags in productivity at 93rd place (16.40 t/ha), trailing South Korea (85.40 t/ha), the USA (56.05 t/ha), and Spain (54.72 t/ha). The low productivity of onions in India can be linked to the large-scale use of open-pollinated varieties (OPVs) seeds, which are cheap and readily available to farmers as compared to high-yielding hybrids.

Most of the research studies on Indian onion diversity have primarily focused on very few local commercial varieties or germplasm, with limited exploration of broader genetic diversity. Limitations of using a small group of genotypes for diversity assessment led to a need for more diverse potential genotypes for hybridization (Dangi *et al.* 2018). Morphological characterization serves as the initial step in the description and genetic resources classification. To overcome the environmental influence and make more effective selection, it is essential to assess both genetic advance and heritability. Cluster analysis is a valuable tool in accurately grouping genotypes, facilitating the selection of suitable candidates for future breeding goals. Principal component analysis (PCA) remains valuable in identifying key traits contributing to genotype differentiation. It allows plant breeders to assess the impact and associations among different traits, aiding in their understanding of genotype characteristics (Kovacic 1994). Characterizing onion germplasm is a crucial step in harnessing the existing genetic variability for cultivar development. The primary focus of our study was to investigate the impact of genetic architecture and environmental factors on biochemical and morphological traits, assess the genetic diversity, and identify

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critical traits for genotype characterization, which would be beneficial for a diverse breeding programme of onion.

MATERIALS AND METHODS

The present study was carried out during 2021 and 2022 at ICAR-Indian Agricultural Research Institute, New Delhi. A total of 83 onion genotypes comprising 45 open-pollinated varieties (OPV), 16 inbreds, 12 hybrids, and 10 breeding lines (BL) were used in this study. The experiment followed a randomized complete block design (RCBD) with two replications. Each replication consisted of a total of 60 plants/genotype, which were spaced at 10 cm (plant to plant) and 15 cm (row to row). The recommended package of practices included the application of nitrogen, phosphorus, and potash (NPK) at a rate of 110:40:60 kg/ha. Half dose of nitrogen and full dose of phosphorus and potash were applied at the time of transplanting, whereas the other half of nitrogen was further split in half and incorporated in to the soil, after 30 and 45 days of transplanting.

Total 7 morphological characters, 4 biochemical traits, and 5 mineral elements were assessed. A hand refractometer (PAL-3 model) was employed to measure the total soluble solids (TSS), reported in degrees Brix ($^{\circ}\text{B}$). The dry matter of the bulbs (%) was assessed using the method outlined by Nieuwhof *et al.* (1973), with minor adjustments made as necessary. Bulb pyruvic acid (BPA) levels were determined using the protocol outlined by Anthon and Barrett (2003), with results reported in micromoles per millilitre ($\mu\text{mol/ml}$). The bulb phenol content (BPC) of the onion bulbs was determined using the Folin Ciocalteu reagent (FCR) method, based on Singleton and Rossi (1965). For mineral profiling, a dried edible portion of the onion bulb from each genotype was used and replicated twice. For each replication, 0.5 gm of the powdered sample was taken and digested in a mixture of 20 ml nitric acid (HNO_3) and 4-Perchloric acid in a ratio of 9:4 in a 500 ml conical flask. 4 minerals (Ca, Fe, Zn and K) in the onion bulb were estimated using an Autosampler Atomic Absorption Spectrometer (Shimadzu, Japan). The sulphur content in the onion samples was determined turbidimetrically using a UV-1900i-UV-Vis double-beam spectrophotometer (Simadzu, Japan). Sulphur levels were determined using turbidity generated with barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) and measured at 420 nm, following Chesnin and Yien's (1950) method.

The analysis of variance and means comparison, along with the estimation of genetic parameters including phenotypic and genotypic coefficient of variance, heritability (broad sense), and genetic advance (GA), was conducted using the "variability 0.1.0" package (Popat *et al.* 2020) in R software (version 4.2.1). Cluster analysis was performed using Ward's D^2 criterion, following the method described by Murtagh and Legendre (2014). PCA, eigenvalue calculations, and PCA visualization were conducted using Past software (version 4.03) (R Core Team 2019).

RESULTS AND DISCUSSION

Genetic variations: The analysis of variance (ANOVA)

for all considered traits revealed a highly significant mean sum of squares, indicating substantial variation among the 83 onion genotypes (Table 1). The highest coefficient of variation was recorded for traits such as BPC (39.27%), iron content (34.78%), and zinc content (29.52%). Equatorial diameter exhibited the lowest coefficient of variation (8.22%), followed by potassium content (9.75%). The genetic coefficient of variance (GCV) ranged from low to high, spanning from 7.43–39.02%. Similarly, the phenotypic coefficient of variance (PCV) was also recorded as low to high, with observations ranging from 8.93–39.36%. The coefficients of variation obtained were in accordance with GCV and PCV, indicating minimum variation in ED and maximum variation in BPC. According to Deshmukh *et al.* (1986), GCV and PCV values exceeding 20% are categorized as high, values falling between 10 and 20% are classified as medium, while those below 10% are considered low. Characteristics including BPC, BPA, neck thickness (NT), average bulb weight (ABW), iron, zinc, and sulphur exhibited high GCV and PCV values, while plant weight (PH), marketable yield (MY), total soluble solids (TSS), dry matter (DM), and calcium traits displayed moderate GCV and PCV values. The findings were consistent with those of Bhoi *et al.* (2020) and Bal (2022), who similarly reported high GCV and PCV in ABW, NT, BPA, and BPC. Low GCV and PCV were recorded in ED and K, while in the case of PD and BSI, both were recorded with low GCV, but PCV values of both traits were classified as moderate. Consistent with our findings, previous studies have also reported moderate GCV and PCV values for traits such as marketable yield (MY) and dry matter (DM) (Santra *et al.* 2017, Bhoi *et al.* 2020), plant height (PH) (Bal *et al.* 2022) and total soluble solids (TSS) (Khosa and Dhatt 2013). However, a low GCV and PCV for equatorial diameter (ED) were reported by Dangi *et al.* (2018). In traits such as plant height (PH), neck thickness (NT), equatorial diameter (ED), polar diameter (PD), marketable yield (MY), total soluble solids (TSS), dry matter (DM), bulb phenol content (BPC), bulb pyruvic acid (BPA), Ca, and K, the difference between PCV and GCV was less than 1, indicating that the genotypic effects and the underlying genes responsible for these traits primarily influence the expression of these traits.

Conversely, for traits such as bulb shape index (BSI), average bulb weight (ABW), iron, zinc, and sulphur, where there was a notable disparity between PCV and GCV, it indicates a substantial influence of environmental factors on the expression of these characters in addition to genotype. Additionally, traits strongly influenced by environmental factors may not be dependable descriptors for morphological characterization (Pandey *et al.* 2008). The selection of superior genotypes based solely on the GCV may not be effective, as it only indicates the degree of variation, and the heritable proportion of variability needs to be taken into account. Therefore, it is suggested that GCV coupled with heritability estimates would be more effective for selecting specific traits. High heritability (broad sense) was observed for all the traits except for iron content. Heritability (broad

Table 1 Analysis of variance, descriptive statistics and genetic variability assessment of quantitative traits among the 83 onion genotypes

Quantitative trait	ANOVA		Descriptive statistics				Estimation of genetic parameters			
	Mean squares (%)		Max.	Min.	Mean	CV (%)	GCV (%)	PCV (%)	h ² B (%)	GA (%)
	Genotypes	Residual								
PH	111.70***	1.30	86.70	44.15	69.79	10.70	10.64	10.77	97.00	15.78
NT	0.047***	0.0009	1.20	0.40	0.66	23.93	22.99	23.48	95.87	0.30
PD	43.39**	5.51	61.30	33.50	45.01	10.35	9.66	10.98	77.43	7.88
ED	35.86**	6.52	60.45	41.65	51.54	8.22	7.43	8.93	69.21	6.56
BSI	0.01***	0.003	1.30	0.70	0.88	10.70	9.19	11.28	66.33	0.13
ABW	300.77***	4.22	99.45	38.55	70.75	17.32	23.71	31.77	55.71	8.07
MY	77.00***	21.90	35.45	7.90	22.13	28.04	17.20	17.45	97.23	24.73
TSS	5.70**	0.11	16.05	6.85	12.25	13.79	13.64	13.92	95.98	3.37
DM	7.67***	0.63	15.25	5.75	10.83	18.14	17.33	18.84	84.63	3.55
BPA	1.65**	0.01	6.65	1.95	3.99	22.83	22.68	22.92	97.92	1.84
BPC	2.60**	0.02	5.70	1.00	2.91	39.27	39.02	39.36	98.32	2.32
Ca	38.28**	1.47	52.70	33.20	40.92	10.69	10.48	10.89	92.59	8.50
K	59.73***	2.38	66.70	40.80	56.07	9.75	9.54	9.93	92.31	10.59
Fe	0.007***	0.003	0.45	0.08	0.18	34.78	25.87	32.02	37.93	0.50
Zn	0.02***	0.001	0.58	0.18	0.37	29.52	28.76	30.22	90.55	0.21
S	0.05***	0.003	0.85	0.20	0.55	29.43	28.27	30.45	86.22	0.29

Max, Maximum; Min, Minimum; CV, Coefficient of variation; GCV, Genotypic coefficient of variation; PCV, Phenotypic coefficient of variation; h²B(%), Heritability in broad sense; GA, Genetic advance; PH, Plant height (cm); NT, Neck thickness (cm); ED, Equatorial diameter (mm); PD, Polar diameter (mm); BSI, Bulb shape index; ABW, Average bulb weight (gm); TSS, Total soluble solids (°B); DM, Dry matter (%); BPA, Bulb pyruvic acid (μ mol/gm fresh weight); BPC, Bulb phenol content (mg GAE/gm fresh weight); Ca, Calcium (mg/100 g); K, Potassium (mg/100 g); Fe, Iron (mg/100 g); Zn, Zinc (mg/100 g); S, Sulphur (%); MY, Marketable yield (t/ha); ns, Non-significant. *, **, *** significant at $P < 0.05$, 0.01 and 0.001, respectively.

sense) ranged from 98.32% (BPC) to 37.93% (Iron). High heritability has been reported for PH, MY, DM, ED, PD, TSS, BPC, and BPA in previous studies (Khosa and Dhatt 2013, Santra *et al.* 2017, Bal *et al.* 2022). Characters with high genetic advance and high heritability are given more consideration in the selection process to achieve improved genotypic values in the new population compared to the base population after one cycle of selection. In this study, the highest genetic advance was recorded in MY (24.73%), while the lowest was observed in BSI (0.13%). MY and PH exhibited both high heritability and high genetic advance. Thus, traits with high heritability and genetic advance can be considered reliable for selection due to their additive gene control. The selection of these traits would be beneficial for their improvement (Panse 1957). Also, traits such as PD, ED, ABW, potassium content, and calcium content exhibited high heritability along with moderate genetic advance, suggesting a significant role of non-additive gene action in the inheritance of these traits.

Cluster analysis: Euclidean distance was computed, and based on Ward's minimum variance method (Ward's D²), 83 onion genotypes were grouped into three distinct clusters. Cluster 1 consisted of 35 genotypes, whereas clusters 2 and 3 comprised 7 and 41 genotypes, respectively (Fig. 1). Cluster 1 showed the lowest values for BSI and the highest values for DM, iron and zinc content. Most of the private

sector hybrids were grouped under this cluster along with a few widely adapted varieties, viz. Punjab Naroha, Pusa Red, Pusa Sobha, Agrifound dark red and Agrifound light red. Cluster 2 is the smallest one, which consisted of genotypes with higher values for PH, PD, ABW, BPA, BPC, Calcium, Potassium, and Sulphur content.

In contrast, it showed a lower value for NT, which is desirable (Table 2). This group primarily consisted of exotic hybrids and breeding lines derived from exotic material, which have intermediate day length requirements for bulbing. Cluster 3 exhibited higher values for ED, TSS and MY. Zinc content was similar for Clusters 2 and 3, while NT values were found to be identical for Cluster 1 and 3. Cluster 3 primarily consisted of well-adapted and extensively cultivated varieties, viz. Pusa Riddhi, Pusa Madhavi, PWF, Bhima Super, Bhima Shweta, etc. Therefore, the presence of well-adapted varieties in cluster 3 showed a higher yield than the other 2 clusters. Manjunathagowda *et al.* (2022) identified five clusters among 62 Indian onion accessions. Arya *et al.* (2017) classified 26 onion accessions into four clusters and suggested that groups were not strongly associated with their geographical origins.

Similarly, Dangi *et al.* (2018) grouped 58 accessions into four clusters, and Luitel *et al.* (2023) grouped 79 onion accessions into three clusters. Our findings support earlier research, suggesting that morphological trait-based clustering

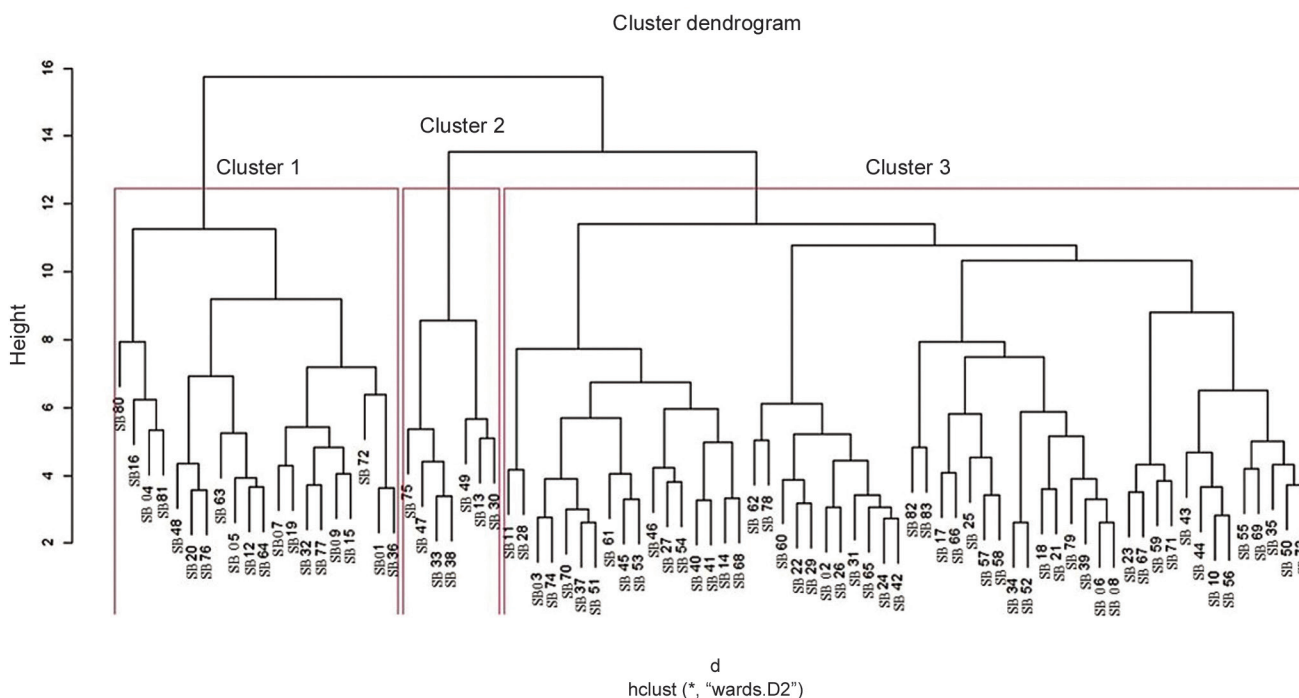


Fig. 1 Dendrogram presenting three main clusters identified using Ward's D^2 cluster analysis (scale: Euclidean distance).

Table 2 Mean and standard deviations for the classification of three onion clusters

Qualitative trait	Cluster 1		Cluster 2		Cluster 3	
	Mean	SD	Mean	SD	Mean	SD
PH	68.98	8.21	75.26	6.29	69.63	6.42
NT	0.66	0.19	0.64	0.09	0.66	0.12
PD	42.23	3.44	52.50	4.70	46.77	3.23
ED	49.65	3.71	50.46	6.54	53.96	3.00
BSI	0.85	0.07	1.05	0.12	0.87	0.08
ABW	64.00	11.12	77.82	11.24	77.26	9.25
MY	19.32	6.30	23.97	6.31	25.05	4.49
TSS	12.28	1.43	9.19	1.48	12.84	1.34
DM	11.06	2.07	8.91	1.87	10.93	1.67
BPA	4.33	0.93	4.49	0.69	3.49	0.67
BPC	3.29	1.18	3.44	0.95	2.36	0.89
Ca	39.56	3.33	42.51	2.23	42.19	5.27
K	55.47	5.28	58.66	4.93	56.27	5.76
Fe	0.20	0.07	0.18	0.05	0.16	0.04
Zn	0.38	0.11	0.38	0.13	0.37	0.11
S	0.56	0.16	0.67	0.12	0.52	0.17

PH, Plant height (cm); NT, Neck thickness (cm); PD, Polar diameter (mm); ED, Equatorial diameter (mm); BSI, Bulb shape index; ABW, Average bulb weight (gm); MY, Marketable yield (t/ha); TSS, Total soluble solids ($^{\circ}$ B); DM, Dry matter (%); BPA, Bulb pyruvic acid (μ mol/gm fresh weight); BPC, Bulb phenol content (mg GAE/gm fresh weight); Ca, Calcium (mg/100g); K, Potassium (mg/100g); Fe, Iron (mg/100g); Zn, Zinc (mg/100g); S, Sulphur (%); SD, Standard deviation.

may not reflect geographical origin. Onion genotypes in Cluster 2, identified through clustering analysis, displayed desirable traits, making them ideal for variety enhancement. These genotypes had the highest PH, PD, ABW, NT, BPC, BPA, calcium, sulphur, and potassium content and can be effectively utilized in breeding programmes aimed at developing improved onion varieties. Onion genotypes clustered in group 3 are suitable for enhancing traits such as ED, TSS, MY, and zinc content.

Principal component analysis: PCA, as a dimensionality reduction technique, effectively reduces the complexity of data by transforming original variables into a smaller set of variables while retaining important information. PCA was employed to confirm the relative contribution of characters to the total variance. In total, 5 principal components (PC1 to PC5) were extracted, each with eigen value exceeding 1, collectively explaining 59.88% of the cumulative variance (Table 3). PC1 and PC2 explained approximately 17.77% and 14.63% of the variance. PCA was done to identify the primary contributors of variation along each axis of differentiation (Fig. 2). In PC1, the greatest contributions to variability were attributed to ABW (23.23%) and MY (20.97%), followed by ED (18.34%). PC1 demonstrated

Table 3 Eigen values, percentage of variance and cumulative variance percentage of the first five principal components based on 16 traits of the 83 onion genotypes

	PC1	PC2	PC3	PC4	PC5
Eigen value	2.84	2.34	1.68	1.47	1.25
Variance (%)	17.77	14.63	10.51	9.17	7.80
Cumulative (%)	17.77	32.44	42.94	52.11	59.88

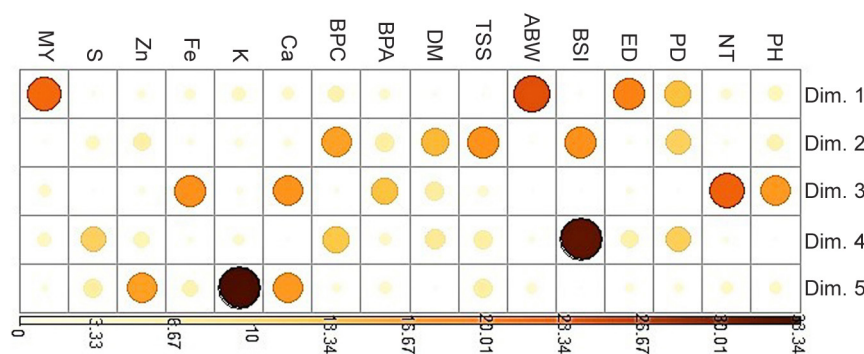


Fig. 2 Principal component analysis (PCA) bi-plot of 16 variables based on their contribution in the first five PCs.

PH, Plant height; NT, Neck thickness; PD, Polar diameter; ED, Equatorial diameter; BSI, Bulb shape index; ABW, Average bulb weight; TSS, Total soluble solids; DM, Dry matter; BPA, Bulb pyruvic acid; BPC, Bulb phenol content; Ca, Calcium; K, Potassium; Fe, Iron; Zn, Zinc; S, Sulphur; MY, Marketable yield.

a positive correlation with the majority of traits, with the exceptions being BSI, TSS, DM, BPA, BPC, iron, and sulphur content. In PC2, the main drivers of variation were Iron (9.47%), BPA (6.14%), and NT (5.93%). PC3 explained 10.51% of the total variation and was mainly associated with NT, iron, PH, and calcium. BSI, BPC, and PD were the primary contributors to the variance observed in PC4 (9.17%), while potassium and calcium content contributed to a variance of 7.80% in PC5.

In conclusion, the traits ABW, MY, BPC, BSI, PD, NT, iron, and sulphur content were identified as significant factors contributing to 66.68% of the variability in the onion germplasm. Hanci and Gokce (2016) documented

that the nine PCs accounted for 71.84% of the variability, with the ABW being a significant contributor. Dangi *et al.* (2018) reported that five principal components (PCs) accounted for 78.5% of the total variation in short-day onion genotypes, with leaf diameter, bulb diameter, and number of leaves identified as major contributors to PC1. Luitel *et al.* (2023) revealed that 6 PCs explained 72.65% of the total variation, and the significant factors were ABW, ED, PD and PH. Similarly, our findings show that ABW and ED are the major contributors to PC1. The positive loading of bulb weight in PC1 observed in this study aligns with the results reported by

Arya *et al.* (2017), where ABW, ED, PD and PH were also identified as major contributing traits in the positive direction. The biplot illustrating the principal component axis 1 and axis 2 for the 83 onion genotypes indicated that higher PC1 and PC2 values are necessary for selecting genotypes with greater ABW, PD, and ED traits (Fig. 3). PCA results corroborated the findings of cluster analysis, with genotypes grouped into three clusters. Genotypes clustered around the origin share similar genetic characteristics, indicating relatedness, while those distant from the origin are considered unrelated. Genotypes within the second cluster were identified as significantly distant from the origin, signifying their substantial dissimilarity with the

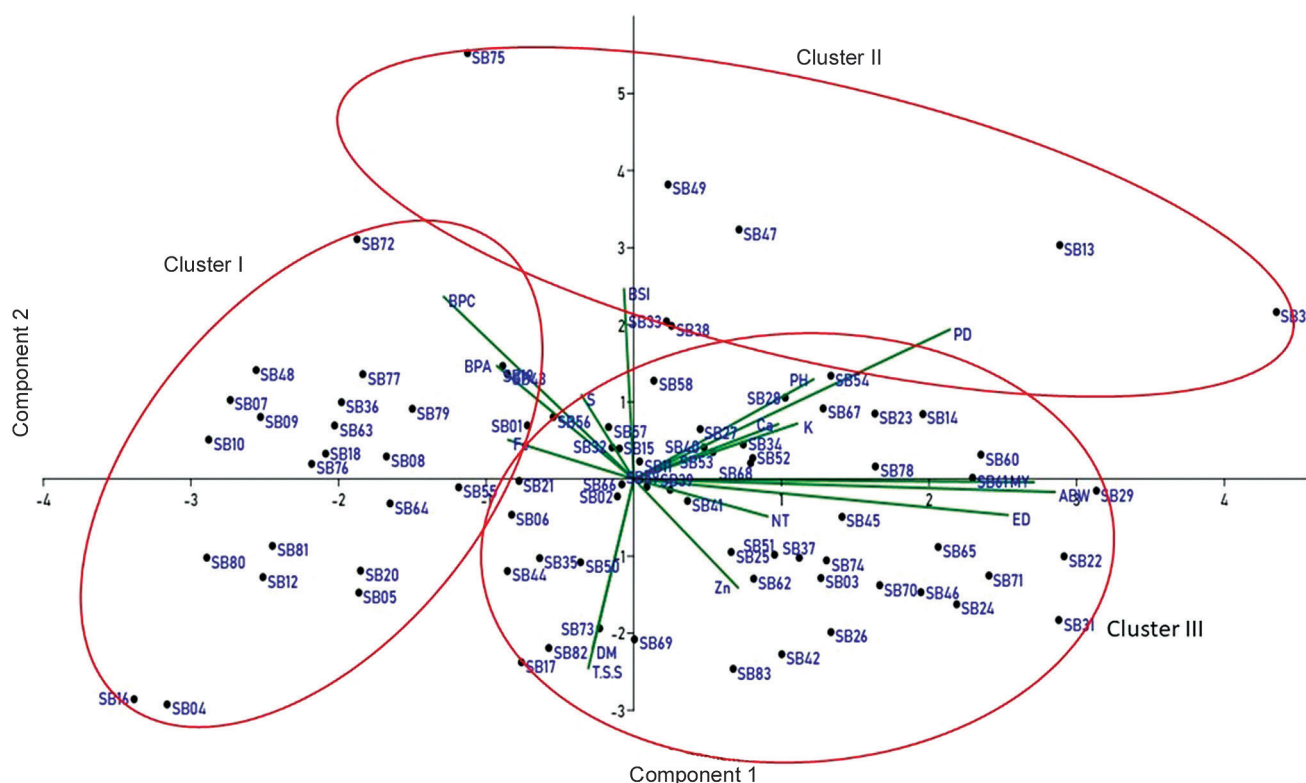


Fig. 3. Principal component analysis (PCA) bi-plot of 83 onion genotypes.

other two groups. Hence, they are considered the most unrelated among the clusters. Therefore, selecting genotypes from the latter group as potential parents holds promise for successful hybridization and the development of heterotic groups in the onion breeding programme.

In conclusion, the current study unveiled substantial genetic variation within the 83 onion genotypes. Evaluation of genetic variability yielded insights into the primary traits contributing to the observed genetic diversity. GCV and PCV were most pronounced for the bulb phenol content, indicating significant variation among individuals. ED showed the lowest GCV and PCV, suggesting a relatively lower level of variation. PH, MY, BPA, BPC, calcium content, and sulphur content exhibited high heritability, signifying a substantial genetic influence. For traits with moderate heritability, selection effectiveness may be constrained, yet still feasible. However, for characters with low heritability, the most effective strategy for improvement would involve hybridization between parents with diverse genetic backgrounds. By crossing genetically distinct parents, breeders can maximize the chances of introducing new combinations of genes and enhancing the expression of desired traits. Therefore, for traits with low heritability, hybridization becomes the preferred approach to achieve improvement in onion varieties.

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