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Genetic divergence and trait contribution analysis in short-day onion (*Allium cepa* L.) genotypes using multivariate approaches

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Abstract
Quantifying genetic divergence is fundamental for exploiting heterosis and broadening the genetic base in onion (*Allium cepa* L.) breeding. In the present investigation, eighteen short-day onion genotypes were assessed using Mahalanobis D^2 statistics to characterize multivariate diversity and define genetic relationships. Tocher's clustering grouped the genotypes into six distinct clusters, reflecting wide genetic dispersion, while the occurrence of two monotypic clusters highlighted the presence of uniquely differentiated genotypes within the population. The maximum inter-cluster distance observed between Cluster I and Cluster III (D^2 = 10,657.99; D = 103.24) indicated exceptionally strong genetic contrast, suggesting that crosses between these clusters would yield maximum heterotic potential. Similarly, substantial divergence among other cluster pairs, such as I-IV and III-VI, further emphasized the breadth of available genetic variability. Trait contribution analysis revealed that yield-associated and bulb architectural traits accounted for more than 92% of the total divergence, confirming their dominant influence on multivariate separation. The relatively low contributions of quality and stress-response traits suggest their lesser role in defining major divergence patterns, although they remain relevant for targeted improvement. Overall, the study demonstrates the presence of strong, structured genetic diversity among the evaluated onion genotypes. The markedly high D^2 values and differentiated cluster composition provide valuable guidance for selecting genetically distant parents, enabling breeders to design crosses that maximize heterosis, generate wide segregation, and accelerate genetic progress in onion improvement under short-day environments.

Keywords: Onion (*Allium cepa* L.), genetic divergence, multivariate analysis, inter-cluster distance, germplasm characterization

Introduction
Onion (*Allium cepa* L.) is a globally significant vegetable crop grown for its culinary, nutritional, and health-promoting attributes. India is one of the leading producers of onion, yet productivity remains highly variable due to genotype-environment interactions, fluctuating climatic conditions, and susceptibility to major pests and diseases. This necessitates the development of stable, high-yielding, and stress-tolerant varieties through systematic breeding programmes (Brewster, 2008) [3]. Success in any crop improvement programme largely depends on the availability of genetically diverse parental material capable of producing desirable recombinants.

Assessment of genetic divergence provides a scientific basis for identifying such diverse parents. Multivariate analytical tools, particularly Mahalanobis D^2 statistics (Mahalanobis, 1936) [10], are extensively employed in horticultural crops for quantifying divergence because they simultaneously evaluate multiple yield, quality, physiological, and stress-related traits (Singh & Chaudhary, 1977) [14]. Clustering based on D^2 values enables breeders to classify genotypes into distinct groups, estimate inter-and intra-cluster distances, and identify genetically distant yet agronomically superior parents for hybridization. In onion—where bulb yield, shape, pungency, maturity, and storage quality are polygenic and environmentally influenced—genetic divergence analysis has proven especially valuable (Havey, 2019; Lyngkhoi *et al.*, 2021) [7, 9].

Despite the availability of diverse germplasm, comprehensive studies integrating yield components, quality parameters, and biotic stress traits in short-day onion genotypes remain

limited. A robust understanding of trait contribution patterns is essential to formulate breeding strategies that combine high yield with consumer-preferred quality attributes and stress resilience. Therefore, the present study aims to assess the genetic divergence among eighteen onion genotypes using Mahalanobis D^2 statistics across fifteen morphological, quality, and yield-related traits.

Materials and Methods

The field experiment was carried out during the *Rabi* season of 2020-21 at the "C" Block Farm, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Kalyani, Nadia, West Bengal (22.89°N, 88.45°E; 9.75 m above mean sea level). The region falls under the humid subtropical climate with mild winters, moderate rainfall, and favourable soil conditions for onion cultivation. The experimental site consisted of sandy loam soil, neutral in reaction (pH 7.1), low in organic carbon, available nitrogen and phosphorus, and medium in potassium, as per soil fertility ratings of NBSS&LUP (2015). Standard cultural operations were undertaken to ensure uniform crop growth throughout the experiment. A total of eighteen onion (*Allium cepa* L.) genotypes—JRO-14-14, NHO-920, PRO-9, L-913, Kasi No.1, Balwan, Rashidpura, Sandeep, Sona, DOGR-1639, PRO-8, DOGR-1625, DOGR-1626, Bhima Shakti, Bhima Kiran, NHRDF Red-2, Phule Samarth and PRO-7—were procured from the All India Network Programme on Onion and Garlic (AICRP-Vegetable Crops), ICAR-Directorate of Onion and Garlic Research, Pune. The experiment was laid out in a Randomized Block Design (RBD) with three replications, following procedures recommended for multi-trait evaluation trials (Gomez & Gomez, 1984) [5]. Seedlings (35-40 days old) were transplanted at 15 × 10 cm spacing in well-prepared plots. Uniform agronomic practices, including basal fertilization, irrigation, weed management, and plant protection measures, were adopted following the cultivation package of practices for Rabi onion recommended by ICAR-DOGR (Khar *et al.*, 2020) [9].

Fifteen agro-morphological, yield, quality, and stress-response traits were recorded from randomly selected plants/plots in each replication. Observations included plant height (cm), number of leaves plant^{-1}, neck diameter (mm), polar diameter (mm), equatorial diameter (mm), average bulb weight (g), days to harvest, total soluble solids (°Brix), pungency, rotting score, splitting incidence, thrips incidence, marketable yield (q ha^{-1}), and total yield (q ha^{-1}). Measurements followed descriptors outlined by UPOV (2010) [15] and DOGR (ICAR-DOGR, 2016) [8].

Data were subjected to analysis of variance (ANOVA) as per RBD to determine the significance of genotypic effects (Panse & Sukhatme, 1985) [12]. Genetic divergence among genotypes was assessed using Mahalanobis D^2 statistics (Mahalanobis, 1936) [10]. Genotypes were grouped into clusters using Tocher's method, and cluster means, inter-and intra-cluster distances, and trait contribution percentages were computed following the multivariate statistical procedures suggested by Singh and Chaudhary (1977) [14]. All analyses were performed using OPSTAT and R (version 4.2.3) for ensuring precision in clustering and multivariate interpretation.

Results and Discussion

Mahalanobis D^2 analysis revealed substantial genetic divergence among the eighteen onion genotypes, resulting in their grouping into six distinct clusters under Tocher's method. The formation of several multi-genotype clusters (Clusters I and II) alongside monotypic clusters (Clusters V and VI) indicates the presence of both broad-spectrum variability and uniquely differentiated genotypes within the evaluated germplasm. Similar clustering behaviour has been frequently reported in onion diversity studies, where genotypes respond differently to short-day environmental conditions, leading to pronounced multivariate dispersion (Abbasi *et al.*, 2023; Lyngkhoi *et al.*, 2021) [1, 9].

The intra-cluster distances varied considerably, with the highest value observed in Cluster II, suggesting substantial heterogeneity among the genotypes grouped within this cluster. In contrast, Clusters V and VI, which contained single genotypes, showed zero intra-cluster distance, reflecting their complete distinctiveness. These monotypic clusters indicate the presence of genotypes possessing rare or uncommon trait combinations, making them valuable sources of novel alleles for broadening the genetic base in breeding programmes (Chalbi *et al.*, 2023).

The inter-cluster distance matrix revealed pronounced genetic separation among clusters, with the maximum divergence recorded between Cluster I and Cluster III (D^2 = 10,657.99; D = 103.24). Such wide genetic separation suggests that hybridization between these clusters would produce high heterotic expression and a broad range of recombinants in segregating generations. Similar conclusions were drawn in earlier multivariate breeding studies emphasizing the importance of selecting parents from genetically distant clusters to maximize heterosis (Arunachalam, 1981; Samsuddin, 1985) [2, 13]. Considerable divergence was also recorded between Cluster I and Cluster IV and between Cluster III and Cluster VI, providing additional scope for constructing high-diversity breeding populations.

Cluster mean comparisons demonstrated clear differentiation among clusters for important agro-morphological and yield-attributing traits. Clusters characterized by higher mean values for bulb weight, bulb diameter, and total yield were positioned farther from clusters recording lower values for the same traits. These patterns confirm that yield components and bulb architecture largely drive multivariate clustering patterns in onion, as also reported by Lyngkhoi *et al.*, (2021) [9] and Abbasi *et al.*, (2023) [1].

Trait contribution analysis revealed that total yield (46.3%), marketable yield (29.4%), and bulb architectural traits (17.0%) together accounted for more than 92% of the total genetic divergence. This dominance of yield-related traits aligns with recent findings where bulb size, bulb weight, and yield attributes were shown to contribute disproportionately to inter-genotypic variation in onion germplasm (Chalbi *et al.*, 2023; Gowd *et al.*, 2024) [4, 6]. Conversely, traits such as TSS, neck thickness, rotting score, and thrips incidence contributed comparatively less to total divergence, suggesting limited influence on the overall genetic structuring despite their agronomic significance.

Taken together, the cluster distribution, magnitude of inter-cluster distances, and the relative contribution of individual traits reveal the presence of substantial and exploitable genetic diversity among the evaluated onion genotypes. The strong divergence between clusters—particularly between Cluster I and Cluster III—provides a robust scientific basis for identifying superior, genetically distant parents for

heterosis breeding. Furthermore, the monotypic clusters highlight the importance of incorporating genetically isolated genotypes to enrich breeding populations with rare and valuable traits. These findings align with current onion breeding strategies emphasizing the integration of diverse genetic backgrounds to achieve long-term genetic gains in yield, adaptability, and quality (Havey, 2019; Brewster, 2008) [7, 3].

Table 1: Cluster mean for 15 different traits

Traits	I	II	III	IV	V	VI
PH (cm)	67.58	60.24	65.33	63.53	58.48	76.87
NOL	7.19	7.88	8.31	7.37	7.57	9.25
ND (mm)	6.18	4.87	4.64	3.63	4.64	5.24
PD (mm)	40.70	44.66	44.88	34.90	43.53	47.72
ED (mm)	44.55	45.30	49.16	47.10	47.29	51.42
AWB (g)	51.36	55.00	67.74	46.53	56.89	72.70
DTH (days)	120.00	115.33	121.50	108.00	116.33	123.50
TI (%)	39.87	43.78	20.33	48.00	17.78	17.00
SBI (%)	32.78	23.42	18.96	49.95	21.70	23.86
TSS (°B)	11.18	12.50	11.15	9.00	9.93	11.95
RS	2.64	2.24	3.42	3.44	3.72	2.88
TS	2.82	2.60	3.61	3.29	3.82	3.42
PA	2.46	4.08	3.62	3.75	3.77	3.65
MY (q/ha)	146.91	160.32	235.23	130.66	177.01	255.80
TY (q/ha)	147.35	162.13	235.88	132.89	178.41	258.21

*PH: Plant height(cm), NOL: Number of leaves, ND: Neck diameter(mm), PD: Polar diameter(mm), AWB: Average weight of bulb(grams), DTH: Days to harvest, TI: Thrips infestation (%), SBI: Stemphylium blight incidence (PDI %), TSS: Total soluble solids(°B), RS: Reducing sugar, TS: Total Sugar, PA: Pyruvic acid, MY: Marketable yield(q/ha), TY: Total yield(q/ha)

Table 2: Composition of different cluster

Cluster	Number of Genotypes	Genotypes
I	6	Bhima Shakti, NHRDF Red -2, Bhima Kiran, L-913, DOGR-1626, PRO-9
II	6	Sona, Phule Samarth, NHO-920, Kasi No.1, PRO-8, NHO-920
III	2	JRO-14-14, Sandeep
IV	2	Balwan, Rashidpura
V	1	DOGR-1639
VI	1	PRO-7

Table 3: Inter (upper half diagonal) and intra (diagonal) cluster D2 and D values

	I	II	III	IV	V	VI
I	376.98 (19.42)	4186.65 (64.7)	10657.99 (103.24)	9200.97 (95.92)	5945.15 (77.1)	1341.55 (36.63)
II		488.69 (22.11)	3087.45 (55.56)	1449.9 (38.08)	1495.67 (38.67)	1318.98 (36.32)
III			151.82 (12.32)	1517.31 (38.95)	840.54 (28.99)	6487.19 (80.54)
IV				266.4 (16.32)	1711.51 (41.37)	4242.58 (65.14)
V					0(0)	3284.4 (57.31)
VI						0(0)

Table 4: Percentage contribution of each character

Traits	Singh statistic	Proportion	Cumulative proportion
TY(q/ha)	2346984.00	0.463	0.463
MY(q/ha)	1490566.00	0.294	0.757
PA	859861.50	0.170	0.926
RS	60324.20	0.012	0.938
TI (%)	58990.69	0.012	0.950
SBI (%)	58150.60	0.011	0.961
ED (mm)	45292.51	0.009	0.970
NOL	43305.73	0.009	0.979
PD (mm)	38793.33	0.008	0.987
TSS (°B)	26109.88	0.005	0.992
AWB (g)	16381.26	0.003	0.995
PH (cm)	13323.35	0.003	0.998
DTH (days)	9011.27	0.002	0.999
TS	3116.66	0.001	1.000
ND (mm)	410.95	0.000	1.000

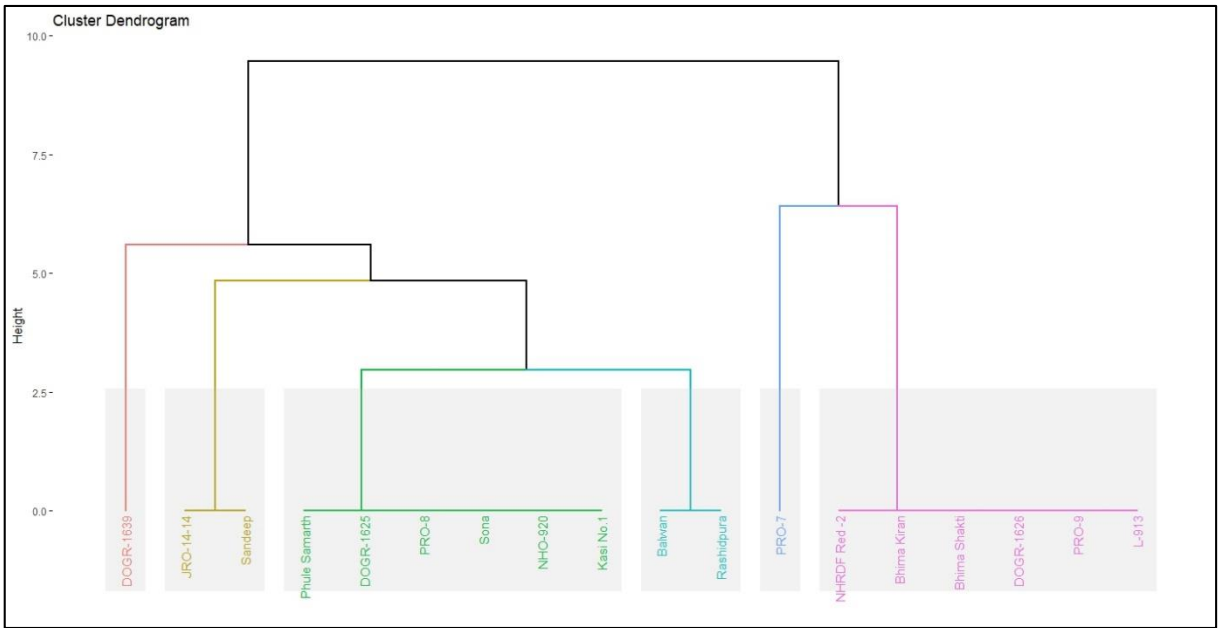

Fig 1: Dendrogram for 18 genotypes

Conclusion

The genetic divergence study based on Mahalanobis D^2 statistics revealed considerable multivariate variability among the eighteen onion genotypes, resulting in their grouping into six distinct clusters. The wide inter-cluster distances, particularly between Cluster I and Cluster III, indicate strong genetic separation and highlight these clusters as ideal parents for exploiting heterosis. The presence of monotypic clusters reflects the uniqueness of certain genotypes, making them valuable for broadening the genetic base. Trait contribution analysis showed that yield and bulb architectural traits were the major drivers of divergence, emphasizing their importance in genotype differentiation. Overall, the findings confirm the availability of substantial exploitable diversity and provide a sound basis for strategic parent selection in onion breeding programs aimed at improving yield, quality, and adaptability.

Conflict of Interest

The authors declare that there is no conflict of interest exist (both financial and non-financial).

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Authors' Contributions

Bandari Naresh: Conceptualized the study, designed the experimental layout, conducted field evaluation, collected data, carried out statistical analysis, interpreted the results and prepared the first draft of the manuscript.

Dr. Umesh Thapa: Provided overall supervision, guided the experimental methodology, contributed to data validation and interpretation, critically reviewed the manuscript, and approved the final version for publication.

Dachani Sruthi: Assisted in literature review, data tabulation, preparation of tables and figures, and contributed to editing and final proofreading of the manuscript.
All authors read and approved the final manuscript

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