

Evaluation of Genetic Diversity among Proton Beam Induced Wheat Mutants through Trait Association and Multivariate Analysis

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Abstract

Mutation induction is a potential tool to create genetic variation in plants for crop improvement for novel traits which are not elsewhere existed in the available germplasm. Mutation induction has achieved great success in wheat breeding. Till date, 264 wheat varieties have been developed through mutagenesis. Among the physical mutagens, gamma rays were most widely used, however, it causes distant ionisation events resulting into spread out damage of DNA by the low linear energy transfer (LET) radiation. In contrast, proton beam which causes high linear energy transfer (LET) radiation, causes clustered damage in the DNA near the site of ionisation and produces a broad spectrum of variation.

In the present study, wheat variety HI 1563 was mutated with 150 Gy proton beam (14 MeV) and subsequent mutant generations were grown at UBKV (M₃ onwards) to assess the genetic variation among key morpho-phenetic traits. Considerable variation was observed for traits like DTH, PH, TPP, SL, SPP, TW, GYPP, BYPP and DTM in M₃ generation. Trait association study indicated association between GYPP with other quantitative traits in the order of BYPP > TPP > PH > SPS > SL > TW > DTH > DTM. Cluster analysis revealed optimum number of 02 clusters where most of the early maturing plants with reduced height were grouped in Cluster I. PCA analysis revealed 03 PCs with Eigen values > 1.00 which contributed more than 65% of the total variation. PCA bi-plot indicated mutant lines with high cos2 values which were situated away from the origin included L1131, L1167, L1093, L1087, L1152, L297, L2536, L54, L1168, L1013, L228, L411, L412, L4638, L592, L675, L332, L424, L208, L209, L1065, L1170, L1180, L78, L1070, L1134 and L1042 and thus most divergent among the lot and could be selected for future breeding programme.

Key Words: Mutation induction, proton beam, Wheat, Trait association, PC analysis

1. Introduction

Wheat (*Triticum aestivum* L.), is an important cereal crop, belonging to Gramineae family of the genus *Triticum*, which is most widely cultivated around the globe. Globally, it provides 20% calories as well as protein of the

total dietary intake to more than around 94 developing countries (Arain *et al.*, 2022). It is also one of the oldest food crops known to humans which originated in South-East Asia (Kumar *et al.*, 2020). Cultivated wheat is an



allohexaploid crop species with chromosome constitution as $2n=6x=42$, genome formula as AABBDD and with vast a genome size of ~17 Gb that arose through a process of polyploidization and hybridisation between the cultivated tetraploid emmer wheat (genome formula AABB) and diploid *Aegilops tauschii* (genome formula DD) (McFadden and Sears, 1946; Kihara *et al.*, 1965; Wang *et al.*, 2013). In India, wheat is the second most staple food crop after rice cultivated on an area 30.46 million hectares with production 107.74 million tonnes and yield 35.21 q/ha (Progress Report, ICAR-IIWBR 2023). With the changing climatic condition as well as resurgence of new pests and diseases has adversely affected its area and production to some extent.

Mutation induction has been widely used as a potential tool for crop improvement to create phenotypic and genotypic diversity in several crops (Ling *et al.*, 2013). Mutation breeding offers several advantages over the traditional method of crop improvement like improvement in a single character of a crop variety while retaining the most of the valuable genetic combination of the wild type, creation of new alleles of gene that are otherwise not present in the genetic resources available with the plant breeders and a non-GMO approach towards genetic enhancement (Das *et al.*, 2014; Talukdar, 2014; Khah and Verma, 2015). Mutation induction has achieved great success in wheat breeding. Till date, 264 wheat varieties have been developed through mutagenesis; mainly by physical mutagen like gamma rays along with chemical mutagen like EMS and also with high linear energy transfer radiation like fast neutrons (Ahumada-Flores *et al.*, 2021). Among the physical mutagens, gamma rays are most widely used to create genetic variation (Arain *et al.*, 2022). However, it causes distant ionisation events and results in spread out damage of DNA by the low linear energy transfer (LET) radiation (Kumar *et al.*, 2020). In contrast, proton beam which causes high linear energy transfer (LET) radiation, resulted into clustered damage in the DNA near the site of ionisation and produces a broad spectrum of variation (Katano *et al.*, 2016). Countries like China, Korea and Japan are already involved in using high linear energy transfer radiation for generating novel mutants in crop plants. However, there are no published reports regarding their use in plant breeding programs under Indian condition.

The present variety HI 1563 was released for late sown irrigated conditions under North Eastern Plain Zone (NEPZ) during 2011 and had yield potential of 37.6 q ha⁻¹ and also multiple disease resistance prevalent in this zone. However, to cope up the changing climatic conditions like uneven distribution of rainfall, occurrence of pre-monsoon shower, terminal heat along with to fight against the occurrence of new diseases like wheat blast, this variety was irradiated with proton beam at BARC, Mumbai and subsequent mutant population was evaluated at UBKV to assess genotypic and phenotypic variability.

Materials and Methods

Experimental material

Fresh seeds of wheat variety (HI 1563) were sent to BARC, Mumbai for irradiation with proton beam in 2020. Seeds were irradiated with 150 Gy proton beam (14 MeV) at Nuclear Agriculture & Biotechnology Division (NA&BT), Bhabha Atomic Research Centre, Trombay, Mumbai, India and the corresponding M₁ generation was grown there. The subsequent M₂ and M₃ generation (600 lines) were grown at UBKV, Pundibari for field evaluation during 2021 and 2022 respectively. The detailed procedure followed for the development of mutant lines were presented in Figure 1.

Experimental site

The seeds of individual M₃ mutant line were sown in a separate row by following ear to row method at the instructional farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, during the *Rabi* 2022. The characteristic feature of the soil of this region is sandy loam with acidic pH. Seeds of each mutant line were sown in single row of 1 m length with a row-to-row distance of 50 cm and standard crop management practices were followed throughout the growing period for raising a healthy crop.

Morphological data

Data were recorded on nine quantitative traits viz. days to heading (days), plant height (cm), number of tillers per plant (nos), spike length (cm), seeds per spike (nos), test weight (g), grain yield per plant (g), biological yield per plant (g) and days to maturity (days) from five randomly selected plants from each mutant line and the mean value of five plants were used for further analysis.



Data analysis

Data analysis performed on the recorded data in this study consisted of descriptive statistics, box plots, frequency distribution curve, trait association, Principal Component Analysis and K-means clustering. The analysis was performed using PAST v 3 software and R software. Descriptive statistics, box plots and Principal Component

Analysis was analysed with PAST v 3 software. PCA bi-plot was constructed for the determination of degree of association among various quantitative traits. Trait association and K-means clustering was performed for selection of mutant lines with maximum value for quantitative traits with R software.

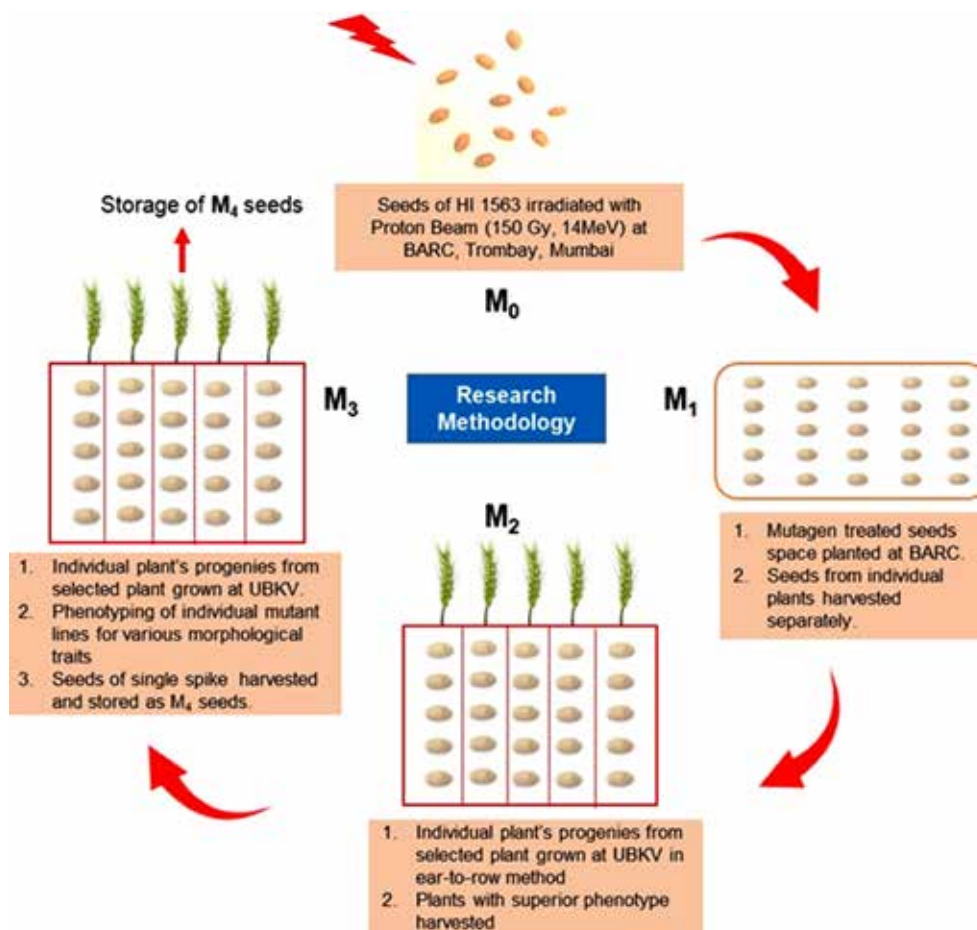


Figure 1. Schematic representation of procedure followed for development of mutant population

Results & Discussion

3.1 Descriptive statistics for morphological traits

Considerable variation was observed among the mutant lines with respect to morphological characters like days to heading, plant height, number of tillers per plant, spike length, seeds per spike, test weight, grain yield per plant, biological yield per plant and days to maturity (Table 1). Days to heading varied from 66 days to 74 days with an average of 69.92 days while plant height ranged from 54.20 cm to 100.40 cm with an average of 76.03 cm. Other traits which varied most included tillers per plant which

varied from 3.00 to 14.80 with an average of 7.67. Spike length ranged from 6.40 cm to 17.60 cm with an average of 10.45 cm whereas seeds per spike varied from 20.00 to 55.60 with an average of 40.63. Test weight ranged from 30.74 g to 49.58 g with an average of 40.07 g. Grain yield per plant varied from 10.00 g to 26.00 g with an average of 15.87 g while biological yield per plant ranged between 22.60 g to 59.60 g with an average of 37.64 g. Days to maturity varied from 106 to 116 days with an average of 110.03 days. Box plots (Figure 2) of these traits also exhibited range of variation along with its average value indicating high variation in traits like SPP and BY.



Thus, the mutant lines under study revealed variation for all the morphological traits studied and be subjected to selection for desirable plant types suitable for this agro-climatic condition. The present variation among different morphological traits is also essential to characterize the genetic diversity of this mutant population which was

analysed later. Importance of genetic variation among different morphological traits in mutant population of wheat was previously described by earlier workers such as Atkinson *et al.*, 2008; Xie *et al.*, 2015; Aliu *et al.*, 2010; Shinwari *et al.*, 2014 and Singh *et al.*, 2014. The present result was in conformity with these earlier findings.

Table 1. Descriptive statistics performed on nine traits for 600 mutant lines

Trait	Range	Mean $\pm$ SE	CV (%)
DTH	66-74	69.92 $\pm$ 0.09	3.23
PH	54.20-100.40	76.03 $\pm$ 0.23	7.34
SL	6.40-17.60	10.45 $\pm$ 0.05	11.94
TPP	3.00-14.80	7.67 $\pm$ 0.08	24.62
SPP	20.00-55.60	40.63 $\pm$ 0.27	16.48
GYPP	10.00-26.00	15.87 $\pm$ 0.13	20.16
TW	30.74-49.58	40.07 $\pm$ 0.13	8.17
BYPP	22.60-59.60	37.64 $\pm$ 0.29	18.55
DTM	106.00-116.00	110.03 $\pm$ 0.09	2.09

[DTH - Days to heading; PH - Plant height; TPP - Number of tillers per plant; SL - Spike length; SPP - Seeds per spike; TW - Test weight; GYPP - Grain yield per plant; BYPP - Biological yield per plant; DTM - Days to maturity]

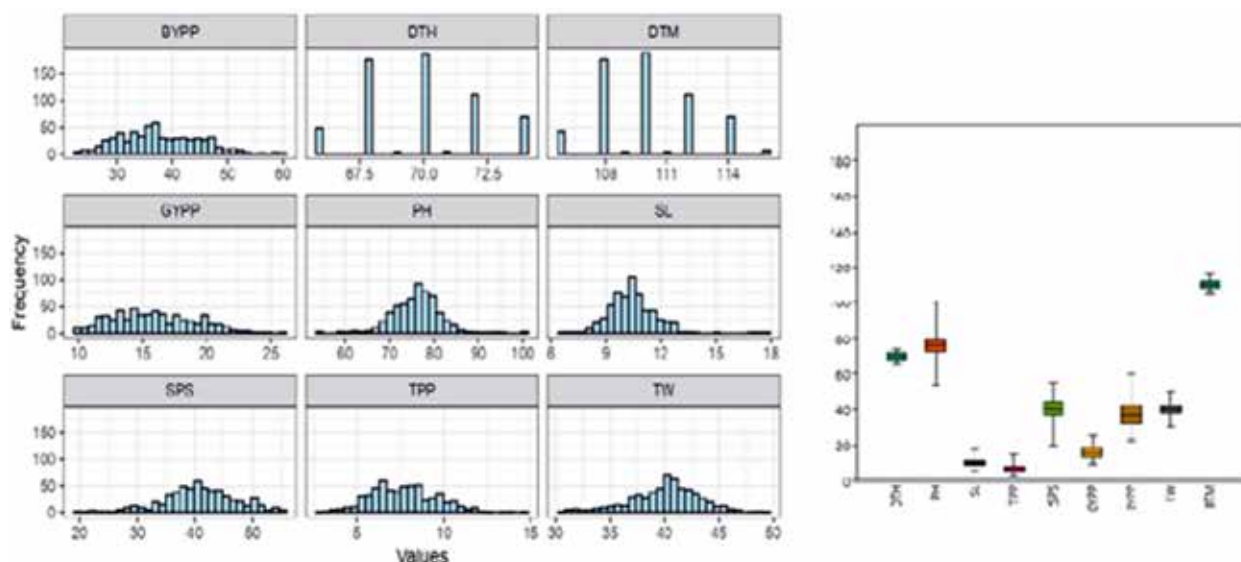


Figure 2. Frequency distribution curve and box plot of nine quantitative traits

3.2 Association study

In the present study, Pearson's correlation coefficient estimated among the mutant lines were presented in Figure 3. Among the yield components GYPP showed significant correlation with PH (0.122), TPP (0.137) and BYPP (0.093) only. However, BYPP showed positive and significant association with PH (0.150), TPP (0.170), GYPP (0.943) and TW (0.093). Other major yield traits

such as TPP showed significant negative association with DTH (-0.209) and DTM (-0.145) and significant positive association with PH (0.412), SPS (0.157), GYPP (0.137), BYPP (0.170) and TW (0.167). Negative association of DTH and DTM with TPP indicated that higher duration in flowering and maturity adversely affected no. of tillers per plant in wheat for the present study. SPP exhibited significant positive correlation with PH (0.215), SL



(0.147), TPP (0.157) and TW (0.245). TW showed positive correlation with PH (0.195), TPP (0.167), SPS (0.245) and BYPP (0.093) and negative correlation with DTH (-0.135) and DTM (-0.108). Positive correlation of SL was found with DTH (0.263), PH (0.431), TPP (0.063), SPS (0.147) and DTM (0.250).

Morphological traits like PH showed positive association with SL (0.431), TPP (0.412), SPS (0.215), GYPP (0.122), BYPP (0.150), TW (0.195) and DTM (0.082). DTH exhibited positive correlation with DTM (0.092) and SL (0.263) and negative correlation with TPP (-0.209), TW (-0.135).

Thus, the presence of association highlighted the significance of selection on the basis of these parameters which could help to identify high yielding superior mutant lines from the very early generations of breeding material. The main objective of plant breeders is focussed around

increasing the yield potential of a particular crop through suitable selection strategies. However grain yield being a complex trait is affected by the interplay of many other traits. Hence it is important to understand the degree and direction of association among various morphological and reproductive traits for their selection to achieve a cumulative yield gain. The presence of association between these key morphological and reproductive traits highlighted the significance of simultaneous selection on the basis of these traits to identify high yielding superior mutants with desirable trait combinations in subsequent generations. Similar trends of correlation of GY with other traits was also reported by Rana et al. (2024) in four different wheat varieties treated with gamma rays and methanesulphonate. Mangi et al., 2016; Fadli et al., 2022; Singh and Negi 2022 found significant correlation between GY and SL while mutation studies in wheat.

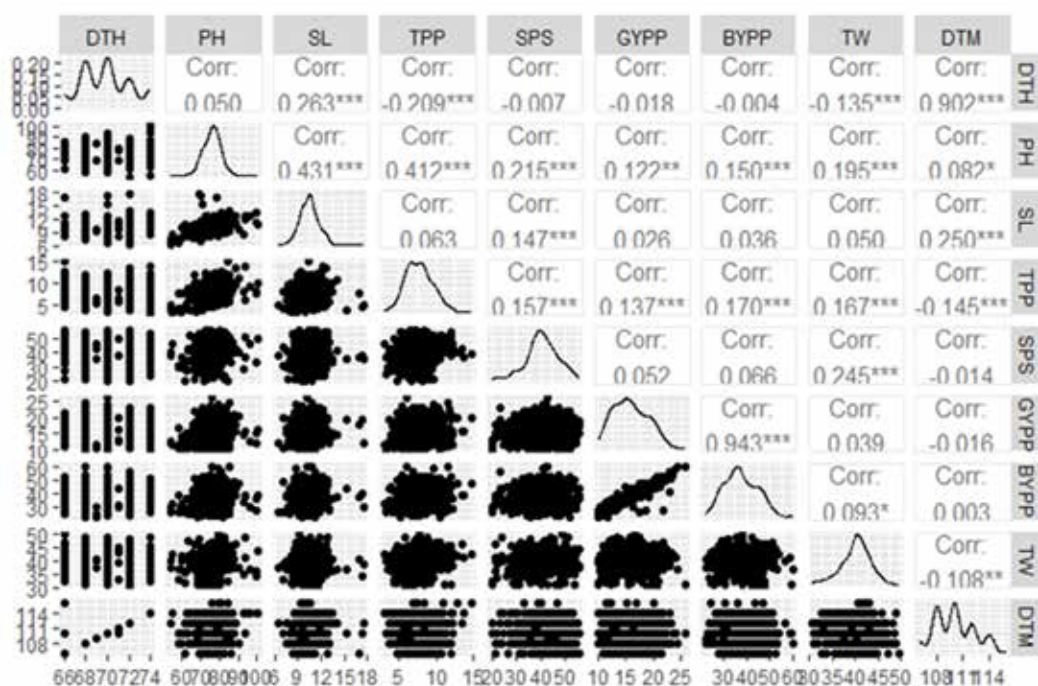


Figure 3. Trait association performed on nine quantitative traits. Lower diagonal represented scatter plots; diagonal represented histograms; above diagonals represented correlation coefficients; *significant at $p \leq 0.05$; **significant at $p \leq 0.01$; ***significant at $p \leq 0.001$.

3.3 Cluster analysis

K-means clustering is a popular approach of separating genotypes/lines based on their contrasting characters (Nagaraja et al., 2023). Through average silhouette method the optimum number of clusters for 600 mutant lines that were evaluated for nine quantitative traits was found to be

two viz., cluster I and cluster II (Figure 4). Cluster I had lesser number of lines (249) in comparison to cluster II (352). Wild type (HI 1563) was found in cluster II; Traits like days to heading, days to maturity and plant height exhibited lower mean values in cluster I as compared to the mean value of wild type (Table 2), thus early flowering



mutant lines with reduced plant height were grouped in cluster I. Similar findings were reported by Nagaraja et al. (2023) for selection of early flowering genotypes from different clusters than wild type.

Table 2: Mean cluster values for cluster I and cluster II along with mean value of wild type

Trait	Cluster I	Cluster II	HI I563
Days to heading	69.65	70.12	70
Plant height	73.38	77.90	84.80
Number of tillers per plant	7.06	8.10	6.00
Spike length	10.25	10.59	12.80
Seeds per spike	35.91	43.97	44.00
Grain yield per plant	14.69	16.71	15.80
Biological yield	34.79	39.66	36.80
Days to maturity	109.73	110.24	100.00
Test weight	38.99	40.83	38.00

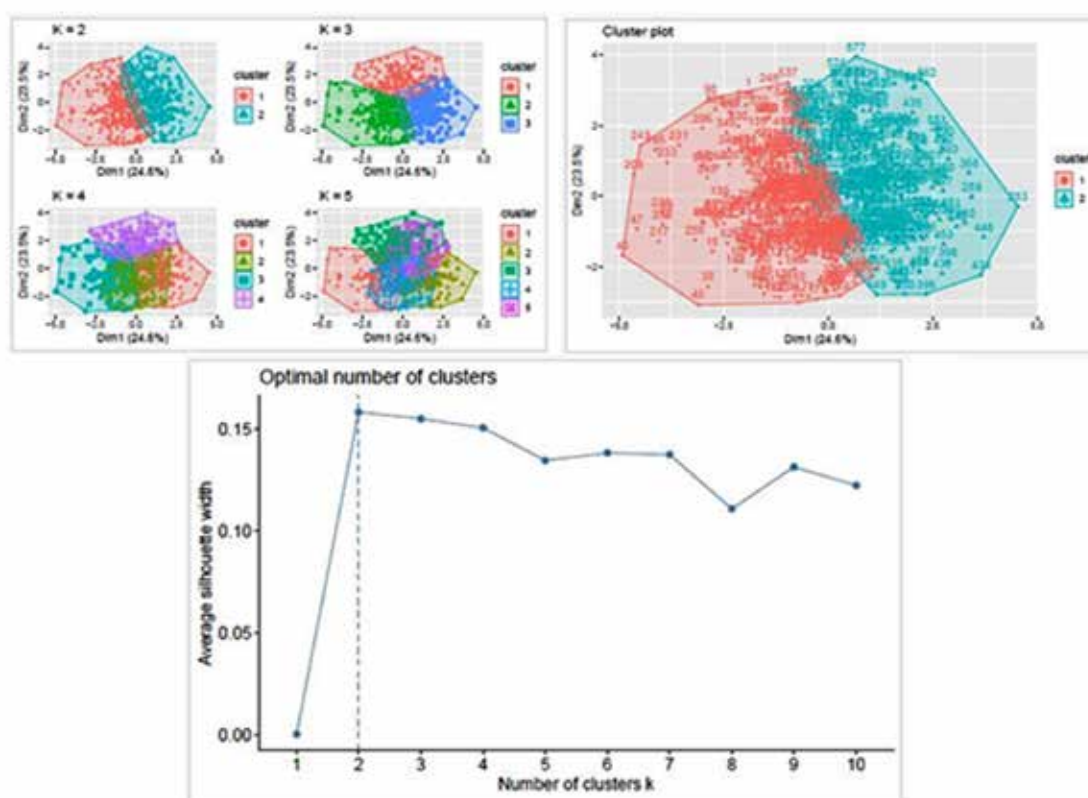


Figure 4. Determination of optimum number of clusters by average silhouette method

3.4 Principal component analysis

Principal component analysis is a multivariate technique of dimensionality reduction of the large original data set into few variables without losing the original information (Jena *et al.*, 2023). The variation present with the studied mutant lines were also assessed using principal

component analysis. In the present analysis, total nine principal components could be formed out of which only three principal component displayed Eigen values greater than one and contributed more than 65% of the total variation among the mutant lines (Table 3). The first principal component accounted for maximum proportion of variance (24.61%) followed by Principal Component



2 (23.46 %) and Principal Component 3 (17.91 %). In the first principal component, traits like biological yield, grain yield, number of tillers per plant, plant height, test weight and seeds per spike were major contributors toward the total variance; hence these traits should be given more preference in the future selection program for development superior mutant lines. Similar results was also reported by Zulfiqar et al. (2021) where the first three principal component contributed more than 65% of the total variation in a gamma rays induced wheat mutant population. Rana et al. (2024) also found PC 1, PC 2 and PC 3 with Eigen values greater than one which explained more than 78%, 79%, 77% and 78% of total variation in four different varieties of wheat treated with different doses of gamma rays and methanesulphonate.

Among the traits, PC 1 exhibited positive effects with plant height (0.54), number of tillers per plant (0.55), spike length (0.23), seeds per spike (0.34), test weight (0.36), grain yield (0.76) and biological yield (0.79). Similarly, in case of PC 2, the characters which showed positive association included days to heading (0.91), plant height (0.29), spike length (0.54), seeds per spike (0.07), test weight (0.38), grain yield (0.10), biological yield (0.11) and days to maturity (0.91). Likewise in the PC 3 had positive effects with plant height (0.52), number of tillers per plant (0.77), spike length (0.39), seeds per spike (0.42)

and test weight (0.38). As most of these traits are major yield components in case of wheat, selection of any of these components would improve the yield potential of the mutant lines in positive direction. Similar results were reported by Niyazi *et al.* (2023) where positive effect of spike length and biological yield was found in case of PC 1.

3.5 PCA biplot

Since the first two components (PC 1 and PC 2) accounted for maximum variation in the population, they were used to construct the PCA bi-plot. The contribution of individual mutant line towards a principal component were presented in Figure 5 as indicated by Cos2 values of these lines. Mutant lines L396, L206, L762, L62, L63, L1055, L218, L283, L80 and L267 had weak contribution (Cos 2 value 0.25) and these mutant lines were clustered at the origin. Whereas mutant lines L1131, L1167, L1093, L1087, L1152, L297, L2536, L54, L1168, L1013, L228, L411, L412, L4638, L592, L675, L332, L424, L208, L209, L1065, L1170, L1180, L78, L1070, L1134 and L1042 had good contribution (Cos2 value 0.75) and these mutant lines were found away from origin in PCA- biplot. Thus these mutant lines with high Cos2 values can be selected for utilisation in future breeding programme. Mangi et al. (2021) reported that the mutant lines that were closely located to each other on a PCA biplot had narrow genetic base.

Table 3. Principal component analysis for nine quantitative traits with Eigen value greater than one for first three components among mutant lines of wheat.

Parameter	PC1	PC2	PC3
DTH	-0.21	0.91	-0.11
PH	0.54	0.29	0.52
SL	0.23	0.54	0.39
TPP	0.55	-0.12	0.77
SPS	0.34	0.07	0.42
GYPP	0.76	0.10	-0.60
TW	0.36	-0.11	0.38
BYPP	0.79	0.11	-0.56
DTM	-0.188	0.91	-0.08
EV	2.21	2.11	1.61
PV	24.61	23.46	17.91
CuV	24.61	48.07	65.98

[DTH - Days to heading; PH - Plant height; TPP- Number of tillers per plant; SL - Spike length; SPP - Seeds per spike; TW -Test weight; GYPP- Grain yield per plant; BYPP - Biological yield per plant; DTM - Days to maturity]



In PCA biplot analysis, all the parameters included in the study were represented as vectors emanating from the origin. According to Hartmann *et al.* (2018), the vectors which were more parallel towards the principal component axis had more contribution towards that component. In the present study, the traits like grain yield and biological yield were closely parallel towards the PC 1, which indicated maximum contribution of these two traits in case of PC 1 (Figure 5). Similarly, PC 2 had closely parallel vectors such as days to heading and days to maturity. Thus, these two traits were mostly contributing towards PC 2. Similar results was also reported by Rana *et al.* (2024) while deciphering induced variability in

four bread genotypes treated with gamma rays and methanesulphonate.

Length of the vector in PCA bi-plot also indicated the extent of variability contributing by the concerned trait (Hartmann *et al.*, 2018). In the present study, traits like days to heading, days to maturity, grain yield and biological yield were noticed with longer vector length, indicating their higher contribution towards genetic variability in comparison to other traits. The close angles between the vectors of days to heading and days to maturity along with grain yield and biological yield revealed that these parameters were positively correlated among each other and thus selection of one parameter will also affect the other trait in positive direction.

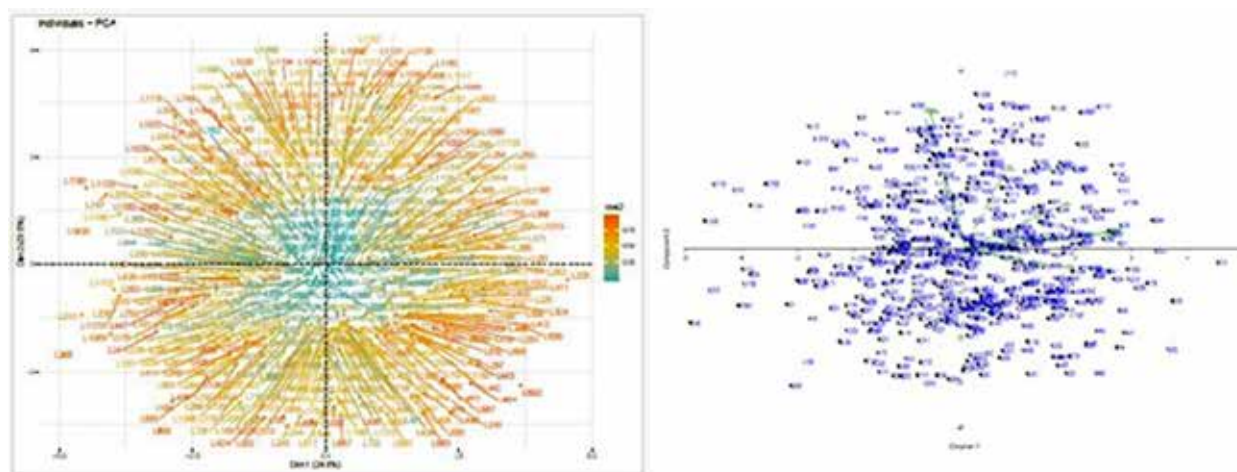


Figure 5. Scree plot and PCA biplot for nine quantitative traits among 600 mutant line

Conclusion

Findings of the present investigation indicated proton beam as a potential physical mutagen to create variability in wheat with respect to phenotypic variation for different morphological traits. Highest variation was found for traits like TPP, GYPP, BYPP and SPP which are the major selection criteria for superior plant ideotype. Trait association study indicated significant association between GYPP with other quantitative traits in the order of BYPP > TPP > PH > SPS > SL > TW > DTH > DTM. Thus indirect selection for these traits could lead to develop superior mutants in the subsequent generation. Cluster analysis revealed optimum number of 02 clusters where most of the early maturing plants with reduced height were grouped in Cluster I. PCA analysis of M_3 generation revealed three principal components which displayed

Eigen values greater than one and contributed more than 65% of the total variation. Among the traits, BYPP, GYPP, TPP, PH, TW and SPP were found as major contributors toward the total variance in PCA analysis, hence these traits should be emphasised in the selection program for developing superior mutant lines. PCA bi-plot indicated that the mutant lines with high cos2 values which were situated away from the origin included L1131, L1167, L1093, L1087, L1152, L297, L2536, L54, L1168, L1013, L228, L411, L412, L4638, L592, L675, L332, L424, L208, L209, L1065, L1170, L1180, L78, L1070, L1134 and L1042 and thus most divergent among the lot and could be selected for future breeding programme.

Author contributions

Conceptualisation of research (SD, RM, BKD & GV); Design of the experiment (TKB and SD); Contribution of



experimental material (BKD and GV); Execution of field experiment and data collection (TKB and SD); Analysis of data and interpretation (RM, SD and TKB); Preparation of manuscript (TKB and SD).

Conflict of interest

No

Declaration

The authors declare no conflict of research

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References

1. Ahumada-Flores S, LR Pando, FIP Cota, EDLC Torres, F Sarsu and SDLS Villalobos. 2021. Technical note: Gamma irradiation induces changes of phenotypic and agronomic traits in wheat (*Triticum turgidum* ssp. durum). *Applied Radiation and Isotopes* 167:109490.
2. Aliu SA and S Fetahu. 2010. Determination on genetic variation for morphological traits and yield components of new winter wheat (*Triticum aestivum* L.) lines. *Notulae Scientia Biologicae* 2: 121-124.
3. Arain SM, MA Sial, and KD Jamali. 2022. Identification of wheat mutants with improved drought tolerance and grain yield potential using biplot analysis. *Pakistan Journal of Botany* 54(1): 45-55.
4. Atkinson MD, PS Kettlewell, PR Poulton, and PD Hollins. 2008. Grain quality in the broadbalk wheat experiment and the winter North Atlantic oscillation. *Journal of Agriculture Science* 146:541.
5. Das P, M Mishra, N Lakra, S Singla-Pareek, and A Pareek. 2014. Mutation breeding: a powerful approach for obtaining abiotic stress tolerant crops and upgrading food security for human nutrition. *Mutagenesis: Exploring Novel Genes and Pathways*, pp. 15-36.
6. Fadli, MUH, MUH Farid, A Yassi, N Nasaruddin, MF Anshori, A Nur, and S Suratman. 2022. Evaluation of the advanced yield trial on tropical wheat (*Triticum aestivum*) mutant lines using selection index and multivariate analysis. *Biodiversitas* 23(1):540-547.
7. Hartmann K, J Krois and B Waske. 2018. E-learning project SOGA: Statistics and geospatial data analysis. Department of Earth Sciences, Freie Universitaet Berlin, 33.
8. Jena NK, PI Vethamoni, T Saraswathi, N Senthil, and D Uma. 2023. Selection criteria and multivariate analysis for identification of Turkey berry (*Solanum torvum*) genotypes for genetic improvement by using correlation and principal components analysis. *Electronic Journal of Plant Breeding* 14(3): 884-892.
9. Khah M and R Verma. 2015. Assessment of the effects of gamma radiations on various morphological and agronomic traits of common wheat (*Triticum aestivum* L.) var. WH-147. *European Journal of Experimental Biology* 5(7): 6-11.
10. Kihara H, H Yamashita and M Tanaka. 1965. Morphological, physiological, genetical, and cytological studies in Aegilops and Triticum collected from Pakistan, Afghanistan and Iran. Results of the Kyoto University scientific expedition to the Korakoram and Hindukush in 1955. In: K. Yamashita (Ed.), cultivated plants and their relatives. Kyoto, pp. 4-41.
11. Kotaro I, Y Kazama, R Morita, T Hirano, T Ikeda, S Usuda, Y Hayashi, S Ohbu, R Motoyama, Y Nagamura and T Abe. 2016. Linear energy transfer-dependent change in rice gene expression profile after heavy- ion beam irradiation. *PLoS One* 11: e0160061.
12. Kumar V, G Vishwakarma, A Chauhan, A Shitre, BK Das, JP Nair, P Surendran, H Sparrow, and AK Gupta. 2018. Use of proton beam as a novel tool for mutations in rice. *BARC NEWSLETTER*.
13. Ling APK, YC Ung, S Hussein, AR Harun, A Tanaka and H Yoshihiro. 2013. Morphological and biochemical responses of *Oryza sativa* L. (cultivar MR219) to ion beam irradiation. *Journal of Zhejiang University- Science B (Biomed & Biotechnol)* 14: 1132-1143.
14. Mangi N, AW Baloch, SM Arain, M Baloch, MN Kandhro, TF Abro, SN Baloch and SN Mari.



2016. Evaluation of advance mutant genotypes and interrelationship analysis of yield and yield associated traits in bread wheat genotypes. *Sindh University Research Journal* 48(4):783-786.
15. Mangi N, AW Baloch, NK Khaskleli, M Ali and W Afzal. 2021. Multivariate analysis for evaluation of mutant bread wheat lines using metric traits. *Sindh University Research Journal* 1(1):29-34.
16. McFadden ES, ER Sears. 1946. The origin of *Triticum spelta* and its free-threshing hexaploid relatives. *Heredity* 37: 81-89.
17. Nagaraja TE, S Bhat, C Nandini, HS Saritha and SG Parveen. 2023. A multivariate approach to assess the genetic diversity in finger millet [*Eleusine coracana* (L.) Gaertn] germplasm. *Electronic Journal of Plant Breeding* 14(4): 1317-1329.
18. Niyazi MK, G Synrem and S Pandey. 2023. Studies on genetic diversity and principal component analysis in wheat germplasm (*Triticum aestivum* L.). *International Journal of Plant & Soil Science* 35(15): 234-243.
19. Progress Report, ICAR-IIWBR. 2023. Director's Report of AICRP on Wheat and Barley 2022-23, Ed: Gyanendra Singh. ICAR- Indian Institute of Wheat and Barley Research, Karnal, Haryana, India. P.90.
20. Rana A, V Rana, S Bakshi, V Kumar, Sood, Priyanka & Anuradha. 2024. Deciphering induced variability, character association and multivariate analysis utilizing gamma rays and ethyl methanesulfonate in bread wheat (*Triticum aestivum* L.) genotypes with differential grain texture. *International Journal of Radiation Biology*. <https://doi.org/10.1080/09553002.2024.2304823>.
21. Shinwari ZK, HINA Rehman and MA Rabbani. 2014. SDS-Page based genetic divergence in safflower (*Carthamus Tinctorius* L.). *Pakistan Journal of Botany* 46: 811-815.
22. Singh J and A Negi. 2022. Character association in M₄ micro mutants in wheat (*Triticum aestivum* L.). *Current Applied Science and Technology* 41(48):123-128.
23. Singh MK, PK Sharma, BS Tyagi and G Singh. 2013. Genetic analysis for morphological traits and protein content in bread wheat (*Triticum aestivum* L.) under normal and heat stress environments. *Indian Journal of Genetics and Plant Breeding* 73: 320-324.
24. Sunil K, N Kumar, S Prajapati and S Maurya. 2020. *Journal of Pharmacognosy and Phytochemistry* 9(2): 1985-1997.
25. Talukdar D. 2014. Mutagenesis as functional biology tool in the improvement of legumes. *Mutagenesis: Exploring Novel Genes and Pathways*, pp. 37-62.
26. Wang J, MC Luo, Z Chen, FM You, Y Wei and Y Zheng. 2013. *Aegilops tauschii* single nucleotide polymorphisms shed light on the origins of wheat D-genome genetic diversity and pinpoint the geographic origin of hexaploid wheat. *New Phytologist* 198(3):925-937.
27. Xie Q. 2015. Physiological and genetic determination of yield and yield components in a bread wheat × spelt mapping population. Doctoral Dissertation, University of Nottingham, Nottingham, UK.
28. Zulfiqar S, S Ishfaq, M Ikram, MA Nawaz and M Rahman. 2021. Characterization of gamma-rays-induced spring wheat mutants for morphological and quality traits through multivariate and GT bi-plot analysis. *Agronomy* 11:2288.

