Estimation of Genetic variability and Frequency Distribution of EMS Mutant derived F₂ Population of Wheat (*Triticum aestivum* L.) for Varied Agronomic Traits

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ABSTRACT: The investigation was carried out at the experimental farm of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, spanning the winter seasons from 2021 to 2023. The experimental material comprised P₁, P₂, and F₂, encompassing 240 lines within the F₂ population derived from the cross mutant line BSM 583 × PBW 343. The experimental was conducted in augmented design, incorporating two commercial cultivars (HD 3298, HD 3226) and two parents as checks for comparative analysis. The findings underscored significant differences in phenotypic values among the measured traits between the two parents and the examined cross. While the means of all studied characteristics surpassed those of the checks, considerable variability in yields and yield components was evident due to the introduced mutation. The averages of measured traits in both F₁ plants and F₂ populations across all crosses closely aligned with those of the respective parents. Notably, the study revealed no consistent reduction in heritability attributable to the mutation. Furthermore, the investigation unveiled substantial genotypic variance for all twelve traits, with coefficients of variance ranging from 1.95 to 37.26%. Remarkably, tiller number (TN) and Biomass exhibited the highest genetic coefficients of variance (GCV) (55.08 %, 58.12%) and phenotypic coefficients of variance (PCV) (55.64 % and 58.32 %), respectively, followed by peduncle length with GCV at 40.83 % and PCV at 42.46 %. Traits such as plant height (PH), tiller number (TN), Flag leaf length (FL), Peduncle length and biomass demonstrated both high heritability and significant genetic advance. In essence, the study underscores the extensive variability introduced by the mutation, impacting yield-related traits, while also emphasizing the heritability and genetic advance observed in key agronomic parameters. In this study, the heritability of yield attributing parameters within an ethyl methanesulfonate (EMS) induced mutant population poses a significant challenge, given the inherently random and spontaneous nature of mutations. In addressing this challenge, our study delves into the examination of heritability and variability within the F2 population of EMS-induced mutants. The objective is to comprehensively assess the potential of these mutants as valuable contributors to the breeding cycle, positioning them as promising donors in enhancing agricultural yields and for crop improvement initiatives.

Keywords: Variability, EMS Mutant population, Heritability, Phenotypic Variance.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) holds a prominent status as the world's primary cereal grain, serving as a dietary staple for over a third of the global population (Shewry *et al.,* 2015). This annual, self-pollinating plant was first domesticated a remarkable 10,000 years ago and is now cultivated across the globe. The crux of worldwide wheat production revolves around two modern species: common or hexaploid bread wheat (*Triticum aestivum*, 2n = 6x = 42, AABBDD) and durum or tetraploid wheat (*T. turgidum* subsp. durum, 2n = 4x = 28, AABB). Notably, it plays a pivotal role in ensuring food security, contributing more than 35% of the cereal calories in the developing world, 74% in the developed world, and 41% globally through direct consumption (Giraldo *et al.,* 2019).

The quest for improved wheat varieties that can thrive in diverse agro-climatic conditions hinges on the extent of genetic diversity within a population for specific traits. As proposed by Rahman *et al.* (2013), the initial step in developing these varieties entails evaluating the genetic variability of available genotypes with regard to the desired characteristics. Grain yield, a multifaceted quantitative trait, is influenced by various yield-contributing factors (Faysal *et al.,* 2022). Consequently, selecting desirable genotypes should consider not only yield itself but also other yield components. Relying solely on yield when selecting wheat varieties can be misleading due to the polygenic nature of wheat yield regulation. Thus, to enhance crop improvement, it is crucial to make effective use of genetic resources and gain insights into both genotypic and phenotypic

correlations, revealing the relationships between different traits and grain yield (Kumar *et al.,* 2022; Mahawar *et al.,* 2022).

The concept of heritability delves into the extent to which the genetic variations seen in a phenotype can be transmitted to the next generation. On the other hand, the study of Genotypic Advance (GAM) pertains to the enhancement in the average genetic characteristics of offspring compared to the parental population (Ajmal and Irshad-ul-Haq 1995). For traits that exhibit both substantial heritability and a noteworthy increase in genetic values as a percentage of the mean, it implies that these traits are influenced by additive genetic factors. As a result, they can serve as valuable selection criteria for enhancing the overall yield of the population. Conversely, traits with low heritability but a substantial genetic advance as a percentage of the mean can also be considered for selection. Low heritability in such cases may be attributed to significant environmental influences, but the potential for genetic improvement is still notable. However, traits that display both low heritability and low genetic advance as a percentage of the mean should be excluded from the selection process, as they are less likely to respond favorably to breeding efforts. Heritability and genetic advance are very valuable for calculating genetic progress in breeding program (Gite *et al.*, 2018).

However, in recent times, the research work has been focused mainly on genetic improvement of introduced wheat than indigenous wheat accessions. These make gradual reducing the variability of Indian wheat landraces and farmers use available local varieties that are low yielding and susceptible to diseases due to a shortage of improved verities. So in this study we have used the mutant line having high micronutrient content and used in the breeding program to check the heritability and genetic advances in a mapping population.

MATERIALS AND METHODS

The research was conducted during the Rabi season of 2023 at the Department of Molecular Biology and Genetic Engineering, housed within the Norman E. Borlaug Crop Research Centre (CRC), affiliated with G. B. Pant University of Agriculture and Technology in Pantnagar, Uttarakhand. Furthermore, research activities took place at the ICAR Off Season Research Station in Keylong, situated in the Lahaul-Spiti district of Himachal Pradesh. The study involved the utilization of one parent from mutant lines BSM-583 and another from the commercial cultivar PBW-343. A total of 240 segregating lines were generated from the cross BSM 583 × PBW-343. These lines were incorporated into an augmented design, featuring four checks, including two parents and two commercial cultivars, namely HD 3226 and HD 3298. Various parameters were meticulously recorded, encompassing aspects such as days to heading

(DTH), days to anthesis (DTA), plant height (PH), number of tillers (TN), flag leaf length (FL), spike length (SL), spikelet number (SN), peduncle length (PL), grain number per spike (GNPS), thousand grain weight (TGW), yield per individual plant (YPP), and biomass. The gathered data underwent rigorous analysis of variance, and genetic parameters were subsequently estimated using the "variability" package (Popat et al., 2020) in R-studio. Additionally, ANOVA of the augmented design was calculated using the "augmentedRCBD" package in R-studio.

RESULT AND DISCUSSION

Descriptive statistics revealed significant and substantial phenotypic variability for yield and yield related traits in the experimental material. Mean value of plant height is 82.79 with standard error of 0.61, standard deviation (SD) is 9.51, co efficient of variation is 6 and skewness and kurtosis is -0.61, 3.78 respectively. Similarly in other agronomic traits descriptive statistics like mean, SE, SD, CV, skewness and kurtosis of spike length (12.98, 0.23, 3.56, 3.88, 0.11 ns, 6.17**); spikelet number (20.38, 0.34, 5.28, 16.13, -.59**, 2.62 ns), tiller number (18.44, 0.64, 10.04, 8.1, 0.94 **, 4.71 **), flag leaf length (17.66, 0.26, 4.11, 9.94, -0.55**, 3.68 ns), peduncle length (6.86, 0.19 2.97, 10.31, 1.63**, 8.29**), biomass (93.23, 3.47, 54.22, 4.48, 0.89**, 4.25**) ; thousand grain weight (34.07, 0.3, 4.75, 1.95, -0.22 ns, 2.49*); days to heading (96.88, 0.26, 4, 2.82, 1.43**, 8.08**); days to anthesis (103.42, 0.24, 3.67, 2.45, 0.89**, 5.49**), Yield per plant (34.23, 1, 15.62, 37.26 , 1.13**, 6.07**) ; grain number per spikelet (81.46, 0.96, 14.92, 20.08, -0.14 ns, 2.71 ns) respectively.

The coefficient of variation was notably low for certain traits, with the lowest recorded for thousand grain weight at 1.95%, followed by days to anthesis at 2.45%, days to heading at 2.82%, and plant spike length at 3.88%. These traits exhibited minimal variability, suggesting robust genetic potential. Conversely, the traits with the highest coefficients of variation were yield per plant at 37.26%, followed by grain number per spikelet at 20.08%, and spikelet number at 16.13%. These higher coefficients of variation imply that these variables were more susceptible to environmental fluctuations.

Furthermore, descriptive statistics for twelve quantitative traits are detailed in Table 1. Notably, positive skewness was observed in parameters such as tiller number, peduncle length, biomass, days to heading, days to anthesis, and yield per plant. This skewness indicates a distribution skewed to the right side. Conversely, negative skewness was noted in plant height, spikelet number, flag leaf length, thousand grain weight, and grain number per spikelet, indicating a distribution skewed towards the left side of the data set.

Table 1: Descriptive Statistical analysis in different agronomic data of newly developed F₂ lines including 4 checks.

Parameter	Mean	SE	SD	CV	Skewness	Kurtosis
Plant Height(PH)	82.79	0.61	9.51	6	-0.61**	3.78/9**
Spike Length(SL)	12.98	0.23	3.56	3.88	0.11 ns	6.17**
Spikelet number (SN)	20.38	0.34	5.28	16.13	-.59**	2.62 ns
Tiller number(TN)	18.44	0.64	10.04	8.1	0.94 **	4.71 **
Flag leaf Length (FL)	17.66	0.26	4.11	9.94	-0.55**	3.68 ns
Peduncle length (PL)	6.86	0.19	2.97	10.31	1.63**	8.29**
Biomass	93.23	3.47	54.22	4.48	0.89**	4.25**
Thousand grain weight (TGW)	34.07	0.3	4.75	1.95	-0.22 ns	2.49*
Days to heading (DTH)	96.88	0.26	4	2.82	1.43**	8.08**
Days to anthesis (DTA)	103.42	0.24	3.67	2.45	0.89**	5.49**
Yield per plant (YPP)	34.23	1	15.62	37.26	1.13**	6.07**
Grain number per spikelet (GNPS)	81.46	0.96	14.92	20.08	-0.14 ns	2.71 ns
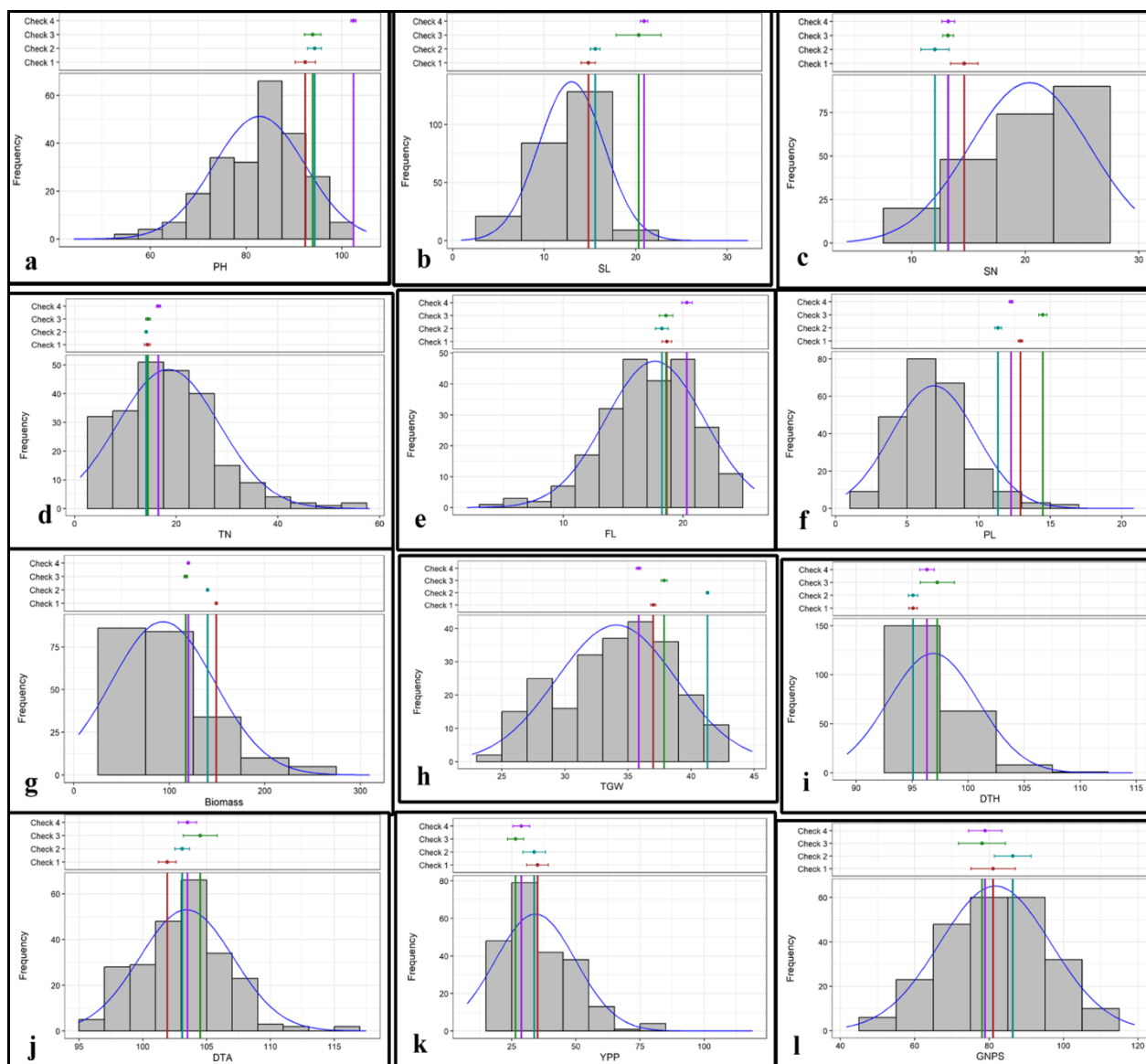
Fig. 1.

The study encompassed the calculation of Genetic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV), as well as the determination of genetic advance and genetic advance as a percentage of the mean for all traits (Fig. 2; Table 2). Following the scale proposed by Sivasubramanian and Madhavamenon (1973), traits such as tiller number (55.08%, 55.64%), flag leaf length (20.05%, 22.83%), peduncle length (40.83%, 42.46%), biomass (58.12%, 58.32%), and yield per plant (26.28%, 45.16%)

exhibited high (>20%) GCV and PCV, indicating substantial variability within the F2 populations for these traits. Conversely, traits like plant height (8.51% and 10.49%), days to heading (2.45%, 3.73%), and days to anthesis (1.98%, 3.15%) displayed low (<10%) GCV and PCV estimates, suggesting limited variability in these traits. Traits with moderate variability (10% to 20%) included 1000 grain weight (13.65% and 13.79%). Other traits, such as grain number per spikelet, exhibited a mix of moderate and low or consistently low estimates for both GCV and PCV.

Notably, PCV estimates were slightly higher than their corresponding GCV estimates across all traits. The smallest difference between PCV and GCV values was observed for tiller number, biomass, and 1000 grain weight, indicating a robust genetic expression in these traits. In contrast, traits like plant height, spike length, spikelet number, grain yield per plant, days to heading, and days to anthesis displayed a wider gap between PCV and GCV values, signifying a notable environmental influence on these traits. Effective selection is achievable for traits with lower environmental fluctuations, making thousand grains weight a crucial selection criterion due to its moderate variability and minimal disparity between PCV and GCV (Table 2).

Table 2: **Genetic Variability analysis of morphological traits in F₂ lines of BSM 583 × PBW 343.**

Parameters	Morphological traits											
	PH	SL	SN	TN	FL	PL	Biomass	TGW	DTH	DTA	YPP	GNPS
Mean	82.79	12.98	20.38	18.44	17.66	6.86	93.23	34.07	96.88	103.42	34.23	81.64
PV	75.44	4.92	20.13	105.21	16.26	8.48	2956.14	22.08	13.09	10.62	238.98	280.49
GV	49.59	NA	10.44	103.12	13.1	7.84	2936.42	21.63	5.63	4.19	80.92	12.18
EV	25.85	19.17	9.69	2.1	3.15	0.64	19.72	0.46	7.46	6.43	158.06	268.31
GCV	8.51	NA	15.85	55.08	20.05	40.83	58.12	13.65	2.45	1.98	26.28	4.27
GCV Category	Low	NA	Medium	High	High	High	High	Medium	Low	Low	High	Low
PCV	10.49	17.09	22.02	55.64	22.83	42.46	58.32	13.79	3.73	3.15	45.16	20.51
PCV category	Medium	Medium	High	High	High	High	High	Medium	Low	Low	High	High
ECV	6.14	33.74	15.27	7.85	10.06	11.67	4.76	1.99	2.82	2.45	36.73	20.06
hBS	65.73	NA	51.86	98.01	80.6	92.45	99.33	97.92	43.01	39.42	33.86	4.34
hBS Category	High	NA	Medium	High	High	High	High	High	Medium	Medium	Medium	Low
GA	11.78	NA	4.8	20.74	6.7	5.55	111.42	9.49	3.21	2.65	10.8	1.5
GAM	14.23	12.19	23.55	112.49	37.96	80.99	119.51	27.86	3.31	2.56	31.55	1.84
GAM Category	Medium	Medium	High	High	High	High	High	High	Low	Low	High	Low

The evaluation of broad-sense heritability and Genetic Advance as a percentage of the mean (GAM) followed the classifications proposed by Robinson (1966) and Johnson *et al.* (1955), respectively. Traits demonstrating high heritability (>60%) along with substantial GAM (>20%) included tiller number (98.01% and 20.74%, respectively), flag leaf length (80.6% and 37.96%, respectively), peduncle length (92.45% and 82.49%, respectively), and thousand grain weight (97.92% and 27.86%, respectively). These findings suggest that these traits exhibit a strong influence of additive gene action, indicating that crop improvement can be effectively achieved through selective breeding (Ogunniyan and Olakojo 2014).

On the other hand, traits with low heritability (<30%) and low GAM (<10%), such as grain number per spikelet (4.34% and 1.84%, respectively), indicate a substantial impact of the environment on the trait. This underscores the need for cautious selection strategies in the future. Plant height displayed high heritability (65.73%) and a moderate GAM (14.23%), pointing towards the presence of moderate non-additive gene action within a portion of the population.

In summary, traits like tiller number, flag leaf length, peduncle length, and biomass exhibited high heritability (>60%), substantial GAM (>20%), as well as high Phenotypic Coefficient of Variation (PCV) and Genetic Coefficient of Variation (GCV) (>20%). These traits have been highlighted in previous studies for their significance in crop improvement, such as grain yield (Ravikanth and Sarma 2017), 1000 grains weight (Lule *et al.,* 2012), plant height, finger length, finger width, and grain yield (Alemu *et al.,* 2020), as well as days to heading, number of productive tillers, plant height, and grain yield (Dagnaw *et al.,* 2022).

Principal component analysis. The graphs depicting Principal Component Analysis (PCA) in our study (Fig. 3) illustrate the relationships among various morphological characteristics. In Fig. 2, the foremost principal component (PC1) emerged as highly significant, explaining 22.53% of the total variance. This was predominantly influenced by factors such as flag leaf length, tiller number, biomass, thousand grain weight, and grain number per spikelet. Simultaneously, PC2 contributed to 16.86% of the variation, with its major influencers being day to anthesis, days to heading, and spikelet number.

The positive and negative loadings in the graphs signify the presence of correlation trends, whether positive or negative, between the principal components and variables, aligning with the observations of Hailegiorgis *et al*. (2011). For our study, the significance of principal components was determined based on Eigen values greater than or equal to 1.0. Notably, we observed that the Eigen values of days to heading, days to anthesis, plant height, spike length, spikelet number, and tiller number exceeded 1 compared to other traits. Consequently, these traits emerge as primary contributors to genetic divergence. Selecting based on these characteristics holds promise for effective yield improvement. Interestingly, our findings align with those of Priya *et al.* (2015).
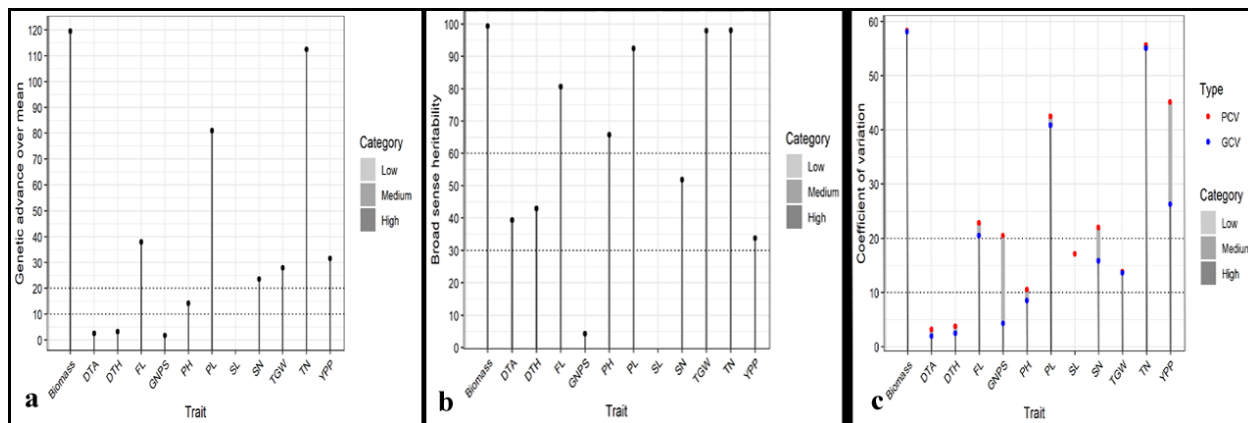
Fig. 2. (a) genetic advances over mean of 240 segregating lines (b) Broad sense heritability and (c) coefficient of variability analysis of different agronomical parameters of 240 segregating lines of F2 population from BSM 583 x PBW 343
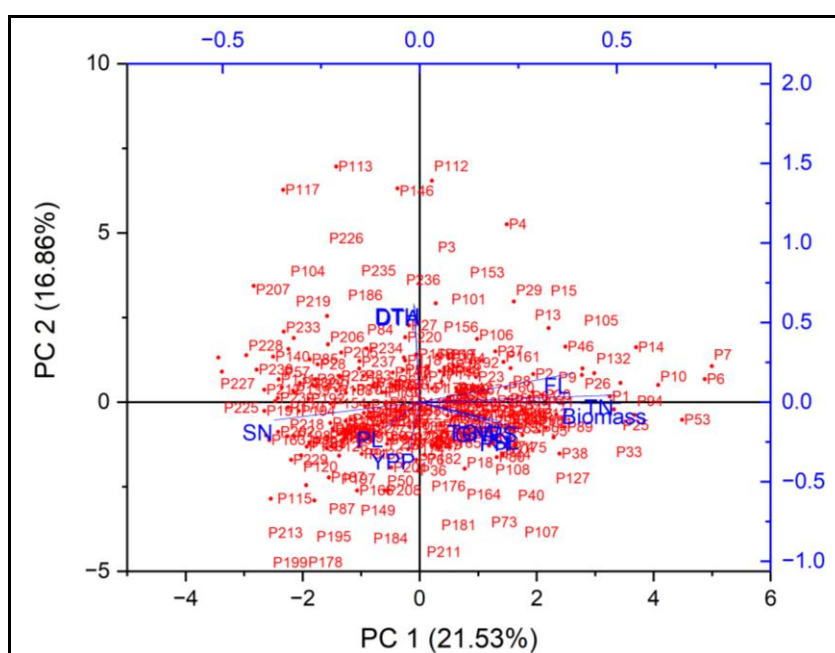
Fig. 3. Principal component analysis (PCA) of various agronomical parameters in F_2 population.

CONCLUSIONS

The current investigation highlights the substantial genetic variation among the F_2 segregating lines derived from EMS, encompassing all the tested traits. This observation provides a valuable opportunity for plant breeders to engage in the meticulous selection and enhancement of these traits. Traits that exhibit high heritability and are easily measurable can be particularly advantageous for expeditious screening of large volumes of planting materials, specifically for the trait of interest. Furthermore, the principal component analysis unveiled that principal components PC1 and PC2 collectively accounted for approximately 30% of the total variations. This outcome serves as additional confirmation of the ample genetic diversity present in the mutant lines, particularly BSM 583, thereby underlining their potential utility in wheat improvement programs.

FUTURE SCOPE

From the above study, by elucidating the heritability patterns and variability inherent in this F_2 population,

we aim to pave the way for informed and strategic integration of these mutants into breeding programs, thereby optimizing their utility and impact in crop improvement initiatives.

Conflicts of Interest. None.

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