

Variability in Indian wheat germplasm for important quality and physiological traits

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ABSTRACT

Knowledge of variability in wheat genotypes is important for proposing crosses intended for development of heterotic combinations with improved stability. The present study consists of 120 wheat genotypes comprised of landraces, genetic stocks, released varieties, and improved genotypes. For the research experiment was conducted field in two rabi seasons (2019–2020 and 2020–2021) at ICAR-IIWBR, Karnal and a total of 120 genotypes were assessed using a randomized block approach for four quality (Zinc, iron, protein content and nitrogen) and six different physiological traits (Normalized difference vegetation index [NDVI]-1, NDVI-2, Soil Plant Analysis development [SPAD]-1, SPAD-2, Canopy Temperature [CT]-1, and CT-2) beside grain yield (GY). The occurrence of significant genetic variability amongst the several genotypes for nutritional and physiological characters indicates the inevitability for utilization of a considerable degree of genetic variation through the process of selection. The genotypic and phenotypic coefficients of variation (Genotypic coefficient of variation and Phenotypic coefficient of variation) exhibited their peak values for the trait GY, trailed by, grain zinc content, nitrogen (%), and SPAD-2. The high heritability values, in conjunction with substantial genetic advances, are indicative of the significance of GY, zinc, nitrogen, and protein content are key traits that hold potential for crop enhancement purposes. The total set of 120 genotypes clustered into 12 discrete sets on the basis of quality and physiological traits using the clustering technique and principal component analysis program available in the Statistical Package for Agricultural Research. Principal components axis 1 to principal component axis 4 unveiled about 71.31% of the total variability. Based on the findings of this study, it can be inferred that the released varieties showed greater performance in terms of grain output, although some landraces displayed higher values for the quality features. Hence, to acquire a comprehensive range of superior quality (Zn, PC, N, and Fe) cum high-yielding segregants, the selected genotypes from clusters “C” and “L” could function as better lines of parentage to organize breeding plans.

1. INTRODUCTION

Globally, wheat (*Triticum aestivum* L.) is the most widely grown cereal crop, belonging to the family Poaceae and genus *Triticum*. Due to its vast territory, great production, and significant role in the food grain trade, it has been referred to as the “King of Cereals.” As the primary source of food and energy with numerous uses, it holds a unique position in day-to-day life. Forty percent of the world’s population depends on this basic cereal crop [1]. India has the position of the second-largest global producer of wheat, besides China, and holds the largest area under cultivation. Wheat holds the position of being the second most prominent food crop in the country,

after rice. It contributes to about 20% of global dietary energy and protein intake [2]. Micronutrients present in grains have a significant function in both plant physiology and human health. The impact of micronutrient insufficiency on human diets is significant and represents a prominent problem for worldwide malnutrition. Hidden hunger in developing nations leads to substantial health problems in the nation and places financial pressure on the healthcare system. The prevalence of undernourishment (PoU) in Asia fell from 8.8% in 2021 to 8.5% in 2022 - a decrease of more than 12 million people, mostly in Southern Asia. However, this is still 58 million above pre-pandemic levels. There were improvements in every subregion except Western Asia, where the PoU increased from 10.2% in 2021 to 10.8% in 2022 [3]. The current scenario presents a challenge to the agricultural scientific community to increase food production to satisfy the needs of a growing global population. The primary cause of malnutrition may be attributed to the insufficiencies of essential nutrients such as Vitamin A, Zinc, Iodine, Folate, and Iron in the diet [4]. Wheat grain contains many micronutrients, especially Zinc (Zn) and Iron (Fe). Zn

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is a very important trace element as it holds significant importance because of its pivotal involvement in several enzymes responsible for the metabolic processes of protein, carbohydrates, auxin synthesis, and to maintain the integrity of the membrane. Fe also being a crucial trace component holds a significant part in the electron conduction chain and cytochrome, in addition to contributing to the inactivation of many enzymes. The prevalence of mineral deficiencies is that more than half of all people on Earth are deficient in two essential minerals: zinc (Zn) and iron (Fe) because of their high dependence on cereal crops, particularly maize, rice, and wheat, as staple foods in their daily dietary intake [5]. Therefore, it is imperative to provide the human population with adequate nourishment for these crucial elements [6,7]. The significance of grain protein content features in wheat breeding programs is paramount since it plays a crucial role in determining bread quality [8]. One essential component of plant nutrition is nitrogen (N), which exerts a significant influence on the vegetative development and grain production of wheat [9-12]. Normalized difference vegetation index (NDVI) and standardized leaf area index (SPAD) are two examples of spectral reflectance indexes, that have been widely recognized as dependable indicators for evaluating the nitrogen (N) condition of agricultural plants [13,14]. The NDVI is a quantitative measure employed to assess the amount of green crop within a given geographical region [15]. The chlorophyll content of a leaf is commonly measured using a reliable instrument known as the SPAD meter, which is widely popular due to its manageable nature and non-damaging measurement capabilities. The SPAD and Green-Seeker devices incorporate active sensors in the form of integrated light sources, enabling their use in various environmental conditions [16]. Kizilgeci *et al.*, 2021, described a strong statistically significant association between SPAD and NDVI measurements and various grain and yield-related characteristics [16]. This finding supports the notion that SPAD and NDVI can serve as reliable indicators for nitrogen deficiency and can assist in the discovery and selection of high-quality wheat varieties, thus contributing to the overall goal of ensuring food security.

Wide variation in nutritional quality and physiological parameters is reported in all the gene pools of wheat that can be utilized as a foundation for the creation of biofortified wheat varieties through breeding techniques [17-19]. According to Velu *et al.*, 2019, certain landraces exhibit considerable potential, with notable grain constituents of Zn and Fe [20]. The choice of parent with the amount of variation is the key factors as a prerequisite for any successful hybridization program that aims at development of promising strains [21]. It is quite helpful to have a firm grasp of important genetic factors including heritability and genetic progress in predicting genetic advancements within a breeding program and greatly enhance the selection criteria. For these reasons, understanding the extent of variability is crucial for any systematic crop breeding program. Correlation coefficients are statistical measures that quantify the strength and direction of associations between independent variables, allowing researchers to better understand the relationships between different characteristics or factors.

In addition, cluster analysis and principal component analysis (PCA) have been suggested by researchers to investigate the variability in wheat [22,23]. Cluster analysis is a useful tool for evaluating important quality variation [24,25] and physiological traits [21,26,27] and the selection of high-performance genotypes. Genotypes have also been analyzed for their morphological similarity [28,29]. The purpose of this research is to assess variability among different quality and physiological traits in wheat accessions and its association with yield and to group the wheat genotypes on the basis of studied traits.

2. MATERIALS AND METHODS

2.1. Plant Material

ICAR-Indian Institute of Wheat and Barley (ICAR-IIWBR) germplasm resource unit provided the study's 120 wheat genotypes. Ninety-five of the 120 wheat genotypes presented were local variations, seven were registered genetic stocks for a particular attribute, fourteen were commercially available kinds or released varieties, three were advanced lines, and one was exotic germplasm.

2.2. Experimental Site and Design

The ICAR-IIWBR farm in Karnal (29°42' N, 76°59' E), at an elevation of 240 m above sea level, was the site of the field experiment for the 2019–20 and 2020–21 winter seasons. The study was conducted using a randomized block design, consisting of two replicates. The experiment involved cultivating each genotype in a three-row plot measuring 1.25 m in length. The spacing between rows was set at 25 cm, while the distance between individual plants within a row ranged from 5 to 6 cm. A fundamental application of N: P:K at a ratio of 50:60:40 kg/ha was administered as a basal dose during the sowing process. During the tillering phase, a top-dressing treatment of 50 kg/ha of nitrogen was applied. The plots were irrigated at intervals of 20–25 days. Manual methods were employed to control weeds. At the Zadoks developmental stages 47 and 71 (flag leaf sheath opening stage and kernel water ripe stage, respectively), two applications of propiconazole (25EC@0.1%) were administered through spraying. The harvesting of crops was done manually through the process of cutting above the ground and each plot was threshed separately.

2.3. Recording of Physiological Traits

2.3.1. NDVI

NDVI 1 was recorded at the anthesis stage and NDVI 2 values 15 days after anthesis were recorded making use of a Green Seeker active hand-held sensor, when the soil and plant foliage is dry, and green seeker sensor tool was laid around noon, about 0.5 m above the canopy, so that the tool is directly above the plot and in the middle of the middle row.

2.3.2. Canopy temperature (CT)

At 7 days after anthesis stage CT 1 was recorded, while at 15 days after anthesis stage, CT 2 was recorded from all the plots placing an infrared thermometer from about 0.5 m in the front of the canopy between 12:00 and 14:00 h, on a sunny and clear day.

2.3.3. SPAD

At the 7 days after anthesis stage SPAD 1, and 15 days after anthesis SPAD 2 were measured from three randomly selected flagged leaves on each plot by the SPAD chlorophyll meter.

2.4. Documenting Grain Sample and Micronutrient Analysis

2.4.1. Grain yield (GY)

It was recorded after the harvesting and threshing of all wheat plants in each plot. GY was measured in grams per plot (g).

2.4.2. Grain protein content(PC):

The NIR instrument was employed to measure the total grain protein content at 12% grain moisture. We calculated the protein content by utilizing the "Infratec1241" grain analyzer. This instrument utilizes near-infrared light to transmit through the seeds. Scan of seed samples collected 100 data points throughout a 7 nm bandwidth and

wavelength range of 850–1050 nm. The results, which represented the grain's protein and moisture levels, were shown as a percentage. At 12% moisture, the protein value was standardized.

2.4.3. Nitrogen estimation

Nitrogen (N) was estimated using the kjeldhal method [30,31]. First, seeds were crushed and a uniform sample was digested in intense sulfuric acid. Distillation involves converting NH_4^+ to NH_3 through the addition of excess base to the acid digestion mixture, then condensing the NH_3 gas through boiling in a receiving solution. Then, distilled vapors trapped in a solution of boric acid (H_3BO_3) and titrated with 0.1 M HCl with 2-3 drops indicator readings were noted and then nitrogen was calculated.

2.4.4. Estimating the zinc and iron content of grains

To estimate the Zn and Fe concentrations in wheat grains, a non-damaging technique “Energy Dispersive X-ray Fluorescence” (EDXRF) was used. Calibration of the EDXRF (model X-Supreme 8000; Oxford Instruments plc, Abingdon, United Kingdom) was accomplished using values derived from glass beads. Aluminum (Al) outer cups with polypropylene inner cups and a 4 m Poly-4 XRF sample film sealed at one end were used for the scans. Samples were shaken gently to disperse grains throughout the cups. When the sample spinner is engaged, the X-Supreme 8000 scans a circle with a diameter of 21 mm. This mode was used for all scans in this study, yielding a total scanned area of 346 mm². After the physiological maturity of the plants, a sample of 10 spikes was chosen at random from each entry, and then, the spikes were threshed in a clean cloth to remove the husk and expose the grain. A grain sample was taken to determine the levels of iron and zinc. At every stage, precautions were taken to prevent metal contamination. This technique is both cost-effective and efficient in terms of throughput. Zn and Fe concentrations were measured in parts per million (ppm).

2.5. Statistical Analysis

A pooled analysis of variance was conducted using 2-year data replications for each character, including Zn, Fe, PC, N, NDVI-1, NDVI-2, SPAD-1, SPAD-2, CT-1, CT-2, and GY. The Phenotypic coefficient of variation (PCV) and Genotypic coefficient of variation (GCV) were computed using the mathematical equation [32]. The heritability in the broad sense (h^2) was estimated using the recommended formula [33], while the methods given by Sáez-Plaza were employed to determine the genetic progress, which represents the expected genetic improvement [34]. Following this analysis, the means values were derived from the data collected over 2 years for each trait and thereafter utilized in the computation of descriptive statistics. Pearson's correlation coefficients among the traits were calculated. The correlation between the PCA score and 11 quality and physiological traits was calculated.

Genotypes were categorized using Statistical Package for Agricultural Research (SPAR1). A sequential F-ratio test was used to compare

different cluster solutions defined by Beale (1952) and explained by Sparks (1973) [35,36]. For each set, we determined its mean, standard deviation, and coefficient of variation [37]. Graphs were drawn using R (data analysis program) [38].

3. RESULTS AND DISCUSSION

3.1. Variability Parameters

A comprehensive evaluation was conducted on a panel consisting of 120 genotypes to investigate the extent of genetic diversity and perform association analysis. The findings suggest that the sum of squares attributed to genotypes exhibited a high level of statistical significance across all examined qualitative and physiological variables [Table 1]. This indicates the presence of ample genetic diversity within the tested material, hence supporting the potential for selection and enhancement purposes. As a result, the material provides promising avenues for research and advancement of the goals. The presence of significant variability in wheat was also reported by various scientists [39,40] for quality traits and the variability for physiological traits [21,41,42]. Table 2 displays the estimated parameters of genetic variability for all different traits.

The average Zn content in the grains was 36.43 ppm, with a range of 24–60.2 ppm, whereas Fe varied from 33.65 ppm to 54.9 ppm having a mean value of 41.57 ppm [Table 2]. This demonstrated the availability of approximate 1.6-fold variability in grain Fe content and 2.5-fold variability in Zn content in the panel [Table 2]. Similarly, the protein content of grains ranged from 8.8% to 16.6%, with a mean of 10.39 and the values of N were 1.29–2.66 %, with 1.66% being the average. NDVI-1 and NDVI-2 had a range of 0.54–0.79 and 0.32–0.65, respectively. CT-1 with a mean value of 20.59°C, ranged from 15.83°C to 23.30°C, whereas CT-2 showed a range of 19.96°C to 25.09°C. SPAD-1 and SPAD-2 showed varied ranges of 28.63–53.23 and 22.39–43.05, respectively.

The environment has an impact on the expression of each nutritional and physiological trait due to larger PCV than GCV. For every trait examined, GCV was a significant contributor to the PCV, indicating that the genotypes are reflected in the phenotype and beneficial in the selection based on phenotypic performance for these traits. The results were in accordance with those of the earlier published reports [43,44].

The observed values for the PCV and GCV ranged from 4.31% to 32.18% and 1.41% to 21.31%, respectively. A review of the data showed that the greatest coefficients of variation, both phenotypic and genotypic, were found in GY, followed by Zn. This suggests that there is a considerable amount of variability present, indicating the potential for genetic enhancement through selective breeding methods. The NDVI and CT demonstrated lower levels of genotypic and phenotypic coefficients of variation. Similar kinds of outcomes have been reported by various workers [45-49].

Table 1: Sum of squares for different traits in wheat accessions.

Source of Variation	Sum of Square											
	DF	Zn	Fe	PC	N	NDVI 1	NDVI 2	SPAD 1	SPAD 2	CT1	CT 2	GY
Replication	1	395.8	328.07	3.138	0.080	0.0354*	0.293	281.54	217.1	61.62	100.22	9067
Genotype	119	4237.6**	2451.28**	238.094**	6.0952**	0.157**	0.236**	2540.5**	2325.91**	85.215**	101.41**	1279996**
Residue	119	647.3	684.87	30.223	0.774	0.1235	0.249	632.62	895.04	122.27	125.72	499410

“***” and “**” Significant at 1% and 5% level. Zn: Zinc content in grain, Fe: Iron content in grain, PC: Protein content in grain, N: Nitrogen content in grain, GY: Grain yield per plot, NDVI1: Normalized difference vegetation index at anthesis, NDVI 2: Normalized difference vegetation index at 15 days after anthesis, CT 1: Canopy temperature at anthesis, CT 2: Canopy temperature at 15 days after anthesis, SPAD 1: Soil plant chlorophyll development at anthesis, SPAD 2: Soil plant chlorophyll development at 15 days after anthesis.

Table 2: Analysis of Genetic variability parameters: Variability, heritability (h^2), GA, GCV, and PCV of different physiological and nutritional traits of wheat accessions.

Traits	Mean±SE	Range	CD at 5%	GCV (%)	PCV (%)	h^2 (%)	GA
Zn (ppm)	36.43±1.65	24–60.2	4.62	10.66	12.44	74	18.83
Fe (ppm)	41.57±1.70	33.65–54.9	4.75	6.55	8.73	56	10.13
PC (%)	10.39±0.36	8.08–16.6	1.00	8.99	10.22	77	16.31
N (%)	1.66±0.06	1.29–2.66	0.16	9.00	10.23	78	16.32
NDVI 1	0.71±0.02	0.54–0.79	0.06	1.41	4.67	9	0.87
NDVI 2	0.54±0.02	0.32–0.65	0.04	5.85	7.17	67	9.84
SPAD 1	38.36±1.63	28.63–53.23	4.57	7.38	9.52	60	11.79
SPAD 2	33.64±1.94	22.39–43.05	5.43	7.29	10.94	44	10.01
CT1	20.59±0.36	15.83–23.30	1.00	3.81	4.53	71	6.60
CT 2	22.29±0.36	19.96–25.09	1.02	3.64	4.31	71	6.34
GY (g)	279.27±81.5	114.28–531.75	128.28	21.31	32.18	44	29.08

GA: Genetic advance, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation.

Heritability determines a trait’s genetic control and transmission to progeny, which affects its selection efficiency. Nitrogen had the highest heritability (h^2) at 78%, while NDVI had the lowest at 9%. The expression of heritability estimates in terms of genetic advancement confers greater advantages [34,50,51]. Traits with high heritability and high genetic advance, such as GY, Zn, N, and PC, indicate that the present variability was additive, which can be better exploited for crop improvement and are likely to respond to direct selection. These findings confirm the results obtained by previous researchers [20,52,53]. Wheat studies have also documented the outcomes of high heritability estimates for various traits [54–57].

3.2. Correlation Analysis

Analyses of the relationship between quality and physiological traits were conducted, and the results are shown in the form of a Chord diagram [Figure 1]. A significant partial correlation of values $r = 0.49$ ($P < 0.001$) and $r = 0.22$ ($P < 0.05$) was observed between PC and Zn, and PC and Fe, respectively. PC and N were found to be highly correlated with a value of $r = 0.99$ ($P < 0.01$). Quality parameters Zn, Fe, PC, and N showed a positive correlation with each other with a significant P -value of 0.001 [19,58–61].

CT-2 showed a negative correlation with GY with $r = -0.38$ ($P < 0.001$). Several other researchers also came to similar conclusions [21,62–64]. The findings also demonstrated that a cooler canopy has the added benefit of increasing grain-filling rate, which in turn promotes yield [65,66].

N and PC showed a confident correlation of value $r = 0.23$ ($P < 0.05$) with SPAD-1. A significant correlation was found between grain protein content and SPAD at anthesis. The results of this study suggest that genotypes with high protein content may be detected in breeding programs by measuring the chlorophyll content. Similar results were obtained by [67,68]. GY showed a positive correlation with, SPAD-1 and SPAD-2 having a value of $r = 0.33$ ($P < 0.001$) and $r = 0.4$ ($P < 0.001$), respectively. Similar results were obtained by [69]. Following our results, their findings show that high-yielding lines can be selected by SPAD reading. Because grain quality parameters (protein content and grain color) were not negatively correlated with GY, it can be possible to select high-yielding genotypes without any decrease in quality parameters, based on SPAD reading. Suggestive correlations for quality parameters in this study can stem from the low genetic effects of

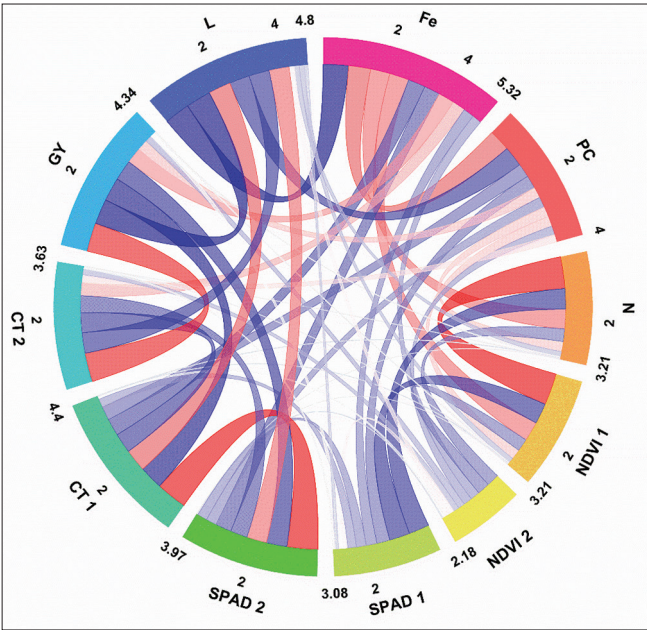


Figure 1: Chord diagram representing Co-relation among various quality and physiological parameters under study.

SPAD readings. Better accuracy is gained from the SPAD-1 reading scan when predicting GY [70,71].

3.3. Cluster Analysis of Genotypes

The cluster analysis software, SPAR1, successfully classified a total of 120 genotypes into 12 distinct clusters denoted as A, B, C, D, E, F, G, H, I, J, K, and L [Table 3]. Cluster “E” included the largest number of genotypes (23), followed by cluster “J” (with 18 genotypes), while Cluster “L” contained just one genotype, and Clusters “H” and “D” contained two and three genotypes, respectively. Cluster “A” comprehended 15 genotypes primarily landraces (11) and released varieties (4) and improved genotype (1). Cluster “B” contained six genotypes. Cluster “C” had 10 wheat genotypes mainly comprising landraces and two released varieties. Clusters “E,” “F,” “I,” and “J” contained mainly landraces and comprised 23, 16, 9, and 18 genotypes, respectively. Cluster “G” comprised 11 genotypes having equal

Table 3: List of genotypes differentiated into clusters on the basis of quality and physiological data.

Clusters	No. of genotypes	Genotypes
A	16	IC145955, DBW187, HUW206, IC321879, IC321886, IC321888, IC321892, IC321910, IC321931, IC322001, IC128300, IC128307, IC128308, IC128338, RAJ3765, IC546937
B	6	IC534350, IC321851, IC321984, KAPARGAON, IC128326, MANGURALLOCAL
C	10	EC556485, DBW88, IC212140, IC212153AMB, IC212161, IC321866, IC321946, IC145564, IC128298, UP115
D	3	IC296432, IC321898, IC128316
E	23	IC138384, IC296681, HTW67, IC212182, IC212190, IC321847, IC321855, IC321899, IC321905A, IC321927, IC321950, IC321958, IC322011A, IC322016, IC322023, IC47017A, IC296742, IC128304, IC128306, IC128310, IC128349, IC111994, IC111993
F	16	IC534337, IC128364, IC128359, IC212142, IC321849, IC321922, IC145559, IC128296, IC128311, IC128313, IC138461, LGM205, LGM225-1, IC145557, IC128285, IC111992
G	11	HI977, IC35117, IC212162, IC296440, IC296491, IC321856, IC321918, K307, IC128344, IC128290, PBW373
H	2	GW173, IC354308
I	9	IC252354, IC252355, IC212151, IC212184, IC321997A, IC47073B, IC128301, IC138456, IC128309
J	18	IC138332, IC138348, IC128367, IC128372, IC212145, IC212176B, IC321869, IC321877, IC321885, IC321937, IC322007A, IC59581, IC145565, IC128277, IC128333, IC128282, IC145605, NP125
K	5	HD2967, HS277, IC321979, PBW550, WH1105
L	1	IC427824

numbers of landraces and released varieties. Cluster “K” comprised 5 genotypes mainly released varieties, various workers [23,72] While researching European winter wheat landraces, researchers found that one cluster was typified by checks. The different clusters were not specified for the distribution of genotypes, as clusters contained a mixture of landraces, released varieties, and other lines [73].

The range of inter-cluster distances observed in the study varied from 2.04 to 11.84. We determined that the minimum spacing between clusters was 2.04. between Cluster “F” and “J” giving sight to closely related genotypes of these clusters. The most diverse groups were found to be “K” and “L” having 11.84 maximum distance followed by clusters “C” and “L” with 11.8, and clusters “E” and “L” with 11.44. Intra-cluster distance is highest (2.29) and lowest (1.509) represented by clusters “A” and “H,” respectively. Cluster “H” congregates only two genotypes [Table 4].

3.4. Cluster Mean Analysis

The cluster analysis demonstrated a substantial level of variability within the examined germplasm, therefore providing a valuable tool for the classification of genotypes. Cluster analysis is a method used to group genotypes based on their similarities. This process aims to create

clusters that have high levels of similarity inside the cluster (intra-cluster homogeneity) and low levels of similarity between different clusters (inter-cluster heterogeneity). Table 5 depicts the value for cluster mean and standard deviation for individual traits, landraces, and released varieties. Cluster “L” had a high value for traits Zn (57.23), Fe (49.85), N (2.62), and PC (16.37), and the lowest average value for NDVI 2 (0.52). Cluster “C” had a high average value for trait NDVI 1 (0.74 ± 0.01) whereas a low value for CT 1 (19.56 ± 0.33), and CT 2 (20.94 ± 0.25). Cluster “E” showed a high mean value for the trait NDVI 2 (0.59 ± 0.02), and the low mean value for PC (9.73 ± 0.58) and N (1.56 ± 0.09). Cluster “G” showed the highest mean value for trait SPAD 1 (42.78 ± 3.73). Cluster “H” had the highest mean value for trait SPAD 2 (37.97 ± 3.75). Cluster “K” with a high mean value for trait GY (378.04 ± 68.02) and had a minimum value for trait Fe (36.61 ± 1.07) similarly, Heidari *et al.*, 2016, also reported lower Fe in commercial wheat than in landraces [74]. Cluster “B” showed low mean values for the traits NDVI 1 (0.67 ± 0.02) and GY (207.16 ± 83.71). The lowest mean value for the trait SPAD 1 (32.7 ± 1.39) was observed in cluster “I”, while cluster “J” showed the lowest value for trait SPAD 2 (31.79 ± 1.34). Clusters “A,” “D,” and “F” all had modest average values for all of the traits. The search and identification of genotypes with enhanced Fe and Zn accumulation prompted a comprehensive assessment of landraces.

3.5. PCA

To better understand sources of variance among wheat genotypes, PCA was performed. The PCA method has been used earlier for yield and quality traits [75-77], while Cairns *et al.*, 2012, used for physiological traits [78]. Of the total principal components obtained, only the first four were considered important as their eigenvalues were above one and explained 71.31% of the variation, it elucidates the framework that underlies the analyzed variables. The findings of our study align with the results documented by Poudel *et al.*, 2019, and Gebremariam *et al.*, 2022, indicating that the four principal components accounted for around 72–76% of the overall variance and possessed Eigen values >1 across the various wheat genotypes [79,80].

Majorly first two axes PC1 and PC2 explained 48.74% of the variation, comparable results were depicted by Pandey *et al.*, 2016 [81]. The sorting of all the accessions into distinct groups was a result of the genetic diversity seen among different genotypes for various parameters.

PC 1 explained a 26.10% variation of the total and was positively influenced by Zn (0.470), Fe (0.316), PC (0.441), N (0.441), CT1 (0.309), and CT2 (0.334). PC 2 contributed 22.64% of the total variation and the highly contributing traits were SPAD1 (0.498), SPAD2 (0.522), and GY (0.379). Similarly to our findings, Ahmad *et al.* (2008) found that the first principal component had the most variability compared to the subsequent components [82].

Using multivariate statistical methods might help choose them centered on graphical plots, such as 2-D and 3-D diagrams, in accordance with their characteristics [24].

The genotype distribution was shown in a Scatter plot between PC1 and PC2. The association between all the features using a traits correlation plot is displayed in Figure 2. The traits were shown as vectors on a PCA [Figure 2; the length of the vector indicates the amount of variance in each characteristic] [48]. Different groups of traits were observed based on the trait biplot (B). The Zn, N, PC, and Fe showed a positive correlation with the first two PCs, while CT 1, CT 2, and NDVI 2 traits were negatively correlated with PC2, and the third group (SPAD 1, SPAD 2, and GY) was negatively correlated with PC1.

Table 4: Inter-and intra-cluster (diagonal) distance for 120 genotypes of Wheat.

Clusters	A	B	C	D	E	F	G	H	I	J	K	L
A	2.291											
B	3.635	2.243										
C	2.901	5.195	2.044									
D	4.419	3.362	4.739	1.831								
E	2.59	3.856	3.128	4.396	2.069							
F	3.361	2.9	4.436	3.005	2.694	1.959						
G	3.631	3.869	2.987	3.624	3.176	3.411	2.24					
H	6.16	4.795	5.979	4.503	6.56	5.885	4.958	1.509				
I	3.048	4.402	4.649	4.908	2.528	3.26	5.108	7.17	1.957			
J	3.527	2.855	4.712	4.112	2.358	2.04	4.042	5.825	2.584	1.863		
K	3.254	4.377	3.178	5.766	3.316	4.565	2.946	6.223	5.212	4.344	1.863	
L	11	8.669	11.8	8.214	11.44	9.951	10.206	6.75	11.092	10.061	11.848	0

Table 5: Mean values of 11 quantitative and physiological traits for 12 clusters revealed by cluster analysis among 120 Wheat genotypes.

Clusters	Zn (ppm)	Fe (ppm)	PC (%)	N (%)	NDVI 1	NDVI 2	SPAD 1	SPAD 2	CT 1	CT 2	GY (g)
A	33.82±3.26	41.76±2.7	10.21±0.57	1.63±0.09	0.68±0.02	0.53±0.03	37.62±2.25	32.85±1.82	19.78±0.62	21.34±0.47	297.09±57.05
B	40.55±3.05	43.2±3.19	11.35±0.61	1.82±0.1	0.67±0.02	0.55±0.03	40.37±2.1	34.75±1.67	20.63±0.42	22.44±0.43	207.16±83.71
C	33.05±3.59	38.83±1.72	10.06±0.53	1.61±0.09	0.74±0.01	0.55±0.03	40.42±2.22	36.91±1.53	19.56±0.33	20.94±0.25	328.59±72.69
D	42.25±1.91	47.69±2.12	11.39±0.68	1.82±0.11	0.74±0.02	0.53±0.02	40.34±2.06	36.4±0.39	20.12±0.59	22.14±0.72	223.92±31.1
E	34.83±2.95	40.86±2.42	9.73±0.58	1.56±0.09	0.72±0.02	0.59±0.02	38.09±2.12	32.97±1.8	20.19±0.41	22.02±0.5	258.65±55.58
F	37.66±2.1	44.55±2.08	10.3±0.54	1.65±0.09	0.72±0.02	0.54±0.03	38.25±1.64	32.94±2.2	20.93±0.42	22.56±0.33	248.2±41.08
G	34.95±3.34	42.66±2.32	10.91±0.67	1.74±0.11	0.73±0.02	0.57±0.02	42.78±3.73	37.76±2.36	20.47±0.28	21.83±0.4	361.77±56.55
H	41.71±0.32	38.45±1.84	13.75±0.57	2.2±0.08	0.73±0	0.52±0.02	41.22±3.23	37.97±3.75	20.39±0.16	21.65±0.27	267.13±61.09
I	37±1.42	42.03±2.68	9.9±0.56	1.58±0.09	0.71±0.02	0.57±0.03	32.7±1.39	28.32±2.21	20.12±0.52	21.77±0.31	209.53±30.17
J	39.07±3.23	40.01±2.28	10.46±0.5	1.67±0.08	0.71±0.02	0.57±0.02	36.16±1.41	31.79±1.34	20.84±0.28	22.62±0.39	213.82±37.07
K	33.07±2.22	36.61±1.07	10.15±0.59	1.62±0.1	0.68±0.03	0.58±0.02	41.81±2.55	37.55±1.67	20.35±0.1	21.68±0.13	378.04±68.02
L	57.23±0	49.85±0	16.37±0	2.62±0	0.71±0	0.52±0	38.6±0	32.89±0	21.07±0	22.11±0	234.66±0
LR	36.68±0.45	41.93±0.33	10.45±0.1	1.67±0.02	0.71±0.01	0.55±0.01	38.05±0.32	33.26±0.29	20.34±0.06	22.02±0.07	264.8±7.51
RV	35.52±0.74	40.26±0.56	10.14±0.19	1.62±0.03	0.70±0.01	0.56±0.01	39.45±0.72	34.99±0.75	20.29±0.11	21.77±0.11	282.85±14.73

LR: Landrace, RV: Released varieties.

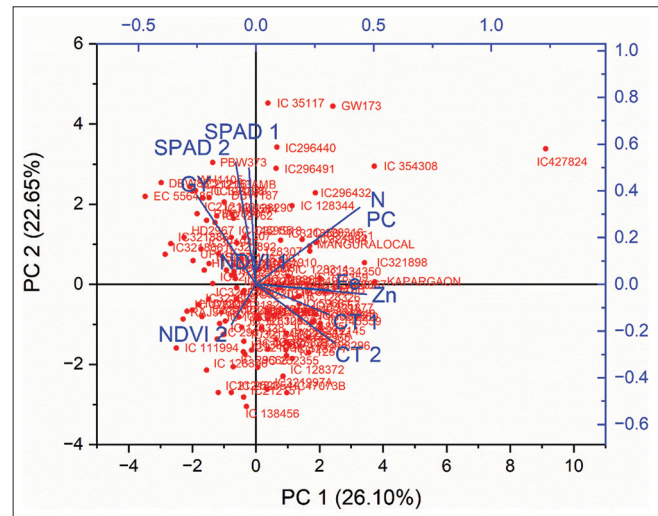


Figure 2: Trait biplot ordination depicting the association among quantitative traits in 120 wheat genotypes assessed across 2 years.

Furthermore, traits such as Zn, N, PC, and Fe had positive correlation with each other as vectors of these traits were in the same direction and acute angle was formed between each other. This was consistent with findings from correlation analyses, which revealed highly substantial positive relationships between many different traits. However, the traits formed obtuse angles between them were negatively correlated such as N with NDVI 2, and GY with CT 1 and CT 2. Similar results were obtained by [59,83].

3.6. Promising Wheat Genotypes

For different quality and physiological traits, a few promising genotypes were identified in the present study [Table 6]. Number of wheat landraces that showed high Zn, these landraces could be considered as adapted strategies for developing improved quality wheat varieties. In the majority of instances, the high-quality genotypes for quality traits (Zn, Fe, N, and PC) were landraces. In addition, Amiri *et al.* (2018) observed that landraces have greater levels of protein and micronutrients [57]. In comparison to released varieties, landraces had more grain micronutrients [84]. Due to increased productivity potential exhibited by released varieties, landraces have been displaced at a rapid pace from their

Table 6: Promising wheat genotypes identified for different quality and physiological traits.

Traits	Criteria	Superior Genotypes
Zn (ppm)	≥42	IC128367 (J), IC321869 (J), IC427824 (L), KAPARGAON (B), IC128316 (D), IC128277 (J), IC128326 (B)
Fe (ppm)	≥46	IC212142 (F), IC296440 (G), IC321898 (D), IC321931 (A), IC321997A (I), IC322001 (A), IC427824 (L), IC47017A (E), KAPARGAON (B), IC145559 (F), IC128296 (F), IC128313 (F), IC128316 (D), LGM205 (F), LGM225-1 (F)
PC (%)	≥13	GW173 (H), IC427824 (L), IC354308 (H)
N (%)	≥2	GW173 (H), IC427824 (L), IC354308 (H)
NDVI 1	≥0.75	DBW88 (C), IC212140 (C), IC212190 (E), IC321856 (G), IC321898 (D), IC321937 (J), IC145564 (C), IC128344 (G), IC128285 (F)
NDVI 2	≥0.61	HINDI62 (E), HS277 (K), IC212184 (I), IC321847 (E), IC321856 (G), IC128344 (G)
SPAD 1	≥44	HTW11 (G), IC296440 (G), IC128298 (C), PBW373 (G), WH1105 (K)
SPAD 2	≥40	GW173 (H), HTW11 (G), PBW373 (G)
CT1	≤19	IC321892 (A), IC145564 (C), IC128309 (I)
CT 2	≤20	EC556485 (C), DBW187 (A), DBW88 (C), IC212153AMB (C), IC212161 (C), IC321879 (A), IC321886 (A), IC321946 (C), IC322016 (E), IC128298 (C), IC128308 (A), IC128338 (A)
GY (g)	≥400	DBW187 (A), DBW88 (C), HD2967 (K), IC296440 (G), IC321856 (G), IC321979 (K), IC128344 (G), Raj3765 (A), WH1105 (K)

The value in parenthesis indicates the cluster number.

respective regions of agriculture. The released varieties demonstrated a higher level of grain output compared to the landraces [Table 5]. The PCA identified important traits that effectively captured the diversity across different wheat genotypes. Therefore, the research offers promising prospects for acquiring a wide range of superior quality (Zn, PC, N, and Fe) cum high-yielding segregants. The selected genotypes from clusters “C” with high yielding potential and “L” with high nutritional value were found to be most distant and may have the potential to be excellent parental lines to develop effective breeding methods.

4. CONCLUSION

The examination of diverse features shown by different individuals is crucial in understanding the manifestation of divergence. Moreover, the evaluation of the assortment of germplasm collections has the potential for targeted breeding objectives. The prioritization of selection in wheat breeding should be given to characters that exhibit a combination of high heritability and high genetic progress. Furthermore, it is proposed that the utilization of multivariate approaches might effectively discern distinct parental lines, hence enabling the production of novel progeny toward future breeding endeavors aimed at enhancing wheat productivity.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

12. PUBLISHER'S NOTE

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