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Selection of New Potential Genotype from Exotic Wheat Germplasm Based on Agronomic Traits and Biochemical Analysis

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ABSTRACT

This study aimed to evaluate the genetic diversity and identify high-yielding wheat *(Triticum aestivum*) genotypes through both morphological and biochemical analyses. A total of 36 wheat genotypes, including three local cultivars and thirty-three exotic genotypes, were analyzed for 16 morphological traits and protein profiles using SDS-PAGE. The genotypes exhibited considerable variability, with the cluster analysis of morphological traits revealing four major clusters, reflecting distinct genetic backgrounds. Significant correlations were observed between several morphological traits, such as stem length, spike length, and total plant height, all of which were closely associated with grain yield. Genotypes like Ma-6, Ma-16, Ma-22, and Ma-30 demonstrated superior performance, with increased spikelet numbers and 1000-seed weight, indicating their potential for high-yielding wheat breeding. The molecular analysis revealed 10 protein bands, 2 of which were monomorphic, and 8 were polymorphic, reflecting high genetic diversity. Four distinct clusters were identified based on protein profiles, confirming the genetic differentiation observed in the morphological data. Specific loci such as L-5 and L-6 exhibited significant variation, which may be linked to traits like disease resistance or drought tolerance. The results highlight the importance of using a multi-trait approach, combining both phenotypic and molecular data, for more accurate assessments of genetic diversity. This study identifies promising genotypes for future wheat breeding programs focused on improving yield, grain quality, and adaptability to diverse environmental conditions. The findings emphasize the value of integrating both morphological and molecular data in wheat breeding efforts to address challenges posed by climate change and other environmental stresses.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple crops globally, playing a critical role in ensuring food security for millions of people. Its high yield, versatility, and adaptability to various agro-ecological conditions make it a key component of the global agricultural landscape (1). However, to meet the demands of an ever-growing population and to overcome challenges posed by climate change, disease pressures, and soil degradation, there is an urgent need for the development of high-yielding wheat varieties with improved resistance to biotic and abiotic stresses (2). The introduction of exotic wheat germplasm offers a promising avenue for genetic improvement by incorporating novel traits into existing local wheat varieties (3).

Exotic wheat germplasm refers to wheat varieties that originate from regions outside the local breeding zones. These genotypes often possess unique genetic variations that contribute to the expression of beneficial traits such as higher yield potential, resistance to pests and diseases, and improved tolerance to adverse environmental conditions (4). Recent studies have indicated that exotic wheat germplasm is a valuable source of genetic diversity, offering a potential solution to the challenges faced by conventional breeding programs (5). This genetic diversity can be leveraged to enhance the adaptability and performance of wheat in diverse environments, particularly under climate stress conditions such as drought, heat, and salinity (6).

The successful incorporation of exotic germplasm into local breeding programs requires a comprehensive understanding of both agronomic and biochemical traits.

Agronomic traits, such as plant height, grain size, tiller number, and disease resistance, are key determinants of wheat productivity (7). Several studies have shown that exotic genotypes often exhibit improved agronomic traits compared to local varieties, including higher grain yield and better resistance to common wheat diseases such as rust, blight, and mildew (8). For example, research has demonstrated that wheat lines derived from exotic germplasm possess higher levels of resistance to wheat leaf rust (*Puccinia triticina*) and stem rust (*Puccinia graminis*), significantly reducing the yield losses caused by these diseases (9).

Biochemical traits, particularly those related to the nutritional quality of wheat, are equally important in determining its suitability for food production. Wheat's biochemical composition, which includes protein content, gluten strength, and starch properties, directly affects its baking and processing qualities (10). Studies have shown that exotic wheat lines can provide valuable insights into the enhancement of wheat's protein content and gluten quality, which are essential for producing high-quality bread and other wheat-based products (11). For instance, high-protein wheat genotypes from Central Asia have been identified as sources of superior gluten quality, contributing to better dough elasticity and bread-making performance (12). Additionally, the starch composition, including amylose and amylopectin ratios, influences the texture and quality of wheat products, and exotic wheat germplasm has been shown to exhibit variation in these traits (13).

With the increasing global demand for wheat, the development of high-quality, disease-resistant, and stress-tolerant wheat varieties is crucial. Exotic wheat germplasm has proven to be an essential tool in enhancing wheat's resilience to environmental stresses such as drought, heat, and salinity, which are becoming more prevalent due to climate change (14). For instance, wheat genotypes originating from regions with arid and semi-arid climates have been found to exhibit higher drought tolerance and water-use efficiency, making them ideal candidates for cultivation in water-scarce regions (15). Similarly, heat-tolerant exotic wheat varieties are being used to develop wheat strains capable of withstanding the increasing temperatures predicted under future climate scenarios (16).

Molecular tools, such as marker-assisted selection (MAS) and genomic selection, have been employed to accelerate the process of incorporating these beneficial traits from exotic germplasm into elite breeding lines (17). These tools enable the precise identification of genes responsible for desirable agronomic and biochemical traits, thus facilitating the efficient selection of high-performing genotypes (18). Recent advancements in next-generation sequencing (NGS) and transcriptomic have further improved the ability to map complex traits in wheat, allowing breeders to make informed decisions when selecting the best exotic genotypes for inclusion in breeding programs (19).

The utilization of exotic germplasm in wheat breeding not only improves productivity but also contributes to sustainable agricultural practices. By enhancing disease resistance, improving drought and heat tolerance, and increasing nutritional quality, exotic wheat genotypes can help mitigate the adverse effects of climate change and ensure a stable food supply for future generations (20). Moreover, the integration of exotic genotypes into wheat breeding programs can enhance genetic diversity, which is essential for the long-term sustainability of wheat production systems (21).

This study aims to select new potential wheat genotypes from exotic germplasm, focusing on the evaluation of both agronomic and biochemical traits. By conducting comprehensive field trials and biochemical analyses, we intend.

MATERIALS AND METHODS

The present study was conducted to assess the genetic diversity and identify high-yielding wheat (*Triticum aestivum*) genotypes, utilizing both morphological traits and biochemical analyses. Thirty-six genotypes were studied, comprising three local cultivars (CV34, CV35, and CV36) and thirty-three exotic genotypes (Ma1 to Ma33). The seeds of these genotypes were obtained from the Seed Bank of the Directorate General of Research and Innovation (DGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. The experiment was conducted at the Botanical Garden and Herbarium, University of Malakand, Khyber Pakhtunkhwa, Pakistan. The primary objectives were to evaluate genetic diversity based on both morphological and biochemical parameters (23).

Morphological Characterization

For morphological characterization, seeds from each genotype were sown in well-prepared seedbeds under controlled environmental conditions in the experimental field of the Botanical Garden and Herbarium, University of Malakand. All recommended crop management practices were followed, including irrigation, fertilization, and pest management, to ensure optimal plant growth. Data collection for morphological traits was initiated after seed germination and continued until harvest. The recorded traits included both qualitative and quantitative characters (24). Qualitative traits included spike color, plant orientation, and seed color, which are visually distinct characteristics that help in classifying and differentiating genotypes. On the other hand, quantitative traits involved measurable aspects such as days to germination (DG), flowering (DFl), fruiting (DFr), and maturity (DM), alongside physical attributes like stem length, leaf length, spike length, total plant height, number of nodes, and internodes. Additional traits like the number of seeds per spike, total plant biomass, and 1000-seed weight were also recorded, providing valuable information on growth and yield potential. These quantitative traits offer a more objective assessment and are essential for identifying performance-related characteristics in wheat breeding programs. Morphological data were recorded and subjected to cluster analysis using the software PC-ORD (25), which was used to to identify genotypes with superior yield potential, disease resistance, and nutritional quality. These genotypes can then be integrated into local breeding programs to develop wheat varieties that are better

adapted to changing environmental conditions and meet the demands of global food security (22).

classify genotypes based on their phenotypic characteristics.

Biochemical Characterization

Biochemical diversity was assessed by analyzing the protein profile of the genotypes using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), a widely used technique for evaluating genetic variation at the molecular level (26).

Seed Preparation and Protein Extraction

Seeds from each genotype were ground into a fine powder using a mortar and pestle. For protein extraction, 0.01 g of the seed powder was transferred into 1.5 mL Eppendorf tubes, and 400 µL of protein extraction buffer (containing 1X Laemmli buffer) was added. Bromophenol blue (BPB) was included in the buffer as a tracking dye. The mixture was vortexes thoroughly to ensure even mixing of the ground seed with the buffer. The homogenized samples were centrifuged at 13,000 rpm for 10 minutes at 4°C. The supernatants were stored at −20°C until further analysis (27).

Electrophoresis

Electrophoresis was performed following the method of Laemmli (1970), with minor modifications. Polyacrylamide gel (15%) was prepared for protein separation. A 20 µL aliquot of the protein extract was loaded into each well of the gel. Electrophoresis was conducted at a constant voltage (100V) until the BPB dye reached the bottom of the gel. After electrophoresis, the gel was stained with Coomassie Brilliant Blue (CBB) for protein visualization (28).

Staining and Destaining

The gel was immersed in a staining solution for 10 minutes and then destained in a solution containing methanol and acetic acid until the background color was removed. The gel was fixed in a solution containing a small amount of ethanol to preserve the protein bands. The gel was stored in distilled water for permanent storage (26).

Data Analysis

The morphological data, including quantitative and qualitative traits, were subjected to statistical analysis using software tools. Days to germination (DG), flowering (DFl), fruiting (DFr), and maturity (DM) were calculated by subtracting respective dates (e.g., sowing date from germination date). Quantitative trait data were analyzed for mean comparisons and standard deviations. The qualitative traits were scored on a binary scale (1 for presence and 0 for absence of a characteristic). The molecular data generated by SDS-PAGE were also recorded using a binary scale, where "1" indicated the presence of a protein band and "0" indicated its absence. The intensity of the protein bands was categorized as major or minor, based on the band's brightness. The data were analyzed using cluster analysis (PC-ORD) to classify genotypes based on both their morphological and biochemical characteristics (25).

RESULTS

Morphological Traits Analysis

Phenotypic Variation among Genotypes

The morphological analysis of 36 wheat genotypes revealed significant intra- and inter-specific variation across 16 traits. Of these, 13 traits were quantitative, while 3 were qualitative. A cluster analysis of these morphological traits was performed using PC-ORD software, resulting in a phylogenetic tree (dendrogram) presented in Figure1. The analysis divided the genotypes into two main lineages at a linkage distance of 12.5%, based primarily on stem length and total plant height. The first lineage (L1) was further divided into three sub-clusters at a genetic distance of 68.5%.

- **Cluster 1** comprised nine genotypes: Ma-1, Ma-6, Ma-7, Ma-17, Ma-18, Ma-23, Ma-26, Ma-28, and CV-35.
- **Cluster 2** included sixteen genotypes: Ma-2, Ma-3, Ma-4, Ma-5, Ma-12, Ma-13, Ma-15, Ma-19, Ma-29, Ma-30, Ma-31, Ma-32, Ma-33, CV-34, CV-36.
- **Cluster 3** contained six genotypes: Ma-16, Ma-20, Ma-21, Ma-22, Ma-25, and Ma-27.

The second linkage (L2) contained only one sub-cluster, **Cluster 4**, which included five genotypes: Ma-8, Ma-9, Ma-10, Ma-11, and Ma-14. The scatter plot in Figure 2, drawn for further confirmation of phylogenetic relationships, also classified the genotypes into four distinct groups based on varietal differentiation. The four clusters were observed to be consistent with those seen in the dendrogram, reaffirming the robustness of the clustering based on morphological traits.

Table 1: Quantitive treats of 36 genotypes of Wheat (Triticum aestivum) revealed significance inter-and intraspecific variations.

V/No	Quantitative traits (cm, mg)												
	DG	DF1	DFr	DM	SpL	LL	SL	TPH	PB	NN	NI	GY	1000SW
Ma-1	29	107	15	173	35.8	71.96	85.8	16.6	3.4	4.4	42.2	44.4	63.5
Ma-2	29	110	10	173	30	70.4	91.6	17.2	3.4	4.4	48.2	21.4	56.1
Ma-3	29	110	10	173	26.6	69.72	88.4	15.2	4	5	37.4	32.2	41.2
Ma-4	34	110	7	182	30.4	72.68	74.8	17.4	3.4	4.4	36	28.4	52.1
Ma-5	34	96	21	173	21.8	69.16	83	12.4	3	4	27.6	7	49.8
Ma-6	34	102	15	173	29.2	70.64	83	10	3	4	40.2	35.2	60
Ma-7	19	109	13	173	27.2	68.24	83.2	8	3.6	4.6	32	32.8	55
Ma-8	19	109	13	173	13.2	65.44	38.4	4.2	4	5	38.8	15.6	36.2
Ma-9	34	110	9	173	9.6	67.12	31.6	4	3.4	4.4	33.2	9.4	55.2
Ma-10	34	110	9	173	10	67.2	28.6	7.2	4	5	37.8	12	39.6
Ma-11	34	102	12	173	17.6	67.72	52.8	5.8	3.4	4.4	32.2	25	50.6
Ma-12	32	104	10	173	28.8	69.56	87.4	15.4	3.2	4.2	32.8	19.8	57.2
Ma-13	32	96	12	173	26.2	67.84	90	9.2	3.4	4.4	37.2	26.2	42.9
Ma-14	22	106	15	173	8.6	64.92	15.2	1.6	3	4	22.2	4.2	23
Ma-15	22	108	11	182	32.8	71.16	92.4	16	3.8	4.8	19.6	22.8	45
Ma-16	22	108	11	173	36.2	70.04	109.2	16.4	3.4	4.4	43.4	24.2	68.2
Ma-17	22	106	18	173	29.4	69.68	99.4	17.2	4	5	43	44.8	42.6
Ma-18	22	108	16	182	28.6	71.32	95.6	17.4	3.4	4.4	39.8	43.2	45
Ma-19	19	109	14	173	29.6	68.92	82.8	20.8	3.6	4.6	33.2	23.8	40.3
Ma-20	17	119	16	173	39.8	72.96	121.2	18.6	3.4	4.4	36	62.4	45.9
Ma-21	17	125	4	173	41.4	72.08	118	15.8	3.2	4	32	29.4	42.1
Ma-22	17	125	10	173	44.2	73.84	119.4	14.6	3.2	4.2	41.6	31.6	69.4
Ma-23	17	119	10	173	33	70.4	89.2	15.2	3.6	4.6	34.8	29.8	56.4
Ma-24	22	108	11	173	34.4	69.68	87	16.4	3.6	4.6	39.4	23.8	61.6
Ma-25	20	122	21	173	41.6	75.52	116.4	15	3.6	4.6	45.6	45	50.5
Ma-26	22	101	14	173	35.4	69.08	90.2	16.8	3.4	4.4	42	47	44.1
Ma-27	22	104	15	173	35.6	69.92	112.6	16.6	3	4	34.2	30.2	65.8
Ma-28	22	118	16	182	28.2	73.24	83.4	18.4	3.6	4.6	38.2	46.8	54.1
Ma-29	22	118	15	182	28	73	72.6	19	3.4	4.4	37.6	17	64
Ma-30	22	122	16	173	27.2	72.04	79.6	14.2	3	4	41.2	16.2	66.2
Ma-31	22	104	16	173	22.6	67.52	100	12.4	4.4	5.4	39.2	14.4	61.1
Ma-32	22	114	16	182	26	72	79.8	14.2	3.2	4.2	35	14.4	55
Ma-33	22	122	16	173	30.4	72.68	68.8	16.4	3.6	4.6	45.6	20	65.4
CV-34	20	94	15	173	32	66.8	84.4	13	3	4	42.6	24.4	45
CV-35	19	107	23	173	40.2	72.44	85.6	18.4	3	4	41.6	29.8	54.5
CV-36	32	96	21	173	37.8	71.96	74.8	15.4	3.6	4.6	32.2	16.8	45.6

Notes: **DG**- days to germination; **DFl**- days to flowering; **DFr**- days to fruiting; **SpL**-spike length; **LL**- leaf length; **SL**- stem length; **TPH**- total plant height; **PB**- plant Biomass; **NN**- number of nodes; **NI**-number of internodes; **GY**-Grain yield; **1000SW**-1000 seed weight

Table 2: Qualitative characters of 36 genotypes of Wheat (*Triticum aestivum*)

V/No	Spike Colour	Plant orientation	Seed Colour
Ma-1	B.Red	Erect	B.Red
Ma-2	Red	Erect	Red
Ma-3	A.White	Erect	A.White
Ma-4	A.White	Semi Erect	A.White
Ma-5	Red	Erect	Red
Ma-6	A.White	Erect	A.White
Ma-7	White	Erect	White
Ma-8	Red	Erect	Red
Ma-9	White	Erect	White
Ma-10	White	Erect	White
Ma-11	Red	Erect	Red
Ma-12	Red	Erect	Red
Ma-13	White	Erect	White
Ma-14	Red	Prostate	Red

Ma-15	White	Erect	White
Ma-16	White	Erect	White
Ma-17	White	Erect	White
Ma-18	A.White	Erect	A.White
Ma-19	B.Red	Erect	B.Red
Ma-20	Red	Semi Erect	Red
Ma-21	White	Erect	White
Ma-22	White	Erect	White
Ma-23	Red	Erect	Red
Ma-24	B.Red	Erect	B.Red
Ma-25	B.Red	Erect	B.Red
Ma-26	A.White	Erect	A.White
Ma-27	White	Erect	White
Ma-28	White	Semi erect	White
Ma-29	White	Erect	White
Ma-30	White	Erect	White
Ma-31	White	Erect	White
Ma-32	White	Erect	White
Ma-33	White	Erect	White
CV-34	White	Erect	White
CV-35	White	Erect	White
CV-36	White	Erect	White

Table 3: Prepared morphological data for cluster analysis

V/No	DG	DF1	DFr	DM	SpL	LL	SL	TPH	PB	NN	NI	GY	1000 SW	Sp C	P O	S C
Ma-1	29	107	15	173	35.8	71.96	85.8	16.6	3.4	4.4	42.2	44.4	63.5	4	1	4
Ma-2	29	110	10	173	30	70.4	91.6	17.2	3.4	4.4	48.2	21.4	56.1	3	1	3
Ma-3	29	110	10	173	26.6	69.72	88.4	15.2	4	5	37.4	32.2	41.2	2	1	2
Ma-4	34	110	7	182	30.4	72.68	74.8	17.4	3.4	4.4	36	28.4	52.1	2	2	2
Ma-5	34	96	21	173	21.8	69.16	83	12.4	3	4	27.6	7	49.8	2	1	2
Ma-6	34	102	15	173	29.2	70.64	83	10	3	4	40.2	35.2	60	3	1	3
Ma-7	19	109	13	173	27.2	68.24	83.2	8	3.6	4.6	32	32.8	55	2	1	2
Ma-8	19	109	13	173	13.2	65.44	38.4	4.2	4	5	38.8	15.6	36.2	1	1	1
Ma-9	34	110	9	173	9.6	67.12	31.6	4	3.4	4.4	33.2	9.4	55.2	3	1	3
Ma-10	34	110	9	173	10	67.2	28.6	7.2	4	5	37.8	12	39.6	1	1	1
Ma-11	34	102	12	173	17.6	67.72	52.8	5.8	3.4	4.4	32.2	25	50.6	1	1	1
Ma-12	32	104	10	173	28.8	69.56	87.4	15.4	3.2	4.2	32.8	19.8	57.2	3	1	3
Ma-13	32	96	12	173	26.2	67.84	90	9.2	3.4	4.4	37.2	26.2	42.9	3	1	3
Ma-14	22	106	15	173	8.6	64.92	15.2	1.6	3	4	22.2	4.2	23	1	1	1
Ma-15	22	108	11	182	32.8	71.16	92.4	16	3.8	4.8	19.6	22.8	45	3	1	3
Ma-16	22	108	11	173	36.2	70.04	109.2	16.4	3.4	4.4	43.4	24.2	68.2	1	1	1
Ma-17	22	106	18	173	29.4	69.68	99.4	17.2	4	5	43	44.8	42.6	1	1	1
Ma-18	22	108	16	182	28.6	71.32	95.6	17.4	3.4	4.4	39.8	43.2	45	1	1	1
Ma-19	19	109	14	173	29.6	68.92	82.8	20.8	3.6	4.6	33.2	23.8	40.3	2	1	2
Ma-20	17	119	16	173	39.8	72.96	121.2	18.6	3.4	4.4	36	62.4	45.9	4	2	4
Ma-21	17	125	4	173	41.4	72.08	118	15.8	3.2	4	32	29.4	42.1	3	1	3
Ma-22	17	125	10	173	44.2	73.84	119.4	14.6	3.2	4.2	41.6	31.6	69.4	1	1	1
Ma-23	17	119	10	173	33	70.4	89.2	15.2	3.6	4.6	34.8	29.8	56.4	1	1	1
Ma-24	22	108	11	173	34.4	69.68	87	16.4	3.6	4.6	39.4	23.8	61.6	3	1	3
Ma-25	20	122	21	173	41.6	75.52	116.4	15	3.6	4.6	45.6	45	50.5	4	1	4
Ma-26	22	101	14	173	35.4	69.08	90.2	16.8	3.4	4.4	42	47	44.1	4	1	4
Ma-27	22	104	15	173	35.6	69.92	112.6	16.6	3	4	34.2	30.2	65.8	2	1	2
Ma-28	22	118	16	182	28.2	73.24	83.4	18.4	3.6	4.6	38.2	46.8	54.1	1	2	1
Ma-29	22	118	15	182	28	73	72.6	19	3.4	4.4	37.6	17	64	1	1	1
Ma-30	22	122	16	173	27.2	72.04	79.6	14.2	3	4	41.2	16.2	66.2	1	1	1
Ma-31	22	104	16	173	22.6	67.52	100	12.4	4.4	5.4	39.2	14.4	61.1	1	1	1
Ma-32	22	114	16	182	26	72	79.8	14.2	3.2	4.2	35	14.4	55	1	1	1
Ma-33	22	122	16	173	30.4	72.68	68.8	16.4	3.6	4.6	45.6	20	65.4	1	1	1
Ma-34	20	94	15	173	32	66.8	84.4	13	3	4	42.6	24.4	45	1	1	1
Ma-35	19	107	23	173	40.2	72.44	85.6	18.4	3	4	41.6	29.8	54.5	1	1	1
Ma-36	32	96	21	173	37.8	71.96	74.8	15.4	3.6	4.6	32.2	16.8	45.6	1	1	1

Notes DG- days to germination; DFl- days to flowering; DFr- days to fruiting; SpL-spike length; LL- leaf length; SL-stem length; TPH- total plant height; PB- plant Biomass; NN- number of nodes; NI-number of internodes; GY Grain yield; 1000SW-1000 seed weight; Sp C- spike color; PO-plant orientation; SC- seed color; HY- High yielding

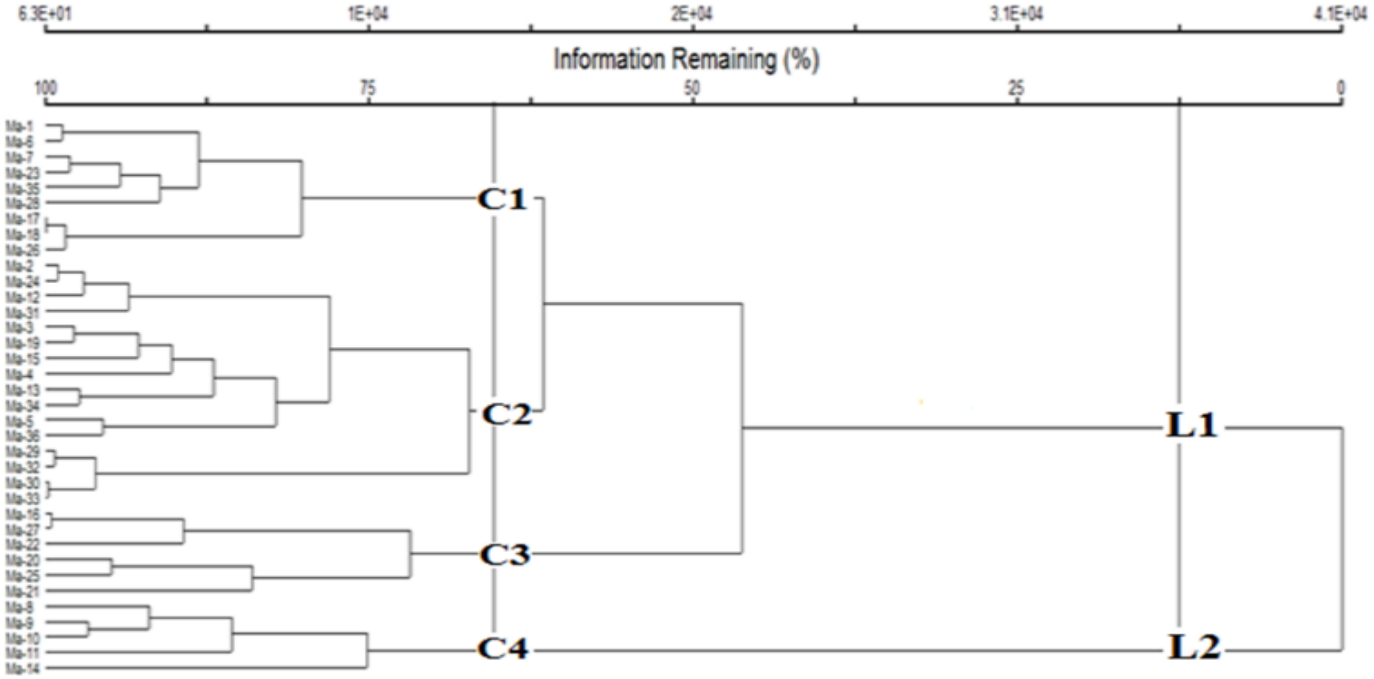

Figure 1: Inter and intra-specific Phylogenetic relationship detected through Morphological traits analysis in 36 different genotypes of Wheat varieties.

Note: CV-34, CV-35, CV-36 indicate genotypes of PR-2004, PR-2005 and PR-2013, Ma-1, Ma-2, Ma-3, Ma-4, Ma-5, Ma-6, Ma-7, Ma-8, Ma-9, Ma-10, Ma-11, Ma-12, Ma-13, Ma-14, Ma-15, Ma-16, Ma-17, Ma-18, Ma-19, Ma-20, Ma-21, Ma-22, Ma-23, Ma-24, Ma-25, Ma-26, Ma-27, Ma-28, Ma-29, Ma-30, Ma-31, Ma-32, Ma-33 indicates Genotypes of 11415, 11413, 11412, 11411,11409, 11408, 11407, 11405, 11402, 11399, 11398, 11397, 11396, 11395, 11394, 11392, 11403, 11391, 11390, 11389, 11388, 11385, 11384, 11381, 11380, 11379, 11378, 11377, 11375, 11374, 11824, 11823 and 113822.

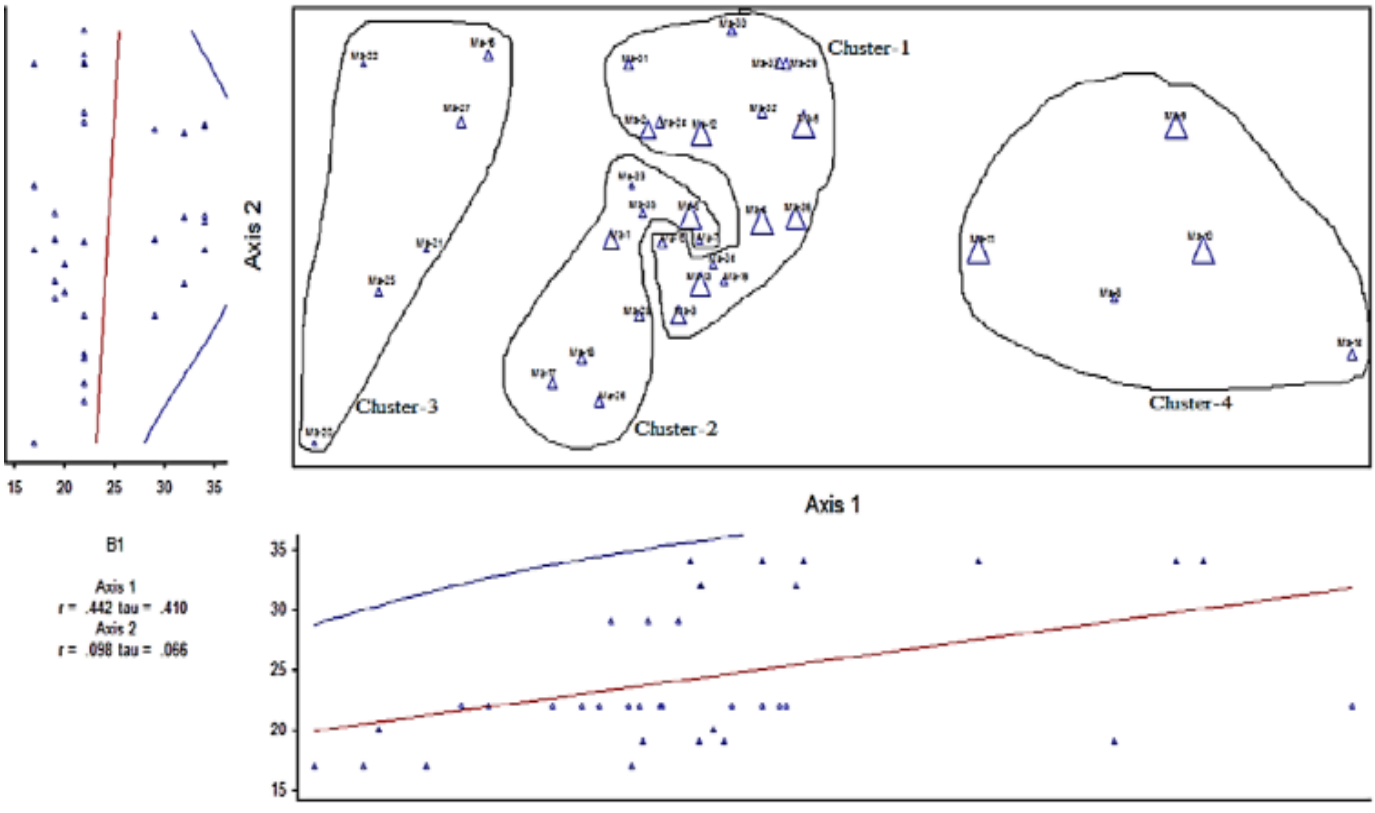

Figure 2: Confirmation of Phylogenetic relationship by scattered plot detected through cluster analysis in 36 different genotypes of Wheat varieties

Correlations among Morphological Traits

The correlation analysis among the 16 morphological traits indicated several significant relationships. The following key correlations were observed:

- **Stem Length** exhibited a strong positive correlation with **Spike Length** (0.862), **Leaf Length** (0.599), and **Total Plant Height** (0.764).
- **Leaf Length** was significantly correlated with **Days to Flowering** (0.738) and **Spike Length** (0.592), suggesting a close association between vegetative growth and reproductive development.
- **Number of Nodes** showed the highest positive correlation with **Plant Biomass** (0.995), indicating that the number of nodes could be a potential indicator of overall plant productivity.
- **Grain Yield** showed a strong positive correlation with **Stem Length** (0.597), **Spike Length** (0.609), and **Leaf Length** (0.481), highlighting the importance of these traits in determining yield (Table 4).A significant negative correlation was found between **Days to Germination** and **Days to Maturity**, indicating that earlier germinating genotypes tend to reach maturity more rapidly (Table 4).

Table 4: Show correlation values among 13 different morphology traits of Wheat

	DG	DF1	DFr	DM	SpL(cm)	LL(cm)	SL(cm)	TPH(cm)	PB(mg)	NN	NI	GY	1000 SW (gm)
DG	1												
DF1	-0.510	1.000											
DFr	-0.127	-0.229	1.000										
DM	-0.032	0.179	-0.031	1.000									
SpL(cm)	-0.435	0.264	0.142	-0.008	1.000								
LL(cm)	-0.225	0.592***	0.214	0.367**	0.738***	1.000							
SL(cm)	-0.412	0.219	0.079	-0.002	0.862***	0.599***	1.000						
TPH(cm)	-0.327	0.260	0.164	0.300**	0.764***	0.709***	0.704***	1.000					
PB(mg)	-0.041	0.059	-0.122	0.022	-0.213	-0.169	-0.071	-0.025	1.000				
NN	-0.020	0.027	-0.081	0.029	-0.232	-0.178	-0.093	-0.031	0.995***	1.000			
NI	-0.128	0.203	0.145	-0.203	0.332**	0.308**	0.306**	0.333**	0.089	0.102	1.000		
GY	-0.312	0.208	0.125	0.063	0.597***	0.481**	0.609***	0.491**	0.049	0.045	0.365**	1.000	
1000 SW (gm)	-0.031	0.283	0.014	0.025	0.395**	0.474**	0.394**	0.354**	-0.186	-0.168	0.425**	0.048	1.000

Notes DG- days to germination; DFl- days to flowering; DFr- days to fruiting; SpL-spike length; LL- leaf length; SL-stem length; TPH- total plant height; PB- plant Biomass; NN- number of nodes; NI-number of internodes; GY-grain yield; 1000SW-1000 seed weight

High-Yielding Genotype Selection

Based on grain yield and 1000-seed weight, two categories of genotypes were identified: high-yielding and low-yielding. High-yielding genotypes were defined as those with a 1000-seed weight greater than 60 grams and grain yield greater than 60 grams per plant. These genotypes included Ma-6, Ma-16, Ma-22, Ma-27, Ma-30, Ma-31, and Ma-32. These genotypes exhibited superior performance primarily due to larger seed size, higher spikelet count per plant, and longer days to maturity. In contrast, genotypes like Ma-2, Ma-4, Ma-7, Ma-8, CV-34, CV-35, Ma-9, Ma-12, Ma-23, and Ma-28 also exhibited good yield potential but due to specific combinations of grain yield components (e.g., number of grains per spike, 1000-seed weight, or early flowering time). where seed size, spike length, and maturity duration were crucial in determining overall yield.

SDS-PAGE Analysis

Genetic Diversity Based on Protein Profiles

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was utilized to evaluate the genetic diversity of the wheat genotypes based on their seed storage proteins. The analysis identified a total of 10 protein bands, of which 2 were monomorphic (B8, B9) and 8 were polymorphic, contributing to 80% polymorphism (Figure 3). The cluster analysis based on SDS-PAGE (Figure 4) data also revealed a clear division of the genotypes into two main linkages at a linkage distance of 16.5%. Linkage 1 contained 25 genotypes, including Ma-1, Ma-3, Ma-4, Ma-5, Ma-6, Ma-7, Ma-12, Ma-13, Ma-15, and CV-36. While Linkage 2, on the other hand, contained three clusters, cluster 2, 3 and 4. **Cluster 2** including Ma-9 and Ma-10

(Figure 4) and **Cluster 3** consisting of Ma-14, Ma-33, Ma-31, and CV-35, respectively. **Cluster 4** containing Ma-28 alone (Figure 4). The scattered plot in Figure 5 confirmed this clustering, showing that the protein profile could clearly differentiate the genotypes that offer valuable insights into genetic relationships within wheat populations

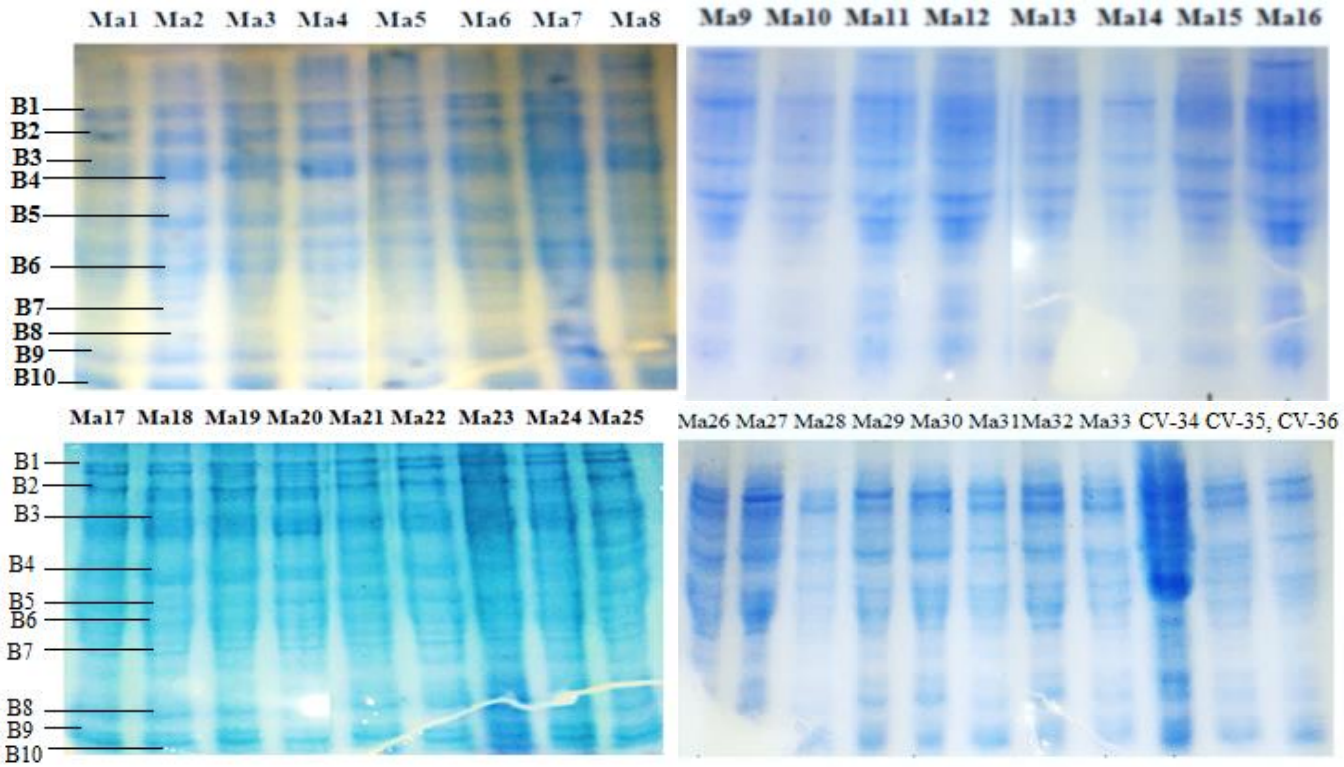

Figure 3: Electrophorogram showing inter and intra-specific locus variation in 36 different genotypes of Wheat varieties

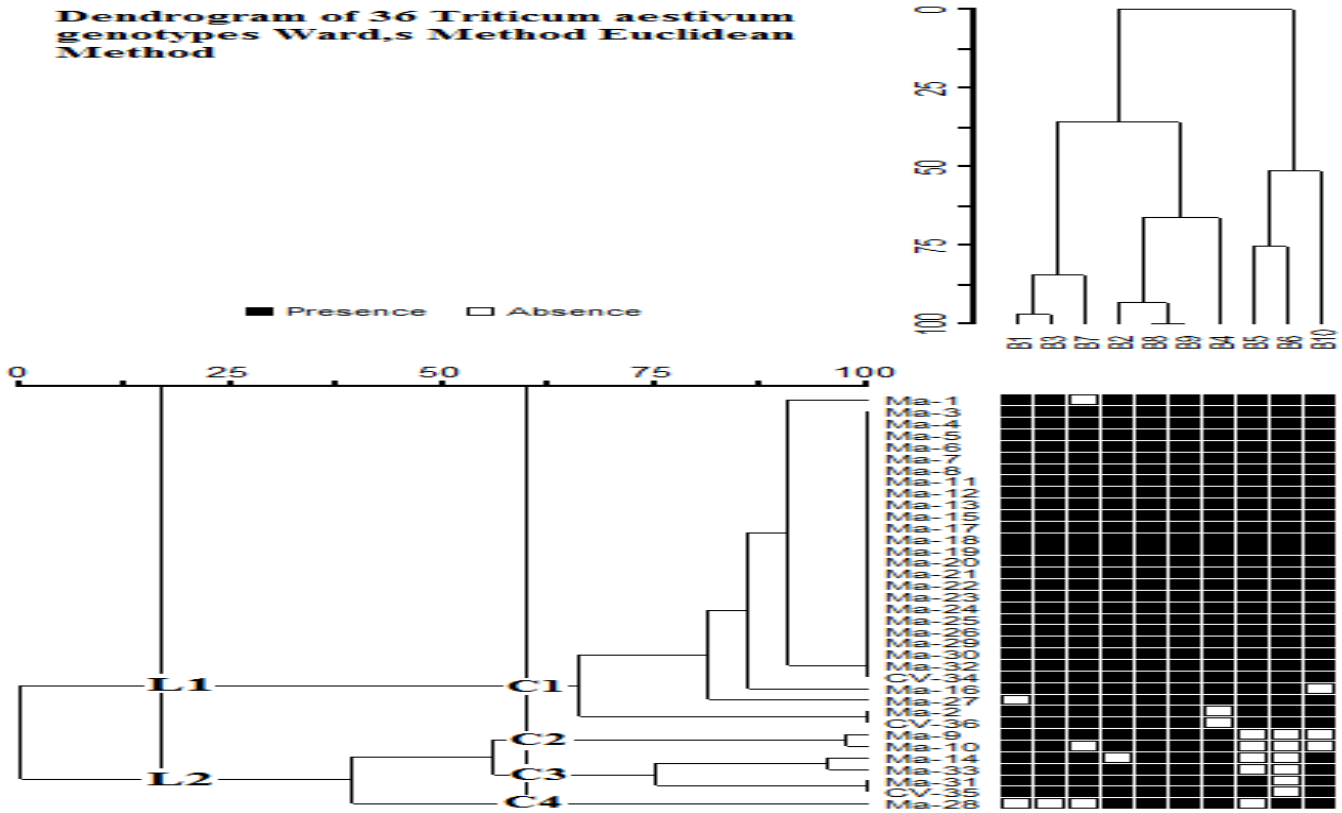

Figure 4: Inter and intra-specific Phylogenetic relationship detected through SDS-PAGE in 36 different genotypes of Wheat varieties.
Note: CV-34, CV-35, CV-36 indicate genotypes of PR-2004, PR-2005 and PR-2013, Ma-1, Ma-2, Ma-3, Ma-4, Ma-5, Ma-6, Ma-7, Ma-8, Ma-9, Ma-10, Ma-11, Ma-12, Ma-13, Ma-14, Ma-15, Ma-16, Ma-17, Ma-18, Ma-19, Ma-20, Ma-21, Ma-22, Ma-23, Ma-24, Ma-25, Ma-26, Ma-27, Ma-28, Ma-29, Ma-30, Ma-31, Ma-32, Ma-33 indicates Genotypes of 11415, 11413, 11412, 11411,11409, 11408, 11407, 11405, 11402, 11399, 11398, 11397, 11396, 11395, 11394, 11392, 11403, 11391, 11390, 11389, 11388, 11385, 11384, 11381, 11380, 11379, 11378, 11377, 11375, 11374, 11824, 11823 and 113822.

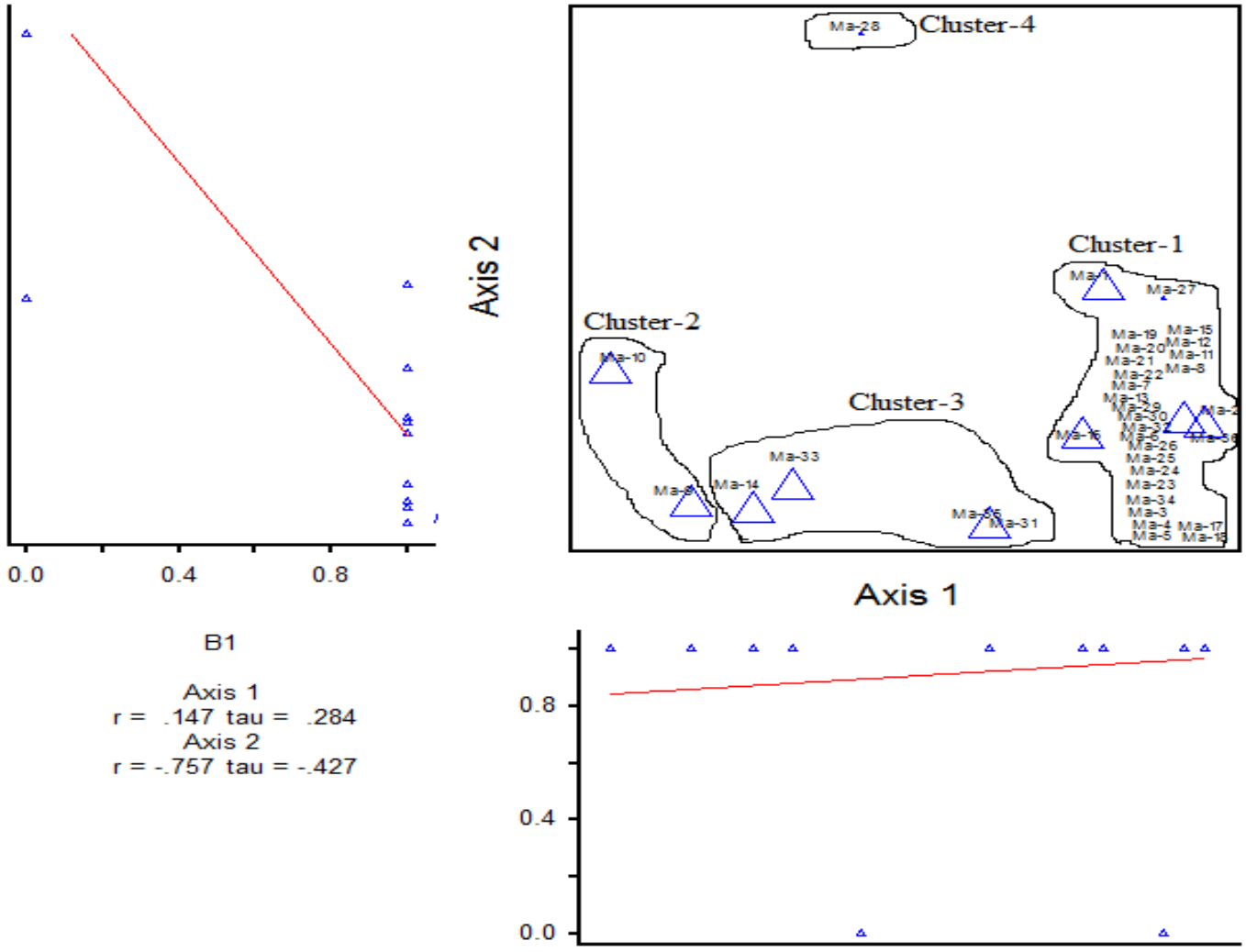

Figure 5: Confirmations of phylogenetic relationship by scattered plot detected through SDS PAGE in 36 different genotypes of Wheat varieties

Locus Variation and Genetic Divergence

Locus-specific variation was also observed across the genotypes. The study detected 10 loci, with loci L-8 and L-9 being monomorphic, showing 100% band presence across all genotypes. The other loci displayed varying levels of polymorphism:

- Loci L-5 and L-6 exhibited the highest variation (16.66%), while Loci L-1, L-3, L-4, and L-7 showed lower variation (5.55% to 8.33%).
- The genetic disagreement at loci L-5 and L-6 was 80%, indicating these loci contribute significantly to genetic divergence.

Table 5: Inter specific locus variation among Wheat varieties

S.NO	Present (%)	Absent %	Variation%	Status	Genetic Disagreat (bands present)
Locus-1 (Band-1)	34 (94.4)	2 (5.55)	(5.55)	Poly	0.944
Locus-2(Band-2)	35 (97.2)	1 (2.77)	(2.77)	Poly	0.972
Locus-3(Band-3)	35 (97.2)	1 (2.77)	(2.77)	Poly	0.972
Locus-4(Band-4)	34 (94.4)	2 (5.55)	(5.55)	Poly	0.944
Locus-5(Band-5)	30 (83.33)	6 (16.66)	(16.66)	Poly	0.833
Locus-6(Band-6)	30 (83.33)	6 (16.66)	(16.66)	Poly	0.833
Locus-7(Band-7)	33 (91.66)	3 (8.33)	(8.33)	Poly	0.916
Locus-8(Band-8)	36 (100)	0 (0)	(0)	Mono	1
Locus-9(Band-9)	36 (100)	0 (0)	(0)	Mono	1
Locus-10	33 (91.66)	3 (8.33)	(8.33)	Poly	0.916
Locus contribution toward genetic disagreement=(poly loci/total loci)100	80.00				

DISCUSSION

The current study aimed to assess both the morphological and molecular diversity of 36 wheat genotypes, providing valuable insights into their genetic variability. A detailed analysis of 16 morphological traits was performed, and the results showed considerable variation, which is consistent with earlier research on wheat diversity. The cluster analysis of these traits revealed a clear division of the genotypes into four major clusters, reflecting distinct genetic backgrounds. This result aligns with the findings of Mahpara (1), who also reported significant morphological diversity among wheat accessions, highlighting the potential of using these traits for breeding purposes. The presence of distinct groupings based on key traits like plant height and stem length is in agreement with similar studies on wheat, where plant height and related traits have been used to classify genotypes based on environmental adaptation (2, 29).

The correlation among different morphological traits was significant, confirming the close interrelationship between stem length, spike length, and plant height. Specifically, the study found a high positive correlation between stem length and spike length (0.862), suggesting that these traits are likely controlled by similar genetic factors. This relationship is supported by the work of Paux (3), who demonstrated the importance of these traits in wheat breeding, particularly when selecting for increased biomass or grain yield. Furthermore, the correlation between total plant height and the number of nodes (0.995) suggests that node number could serve as a useful indicator in selecting for high-yielding genotypes, as nodes are related to the overall productivity of the plant (30, 4).

The correlation of leaf length with days to flowering and spike length is consistent with findings from Johansson (2021), who showed that early flowering and long spikes are often associated with high yield potential (5, 31, 32). These results are also in line with earlier studies where leaf length was linked to photosynthetic capacity and ultimately to the plant's overall growth performance (6). Furthermore, the study revealed that the number of internodes was significantly correlated with spike length, suggesting that these traits may be inherited together and could be considered when selecting for improved wheat performance (33).

In terms of high-yielding genotypes, the analysis revealed that genotypes such as Ma-6, Ma-16, Ma-22, and Ma-30 exhibited higher grain yields due to increased spikelet numbers and 1000-seed weight. These findings correspond with earlier studies, such as those conducted by Bapela (8), who identified similar relationships between yield and seed weight in wheat. Notably, the genotypes that demonstrated superior performance in terms of yield were those with longer spikes and larger seed weights. This is consistent with earlier reports, which indicated that seed weight and spike size are crucial determinants of wheat yield under various environmental conditions (34, 35). The varieties identified in this study, including Ma-6 and Ma-30, showed strong performance not only in seed weight but also in terms of other yield components, confirming the value of selecting for multiple traits when breeding for high-yielding wheat varieties.

The SDS-PAGE analysis of the seed storage proteins provided additional insights into the genetic diversity of the wheat genotypes. A total of 10 bands were detected, of which 2 were monomorphic, indicating a conserved genetic background for these loci, while the remaining 8 bands were polymorphic. This high level of polymorphism is in line with the findings of Swarup (10), who noted that polymorphic protein bands are valuable for distinguishing between different wheat genotypes and assessing their genetic diversity. The molecular analysis revealed that the genotypes grouped into four distinct clusters based on their protein profiles, similar to the clustering observed in the morphological data. This suggests that the phenotypic traits and molecular markers provide complementary information, which can be leveraged for effective selection in breeding programs (36).

The variation observed at specific loci, such as L-5 and L-6, showed considerable genetic disagreement, highlighting their potential for further investigation. These loci exhibited the highest degree of variation, which may be associated with important agronomic traits like disease resistance, drought tolerance, or grain quality. This finding is consistent with the work of Bacala (11), who emphasized the role of protein profiles in assessing the genetic diversity of wheat germplasm. Loci such as L-5 and

L-6, which showed significant variation, could be potential markers for these traits, aiding in the development of wheat varieties that are better suited to challenging environmental conditions.

Moreover, the protein profile analysis demonstrated that some genotypes, such as Ma-1, Ma-3, and Ma-5, clustered together due to similar protein banding patterns, suggesting a close genetic relationship. This observation confirms the utility of SDS-PAGE in evaluating genetic diversity, as noted by Hasan (12), who demonstrated the usefulness of protein profiling in identifying genetic variation in wheat. The results from this study indicate that SDS-PAGE can effectively complement traditional morphological analyses, providing an additional layer of genetic information that can assist in wheat breeding.

The protein profiling results also confirmed the presence of genetic variation across the wheat genotypes, consistent with previous reports by Kumar (13), who found similar levels of polymorphism in wheat protein profiles. The clusters identified through SDS-PAGE provide further support for the differentiation of wheat accessions based on their molecular and morphological traits, thus confirming the robustness of this method for assessing intra- and inter-specific genetic diversity. The findings of this study highlight the potential of combining both phenotypic and molecular data to improve the accuracy of genetic diversity assessments, which is crucial for breeding programs aimed at developing high-yielding and stress-resistant wheat varieties.

CONCLUSION

By analyzing 36 wheat genotypes, focusing on both morphological and molecular traits, our studies revealed significant genetic variation. Key morphological traits like stem length, spike length, and total plant height were strongly correlated with grain yield, suggesting their importance in wheat breeding. The molecular analysis through SDS-PAGE confirmed substantial genetic diversity among the genotypes, with polymorphic protein bands helping classify them into distinct genetic groups. Several loci were identified as contributing to genetic divergence. High-yielding genotypes such as Ma-6, Ma-16, Ma-22, and others showed superior grain yield and 1000-seed weight, indicating their potential for breeding programs. The combination of morphological and molecular data offers a comprehensive approach to identifying promising genotypes and understanding wheat genetic resources. These findings will support the development of high-yielding, adaptable wheat varieties for diverse environments.

Recommendations and Limitations

While this study provides valuable insights into the morphological and molecular diversity of wheat genotypes, it is important to consider the limitations that could affect the generalization of the results. The study was conducted on a limited number of genotypes, and expanding the sample size to include a broader range of wheat varieties, especially those from diverse geographical regions, could provide more comprehensive results. Furthermore, the study primarily relied on SDS-PAGE for molecular analysis, which, although effective, has limitations in resolution and the detection of specific gene variations. Future studies could employ more advanced techniques such as next-generation sequencing or other high-resolution molecular markers to provide a deeper understanding of the genetic underpinnings of wheat traits. Additionally, field trials under varying environmental conditions would help assess the stability of the identified high-yielding genotypes, ensuring their practical applicability in different agro-climatic zones.

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