

## ANCESTRY AND GENETIC DIVERSITY STUDIES AMONG PRIMITIVE AND MODERN WHEAT SPECIES BASED ON PHENOTYPIC DATA POOL

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### Abstract

The history of modern wheat and its relation with ancestral species have remained major concern for the evolutionist. Evolutionary path of wheat ancestors and modern wheat were studied using phenotypic data pool. Twenty three wheat species including ancestral and modern species were characterized for basic traits. Replicated phenotypic data on panel of 23 species were recorded, analyzed, pooled and dendrogram was developed. The clustering of species divided the 23 species into two main groups A and B. Group A contained four species *Aegilops biuncialis*, *Aegilops ovata*, *Aegilops vavilovii* and *Aegilops variabilis*. The group B consisted of 19 species. Group B had four sub clusters. First sub-cluster had *Triticum monococum*, *T. polonicum*, *Hordeum vulgare-1*, *H. vulgare-2* and *T. compactum*. Second sub-cluster had *T. durum*, *T. sphaerococum-1*, *T. sphaerococum-2*, *T. sphaerococum* and *T. aestivum*. Third sub-cluster had *T. dicocum*, *T. spelta*, *T. dicoccoides* and *Triticale*. Fourth Sub-cluster had *T. turgidum-1*, *T. turgidum-2*, *T. turgidum-3*, *T. turgidum-4* and *T. turgidum-5*. The species in the same sub cluster such as of sub-cluster-1 were more similar to each other compared to other sub clusters viz., 2, 3, 4 and 5. Hence modern wheat i.e., *Triticum aestivum* is lying in sub-cluster 2 in the group of *Triticum sphaerococum* and *Triticum durum* species. The findings showed that *Triticum durum* has lineage of ancestral history with modern wheat. Exploration of evolutionary path of modern wheat *Triticum aestivum* L., will be helpful in successfully improving wheat crop for various diverse traits.

**Key words:** Wheat evolution, Origin, History, Ancestral lines, *Triticum aestivum* L., Data pool.

### Introduction

Wheat (*Triticum aestivum* L.) is the most important crop of family poaceae. It is a crop that is commonly grown throughout the world. Humans mostly utilize it to make food in a variety of forms e.g., bread, chapatti, biscuits, cookies, cakes and other baked foods. Wheat crop is cultivated in Pakistan over an area of 8.86 thousand hectares from which country got production of 28 million tones and the share of Sindh province was 4.4 million tons (Anon., 2022-23, Anon., 2020-21). The average production in Pakistan is 32 maunds/acre and the average yield in Sindh province was recorded 34.4 maunds/acre (Anon., 2020-21).

Wheat (*Triticum* spp.) is one of the founding crops that most likely sparked the Neolithic shift to sedentary agricultural communities in the Fertile Crescent more than ten thousand years ago (Avni *et al.*, 2017). Its first cultivated form was diploid with genome AA which was known as einkorn wheat, the donor of A genome in wheat. *Triticum urartu* is the ancestor of wheat's A genome, while a near cousin of *Aegilops speltoides* is assumed to have given rise to wheat's B genome. The tetraploid species *Triticum turgidum* ssp., *dicoccoides*, sometimes known as wild emmer, was created by natural hybridization between these progenitors less than one million years ago. It has the AABB genomic structure (Marcussen *et al.*, 2014). Tetraploid wheat with the genome AABB, known as emmer wheat, and its genetic relationships suggested that Turkey's southern-eastern region was its area of origin (Dubcovsky & Dvorak, 2007). When *T. tauschii*, the D genome donor, crossed with a natural diploid species, *T. aestivum*, a hexaploid

wheat with six genomes (AABBDD) and also known as bread wheat, was formed (Salamini *et al.*, 2002). It is assumed that the sowing of wheat was started from at least nine thousand (9000) years ago from Eastern side of Turkey, where common bread wheat (hexaploid having 42 chromosomes) was produced (Feldman, 2001). It is believed that there have only been a small number of free crossings between *T. aestivum* progenitors, possibly leading to a failure in positive genetic diversity. Currently, the genetic basis of bread wheat (*Triticum aestivum* L.) may have been limited as a result of recent plant breeding and domestication, which could jeopardize future crop improvement both globally and in Pakistan (Salamini *et al.*, 2002). It has been believed that wheat ancestral/wild progenitors possess useful genes which can be helpful in increasing genetic diversity and to incorporate desirable genes for drought, heat, salinity, diseases and climate resilient into modern cultivars. These wheat relatives can help the breeders to compensate genetic erosion and introduce new varieties for changing climate change agri-eco system. Hence, such biotic and abiotic stress tolerance traits will be useful for the yield associated genetic progress in the plant. Aghaei *et al.*, (2008) also studied wheat wild relative and various wheat species and found huge genetic variation among these species for various phenotypic trait. However, their studies could not explore evolutionary perspective. The objectives of the present studies include evaluating the diversity, characterization of wheat ancestors and modern wheat cultivars to facilitate its effective utilization into wheat breeding programs and also assess evolutionary pathway based on phenotypic data.

## Material and Methods

The seeds were obtained from wheat breeding section of Nuclear Institute of Agriculture (NIA) Tandojam and the studies were conducted in field at experimental farm, Nuclear Institute of Agriculture (NIA) Tandojam. The experiment was laid out using randomized complete block design (RCBD) and replicated thrice during wheat cropping season 2021-2022. Each species was sown in 2 rows x 3m row length and row to row distance were maintained as 30cm. List of wheat wild, ancestral and modern wheat species included under study is as given in Table 1.

Data on different Agro-morphological especially yield associated characters viz., Leaf area (cm<sup>2</sup>), days to heading, days to maturity, spike length (cm), plant height (cm), number of grains per spike and seed index (100 seed weight (g)) were recorded and analyzed. These characters were recorded as Leaf area (cm<sup>2</sup>) was obtained by multiplying the product of leaf length (L) and width (W) for measuring leaf (cm<sup>2</sup>), days to heading was counted from number of days from sowing to when 50% of the plants of each plot headed., days to maturity was counted from number of days from sowing to when 50% of the plants of each plot matured and turned yellow. Spike length (cm) was recorded from the base to the top of spike excluding awns, plant height (cm) was recorded from the ground level to the tip of the spike without awns at maturity, number of grains per spike were counted after threshing the main spike of every individual plant spike and seed index were obtained by the weight of 100 grains using electronic balance. Data collected was analyzed statistically. ANOVA (analysis of variance), DMRT (Duncan multiple range test), clustering and correlations were computed using software statistix 8.1. Clustering was generated with the help of MATLAB statistical program.

## Results and Discussion

Mean square for analyses of Variance (ANOVA) was carried out and results were presented in (Table 2). The results showed a significant differences among the species for all the characters which were studied viz. Leaf area, days to heading, days to maturity, plant height, spike length, no. of grains per spike and 100 grain weight, which showed that ample genetic variation existed in the traits among wheat species.

Data for various morphological traits of all diploid, tetraploid and hexaploid wheat species is given in Table 3. Data for leaf area showed that *Aegilops ovata* had smallest leaf area compared to other species. Maximum leaf area was observed in *T. turgidum-5*. The leaf area of *T. aestivum* was very close to the leaf area of *T. sphaerococum-2*, this showed similarity for leaf area among both species. The early heading and maturity were observed in species *T. aestivum* followed by *T. durum*, while *A. vavilovii* and *A. ovata* were found late for heading and maturity respectively. *T. aestivum* and *T. sphaerococum* were found more similar to each other in terms of spike length compared to the rest of species. The maximum height was observed in *T. turgidum-5* and minimum in *Aegilops ovata*. Plant height of *T. aestivum* was immensely near to the height of *T. compactum* and *T. sphaerococum*. The hexaploid wheat i.e., *T. aestivum* had maximum number of grains per spike and minimum grains per spike were produced by *A. ovata* and *A. vavilovii*. Less difference in number of grains per spike was observed between *T. durum* and *T. aestivum*. Seed index (100 grain weight) was maximum in *T. polonicum* followed by *T. durum*.

**Table 1. List of wheat wild, ancestral and wheat modern specie used for this study.**

S. No.	Name of wheat specie/ancestral line	S. No.	Name of wheat specie/ancestral line
1.	<i>Aegilops biuncialis</i>	13.	<i>Triticum durum</i>
2.	<i>Aegilops ovata</i>	14.	<i>Triticum polonicum</i>
3.	<i>Aegilops variabilis</i>	15.	<i>Triticum spelta</i>
4.	<i>Aegilops vavilovii</i>	16.	<i>Triticum sphaerococum</i>
5.	<i>Triticum.monococum</i>	17.	<i>Triticum compactum</i>
6.	<i>Triticum dicocum</i>	18.	<i>Triticum sphaerococum 1</i>
7.	<i>Triticum dicoccoides</i>	19.	<i>Triticum sphaerococum 2</i>
8.	<i>Triticum turgidum 1</i>	20.	<i>Triticum aestivum</i>
9.	<i>Triticum turgidum 2</i>	21.	<i>Hordeum vulgare-1</i>
10.	<i>Triticum turgidum 3</i>	22.	<i>Hordeum vulgare-2</i>
11.	<i>Triticum turgidum 4</i>	23.	<i>Triticale</i>
12.	<i>Triticum turgidum 5</i>	-	---

**Table 2. Mean squares from analysis of variance (ANOVA) of various quantitative characters in ancestors and modern wheat cultivars.**

Source of variation	DF	Leaf area (cm <sup>2</sup> )	Days to heading	Days to maturity	Plant height (cm)	Spike length (cm)	No. of grains per spike	100 Grain weight (g)
Replicate	2	0.00261	1.319	4.478	0.31	0.0314	1.087	0.01316
Wheat species	22	731.995***	506.726***	142.292***	4287.66***	47.000***	574.589***	6.483***
Error	44	4.630E-04	1.046	0.842	0.05	0.0092	2.1781	0.10828
Total	68							

**Table 3. Phenotypic data pool of various ancestral and modern wheat species.**

Genotypes	Leaf area (cm <sup>2</sup> )	Days to heading	Days to maturity	Spike length (cm)	Plant height (cm)	No. Grains per spike	100-grain weight (g)
<i>Aegilops biuncialis</i>	7.1 <sup>u</sup>	109.0 <sup>cde</sup>	147.0 <sup>ab</sup>	6.3 <sup>j</sup>	45.7 <sup>r</sup>	4.3 <sup>m</sup>	1.0 <sup>l</sup>
<i>Aegilops ovata</i>	3.4 <sup>w</sup>	113.0 <sup>ab</sup>	149.0 <sup>a</sup>	2.5 <sup>m</sup>	40.4 <sup>s</sup>	3.3 <sup>m</sup>	1.3 <sup>kl</sup>
<i>Aegilops variabilis</i>	6.6 <sup>v</sup>	106.0 <sup>efg</sup>	145.0 <sup>b</sup>	2.8 <sup>m</sup>	55.0 <sup>q</sup>	4.3 <sup>m</sup>	1.9 <sup>jkl</sup>
<i>Aegilops vavilovii</i>	13.4 <sup>t</sup>	114.0 <sup>a</sup>	148.0 <sup>a</sup>	12.0 <sup>c</sup>	46.3 <sup>r</sup>	3.3 <sup>m</sup>	4.5 <sup>cd</sup>
<i>Triticum monococum</i>	21.2 <sup>s</sup>	103.0 <sup>gh</sup>	131.0 <sup>k</sup>	8.7 <sup>g</sup>	107.3 <sup>l</sup>	21.0 <sup>hij</sup>	2.2 <sup>ijk</sup>
<i>Triticum.dicocum</i>	39.6 <sup>k</sup>	102.0 <sup>h</sup>	136.0 <sup>fgh</sup>	11.7 <sup>c</sup>	129.3 <sup>g</sup>	18.6 <sup>ij</sup>	2.9 <sup>fghij</sup>
<i>Triticum dicoccoides</i>	45.6 <sup>f</sup>	97.0 <sup>i</sup>	138.0 <sup>def</sup>	10.0 <sup>e</sup>	149.7 <sup>e</sup>	22.6 <sup>ghi</sup>	2.5 <sup>ghij</sup>
<i>Triticum.turgidum</i> 1	46.7 <sup>e</sup>	112.0 <sup>abc</sup>	139.0 <sup>cde</sup>	7.3 <sup>h</sup>	155.3 <sup>d</sup>	31.3 <sup>def</sup>	2.7 <sup>fghij</sup>
<i>Triticum turgidum</i> 2	48.9 <sup>d</sup>	112.6 <sup>ab</sup>	140.0 <sup>cd</sup>	9.3 <sup>f</sup>	158.7 <sup>c</sup>	28.6 <sup>ef</sup>	3.2 <sup>fghi</sup>
<i>Triticum turgidum</i> 3	51.7 <sup>b</sup>	110.0 <sup>bcd</sup>	140.0 <sup>cd</sup>	9.1 <sup>f</sup>	160.3 <sup>b</sup>	32.6 <sup>de</sup>	3.4 <sup>efg</sup>
<i>Triticum turgidum</i> 4	49.9 <sup>c</sup>	107.0 <sup>def</sup>	137.0 <sup>efg</sup>	11.7 <sup>c</sup>	159.3 <sup>c</sup>	30.0 <sup>ef</sup>	2.3 <sup>hij</sup>
<i>Triticum turgidum</i> 5	58.8 <sup>a</sup>	111.0 <sup>abc</sup>	141.0 <sup>c</sup>	11.1 <sup>d</sup>	163.0 <sup>a</sup>	31.0 <sup>def</sup>	3.1 <sup>fghi</sup>
<i>Triticum durum</i>	35.4 <sup>m</sup>	83.0 <sup>l</sup>	131.0 <sup>k</sup>	6.1 <sup>j</sup>	101.3 <sup>o</sup>	42.6 <sup>b</sup>	5.6 <sup>b</sup>
<i>Triticum spelta</i>	44.0 <sup>h</sup>	85.0 <sup>kl</sup>	132.0 <sup>jk</sup>	12.0 <sup>c</sup>	99.7 <sup>p</sup>	23.6 <sup>gh</sup>	7.7 <sup>a</sup>
<i>Triticum compactum</i>	42.3 <sup>j</sup>	111.0 <sup>abc</sup>	145.0 <sup>b</sup>	14.3 <sup>b</sup>	122.0 <sup>h</sup>	12.3 <sup>l</sup>	2.7 <sup>fghij</sup>
<i>Triticum polonicum</i>	22.2 <sup>r</sup>	85.0 <sup>kl</sup>	132.0 <sup>jk</sup>	5.3 <sup>l</sup>	112.3 <sup>i</sup>	38.6 <sup>bc</sup>	2.6 <sup>fghij</sup>
<i>Triticum sphaerococcum</i>	34.0 <sup>n</sup>	88.0 <sup>jk</sup>	133.0 <sup>ijk</sup>	17.0 <sup>a</sup>	136.7 <sup>f</sup>	32.0 <sup>de</sup>	3.3 <sup>fgh</sup>
<i>Triticum sphaerococcum-1</i>	42.9 <sup>i</sup>	85.0 <sup>kl</sup>	134.0 <sup>hij</sup>	6.3 <sup>j</sup>	109.0 <sup>k</sup>	27.0 <sup>fg</sup>	3.5 <sup>def</sup>
<i>Triticum sphaerococcum-2</i>	28.9 <sup>p</sup>	83.0 <sup>l</sup>	136.0 <sup>fgh</sup>	5.7 <sup>k</sup>	99.3 <sup>p</sup>	33.0 <sup>de</sup>	2.9 <sup>fghij</sup>
<i>Triticale</i>	30.0 <sup>o</sup>	84.0 <sup>l</sup>	137.0 <sup>efg</sup>	6.0 <sup>jk</sup>	103.0 <sup>mn</sup>	34.6 <sup>cd</sup>	3.0 <sup>fghi</sup>
<i>Hordeum vulgare-1</i>	39.0 <sup>l</sup>	90.0 <sup>j</sup>	136.0 <sup>fgh</sup>	9.1 <sup>f</sup>	102.7 <sup>n</sup>	17.0 <sup>jk</sup>	5.2 <sup>bc</sup>
<i>Hordeum vulgare-2</i>	45.5 <sup>g</sup>	105.0 <sup>fgh</sup>	135.0 <sup>ghi</sup>	6.7 <sup>i</sup>	103.5 <sup>m</sup>	14.0 <sup>kl</sup>	2.4 <sup>ghij</sup>
<i>Triticum aestivum</i>	27.6 <sup>q</sup>	72.0 <sup>m</sup>	118.0 <sup>l</sup>	17.0 <sup>b</sup>	111.3 <sup>j</sup>	58.6 <sup>a</sup>	4.4 <sup>cde</sup>
Mean	34.1	98.6	124.4	9.9	117.2	30.3	3.3

The data for morphological observations was subjected for cluster analysis and the dendrogram was constructed which distributed all the 23 species (ancestral, wild and modern wheat species) into two main A and B groups (Fig. 1). The first group i.e., Group A consisted of 4 species which all belonged to the genus *Aegilops*, It included *Aegilops biuncialis*, *A. ovata*, *A. vavilovii* and *A. variabilis*, among them *A. biuncialis* and *A. ovata* showed 95% of similarity index with each other, while *A. vavilovii* and *A. variabilis* showed the 90% of similarity with *A. biuncialis* and *A. ovata*. Prazak (2013) similarly identified high levels of genetic similarity within *Aegilops* species, emphasizing their evolutionary closeness. The second group B contained 19 species which were further divided into 4 sub-clusters. Group B divided into four sub clusters. Sub-clusters 1 had 5 species which included *T. monococum*, *T. polonicum*, *Hordeum vulgare-1*, *T. compactum* and *Hordeum vulgare-2* as elucidated by Zhao *et al.*, (2017). Sub-cluster 2 had also 5 species viz., *T. durum*, *T. sphaerococcum* 1, *T. sphaerococcum* 2, *T. sphaerococcum* and *T. aestivum*. Sub-cluster 3 had 4 species also viz., *T. dicocum*, *T. spelta*, *T. dicoccoides* and *Triticale*. There were reports that *T. spelta* developed viable F1 hybrids with *T. aestivum*, which had perfect chromosomal pairing at meiosis, and was hexaploid in most morphological features (Zhao *et al.*, 2017). Sub-cluster 4 had also 5 species *T. turgidum-1*, *T. turgidum-2*, *T. turgidum-3*, *T. turgidum-4* and *T. turgidum-5*. Feldman *et al.*, (2015) offered compelling support for the proposition that the diploid species contributed to the bread wheat B subgenome sharing genetic affinities with *A. speltooides*. Also, the findings of Li *et al.*, (2022) provided evidence that the diploid species contributing to the bread wheat B subgenome were in a good relation to *A. speltooides*, and these findings were consistent with our results. Furthermore, Rufati *et al.*, (2022) reported that the clear separation of species into different clusters and sub-clusters were important for comprehending

the evolutionary history and breeding potential of these species. This further affirms the significance of our findings.

Our findings provided a clear clustering of species, based on similarity, which supported the evidence emphasized by, Rufati *et al.*, (2022), held profound significance for unraveling the evolutionary history and harnessing the breeding potential of these diverse wheat species.

The correlation matrix showed a significant ( $p \leq 0.05$ ) and positive correlation among the various attributes (Table 4). The highest positive correlation ( $r = 0.8844$ ) was found between leaf area and plant height. The plants with greater leaf areas tend to have increased plant height. It showed the connection between leaf development and overall plant growth (Tanveer *et al.*, 2022). This correlation was also supported by the work of Gaju *et al.*, (2016) and Shaheen *et al.*, (2023), who highlighted the importance of leaf size in photosynthetic efficiency and plant growth. Days to maturity was positively and highly significantly correlated ( $r = 0.7900$ ) with days to heading. These results suggested that the less days to heading were likely to mature earlier and vice versa as discussed by Khadka *et al.*, (2020) and Hussain *et al.*, (2023). This relationship is further corroborated by Tsenov, N. (2009), who found that early heading generally led to earlier maturation in cereal crops, a critical factor in determining the overall yield. Number of grains per spike showed a positive and significant association with plant height ( $r = 0.5671$ ) a finding that resonates with the research of Arain *et al.*, (2018) who observed similar trends in wheat and it was highly negative but significantly correlated with days to maturity ( $r = -0.8052$ ) and days to heading ( $r = -0.6504$ ). The time to maturity increases or the spike length grows longer, the number of grains per spike tends to decrease (Safeerul-Hassan *et al.*, 2004).

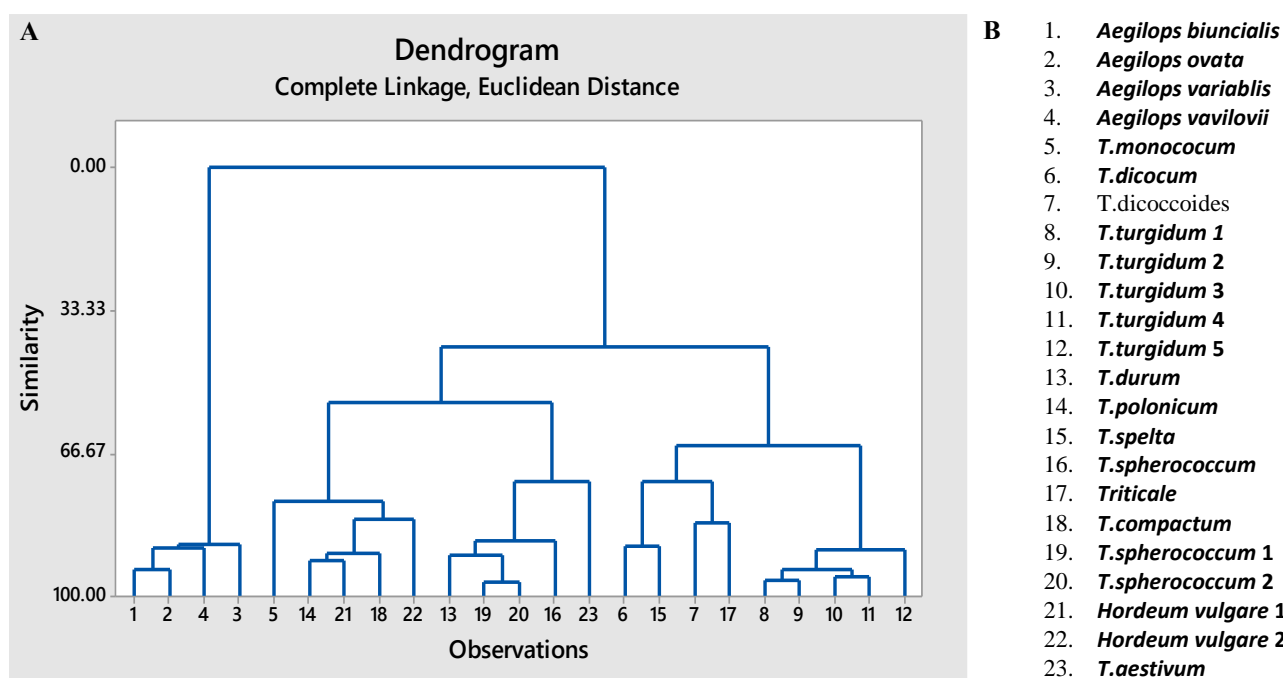


Fig. 1. Similarity index of wheat ancestor and modern cultivars by applying Dendrogram graphics.

**Table 4. Correlation coefficient (r) among of various quantitative characters in ancestors and modern wheat species.**

	LA	DH	DM	SL	PH	GPS
DH	0.0099					
DM	-0.2709	0.7900****				
SL	0.3894	-0.1303	-0.3694			
PH	0.8844***	0.0230	-0.3331	0.4279		
GPS	0.4264	-0.6504***	-0.8052***	0.3127	0.5671**	
100 GW	0.3179	-0.4890	-0.4412	0.3671	0.0762	0.3385

LA= Leaf area, DH= Days to heading, DM= Days to maturity, SL= Spike length, PH= Plant height, GPS= Grain per spike, 100GW= 100 grain weight

## Conclusion

Modern wheat *T. aestivum*, *T. sphaerococcum* and *T. durum* were found lying in same cluster based on phenotypic data pool. Hence, they are more closely related to each than other species. This finding gives us clue that *T. durum* has lineage of ancestral history with nowadays wheat. Further pedigree analyses of *T. durum* will explore other ancestors involved in evolution of modern-day wheat. This finding of evolutionary path of modern wheat *Triticum aestivum* L., will be helpful in successfully improving wheat crop for higher yield, drought, heat, salinity, diseases and climate resilience.

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