The Changes in Coleoptile Length and Root System Architecture During Wheat Polyploidization

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Abstract

Polyploidy, the doubling of chromosomes, has significantly shaped the evolution of flowering plants, including wheat. Yet, its impact on crucial traits like coleoptile length and root development remains unclear. This study compared these traits in wheat varieties with different ploidy levels, focusing on coleoptile length and root system diversity. Five genotypes/cultivars were used for each of einkorn (*Triticum monococcum*), durum (landraces and cultivars, *Triticum durum* and *Triticum turgidum*), and bread wheat (*Triticum aestivum*). The coleoptile length and root morphological measurement procedures were conducted using a blotter-paper germination protocol. The experiment was designed according to a completely randomized design with three replications. The results revealed that domestication and selection pressures have influenced wheat's coleoptile length and certain root system characteristics. Interestingly, polyploidy appears to have a mixed bag of effects. It enhances root angle and seminal root numbers. However, total root length and coleoptile length are negatively affected. Importantly, the longest root remains unaffected. This divergence in root traits highlights the complex interplay between polyploidy and plant morphology. Understanding these trade-offs is crucial for plant breeders. To combine polyploidy's desirable robustness with optimal root systems, wild relatives and modern wheat varieties need to be strategically integrated into breeding programs. This will allow for the recovery of valuable traits that separated during wheat's evolutionary journey.

1. Introduction

Polyploidy, the occurrence of multiple sets of chromosomes, has played a crucial role in the evolution of flowering plants. Estimates suggest that 50-70% of angiosperm species originated through polyploidization (Soltis et al., 1993; Masterson, 1994; Ozkan et al., 2001). This phenomenon has also significantly impacted wheat evolution, as evidenced by the discovery of its diverse chromosome numbers (Sakamura, 1918; Sax, 1918). Wheat belongs to the *Triticum* genus and is one of the most important cereal species in the world, serving as a staple crop for human consumption on almost all continents (Williams, 1993). It was the first domesticated food crop, appearing approximately 8,000 to 12,000 years ago in the Fertile Crescent (Heun et al., 1997; Özkan et al., 2002) and playing a foundational role in the agricultural revolution (Harlan, 1992; Zohary and Hopf, 2000). Wheat comprises three polyploid series: diploid species with 14 chromosomes ($2n = 2x = 14$), tetraploids with 28 chromosomes ($2n = 4x = 28$), and hexaploid with 42 chromosomes ($2n = 6x = 42$). Among the diploid wheat species, einkorn (*Triticum monococcum*, $A^m A^m$) holds a unique position. It played a pivotal role in establishing agriculture in this region and remains the only diploid wheat species with both wild (*Triticum boeticum*) and domesticated forms. Interestingly, einkorn shares a close genetic relationship with *Triticum Urartu* ($A^u A^u$), the A genome donor of both tetraploid durum wheat (*Triticum turgidum* ssp. *durum*) and hexaploid bread wheat (*Triticum aestivum*) (Marcussen et al., 2014). Durum wheat itself is a tetraploid species (AABB) derived from domesticated emmer wheat (*Triticum turgidum* ssp. *dicoccum*) approximately 10,000 years ago (Dubcovsky and Dvorak, 2007). Emmer wheat, in turn, evolved from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*). Wild emmer possibly evolved from natural hybridization between *Triticum urartu* and *Aegilops speltoides* (BB). Finally, bread wheat (*Triticum aestivum*, AABBDD) emerged only 8,500-9,000 years ago as a young hexaploid species. Its formation resulted from a hybridization event between domesticated free-threshing tetraploid wheat (*Triticum turgidum* ssp. *dicoccum*, AABB) and *Aegilops tauschii* (DD), the diploid donor of the D subgenome (Levy and Feldman, 2022). These discoveries and knowledge not only illuminate the remarkable diversity of wheat but also pave the way for further research on polyploidy and its role in shaping wheat's evolution.

Sustainable yield improvement is a common goal among breeders and farmers. However, wheat is frequently grown under rainfed conditions, where drought and heat stress significantly reduce grain yield (Araus et al., 2002; Condon et al., 2004). Drought stress can particularly limit wheat yield during critical stages like heading and grain-filling (El Hafid et al., 1998; Araus et al., 2003; Royo et al., 2006). Tolerance to drought is a complex trait influenced by multiple factors, and coleoptile length and root morphology and biomass may be important approaches to minimize the adverse effects of climate change on wheat yield stability.

Ensuring a stable maximum yield requires creating an even and optimal number of plants per unit area. Achieving optimal seedling establishment in dry areas presents a major challenge for farmers. Temperature and water/moisture are the two most critical environmental factors determining the success of seedling establishment in dry soil (Jame and Cutforth, 2004; Cochrane et al., 2015). Deep sowing in arid and hot regions can provide access to ideal temperatures and humidity, leading to a higher seed germination rate and seedling establishment (Mahdi et al., 1998; Mohan et al., 2013). Deep sowing also minimizes damage to seeds from freezing temperatures and protects them from pests (Brown et al., 2003). An optimal and suitable sowing time is crucial for wheat cultivation (Coventry et al., 1993; Shackley and Anderson, 1995; Mahdi et al., 1998; Rahman et al., 2009; Baloch et al., 2012). In dry areas, sowing is often delayed if the soil lacks sufficient moisture to initiate seed germination. Therefore, sowing is postponed

until the beginning of the rainy season, allowing the soil to accumulate enough moisture. Conversely, when seeds are sown deeper, the reserved water/moisture in deeper soil layers allows farmers to plant wheat at the standard sowing time (Mahdi et al., 1998; Schillinger et al., 1998), thus preventing potential yield losses.

Climate change and extreme environmental events necessitate the development of novel genotypes/cultivars with enhanced stress tolerance. Due to increased drought pressure, cultivars with longer coleoptiles are needed to ensure optimal seedling emergence in dry areas (Rebetzke et al., 2007). The coleoptile, a protective sheath that facilitates seedling emergence in monocots, is significantly influenced by genetics control (Rebetzke et al., 2004; Rebetzke et al., 2007; Murphy et al., 2008). Genotypes with longer coleoptiles can be planted deeper, allowing seedlings to bypass the dry and hot soil layers. However, deep sowing of genotypes with shorter coleoptiles can result in poor seedling establishment and negatively impact grain yield (Mahdi et al., 1998; Rebetzke et al., 2005; Rebetzke et al., 2007). Therefore, developing novel cultivars with longer coleoptiles is crucial.

Wheat harbours a hidden world beneath the surface - its root system. This intricate network, comprised of seminal (embryonic) and nodal (crown or adventitious) roots, plays a critical role in the entire lifecycle of the plant, influencing growth and development at every stage (Kirby and Appleyard, 1987; Chochois et al., 2015; Sinha et al., 2018). Seminal roots, the early pioneers, demonstrate remarkable resilience in the face of drought. Their superior depth-penetrating abilities enable them to tap into the vital water reserves in the deeper soil layers, where shallower nodal roots might struggle (Araki and Iijima, 2001; Manske and Vlek, 2002; Maccaferri et al., 2016). Until the fourth leaves emerge (Zadoks Scale, Stage 1.4) (Zadoks et al., 1974), these pioneering roots serve as the sole anchors for the young plant, highlighting their

crucial role in establishing a strong foundation for growth (Esau, 1965). The architecture of the root system itself holds immense significance for nutrient and water acquisition. Genotypes with deep, narrow, and densely packed roots excel at extracting water and nitrogen from deeper soil layers throughout the season (Manschadi et al., 2008; Uga et al., 2013; Borrell et al., 2014). In contrast, genotypes with shallower, wider-angled roots demonstrate a knack for retrieving nutrients like phosphorus from areas closer to the surface (Miguel et al., 2015). This diversity in root architecture underscores its potential as a key selection factor for breeding wheat cultivars with superior nutrient and water uptake capabilities. Unlocking the full potential of wheat under unfavorable climatic conditions demands careful manipulation of plant traits that support its resilience. Ensuring proper development from germination to physiological maturity becomes crucial for equipping wheat to withstand environmental challenges.

Coleoptile length and root architecture emerge as the key contributors to plant success. Recognizing the crucial role of these traits in mitigating environmental stresses, this study aims to screen three important wheat species: einkorn, durum, and bread wheat. This comprehensive assessment will compare the coleoptile length and root architectural traits of wheat at three different ploidy levels.

2. Materials and Methods

2.1. Plant materials

Plant material consisted of a total of 20 genotypes, comprising 5 genotypes of einkorn wheat, one is a first-registered einkorn cultivar, one is an unregistered landrace that cultivated in Kastamonu province, in Türkiye, and the other three are advanced einkorn lines, 5 durum wheat landraces (*Triticum turgidum* L.), 5 modern durum wheat cultivars (*Triticum turgidum* L. *durum* Desf.), and 5 modern bread wheat cultivars (*Triticum aestivum* L.). Modern wheat cultivars were chosen based on their popularity in Türkiye

and around the world, while the landraces were chosen to represent different locations in Türkiye. The list of all genotypes is given in Table 1.

Table 1. The landraces and cultivars used in the experiment, categorized by their ploidy levels

Ploidy levels	Genome	Species	Cultivar/Genotype name
Diploid, 2n=2x=14	A^mA^m	Einkorn Wheat, *Triticum monococcum*	Monlis
Diploid, 2n=2x=14	A^mA^m	Einkorn Wheat, *Triticum monococcum*	ID1623
Diploid, 2n=2x=14	A^mA^m	Einkorn Wheat, *Triticum monococcum*	ID432
Diploid, 2n=2x=14	A^mA^m	Einkorn Wheat, *Triticum monococcum*	ID347
Diploid, 2n=2x=14	A^mA^m	Einkorn Wheat, *Triticum monococcum*	Kastamonu Landrace
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat landrace, *Triticum turgidum L.*	TR-81356-Konya
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat landrace, *Triticum turgidum L.*	TR-81259-Malatya
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat landrace, *Triticum turgidum L.*	TR-81273-Ankara
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat landrace, *Triticum turgidum L.*	TR-47949-Kars
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat landrace, *Triticum turgidum L.*	TR-80984-Eskişehir
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat, *Triticum durum* Desf.	Balcalı-2000
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat, *Triticum durum* Desf.	Çeşit-1252
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat, *Triticum durum* Desf..	Zenit
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat, *Triticum durum* Desf.	Sevevo
Tetratploid, 2n=2x=28	A^uA^uBB	Durum wheat, *Triticum durum* Desf.	Havidi
Hexaploid, 2n=2x=42	A^uA^uBBDD	Bread wheat, *Triticum aestivum* L.	Pandas
Hexaploid, 2n=2x=42	A^uA^uBBDD	Bread wheat, *Triticum aestivum* L.	Özkan
Hexaploid, 2n=2x=42	A^uA^uBBDD	Bread wheat, *Triticum aestivum* L.	Adana-99
Hexaploid, 2n=2x=42	A^uA^uBBDD	Bread wheat, *Triticum aestivum* L.	Seri-82
Hexaploid, 2n=2x=42	A^uA^uBBDD	Bread wheat, *Triticum aestivum* L.	Gönen-98

2.2. Evaluation of coleoptile length and root architecture traits

The coleoptile length and root architecture measurement procedures were conducted at Şırnak University's Agriculture Faculty. The experiment was designed according to a completely randomized design (CRD) with three replications. Coleoptile lengths for durum genotypes were determined using a blotter-paper germination protocol adapted from Hakizimana et al. (2000). Standard filter paper (400 × 400 mm) was used for the experiment. Six seeds of equal size were selected for each genotype and lined up 2 cm upper on centre of a germination paper (22 cm away from bottom) with a spacing of 5 cm between them. The seeds were placed germ-side down. The protocol was slightly modified by using double-sided sticky tape to attach the seeds to the germination paper and keep them steady during rolling. After placing all the seeds on the line, another germination papers were placed on the first and then rolled horizontally from left to right. The rolled germination papers were secured with rubber bands, covered with a black plastic bag, and placed vertically in a tray filled with approximately 20 cm of distilled water to soak from the bottom of the rolls. Subsequently, the saturated samples were removed from the tray and placed on a grid tray to drain excess water. The samples in the grid trays were placed in a dark refrigerator at 4°C for two days to break any potential dormancy. The samples were then transferred to a growth chamber at 15°C with 80% relative humidity for four days, followed by 7 days at 20°C, at completely dark mode. During this period, the samples were soaked in distilled water every few days if they appeared dry. In total, the experiment ran for twelve days, after which the samples were removed from the growth chamber for image collection. All samples were quickly

photographed to minimize timing-related errors among replicates. Images were then transferred to a computer environment for measurement of coleoptile lengths and root traits using ImageJ image analysis software (Rueden et al., 2017). Coleoptile lengths were measured as the distance from the scutellum to the tip of the coleoptile, as described by Rebetzke et al. (2004). Root measurements included root growth angle (RA), seminal root number (SRN), total seminal root length (TRL), and longest root length (LRL). The root growth angle was measured as the angle between the first pair of roots around the primary root as described by Bektas and Waines (2020).

2.3. Basic statistical analysis

Analysis of variance (ANOVA) was performed on coleoptile length and root architecture traits, followed by Pearson's correlation coefficient analysis between coleoptile length and root traits using JASP software, version 0.11.1 (Love et al., 2019; Team, 2019). The Broad-sense heritability and coefficient of variation (CV) were computed using the "metan" R package (Olivoto and Lúcio, 2020). Means for each species over the two trials were subjected to multiple comparison testing by using Tukey's HSD ($P = 0.05$) and bar plots with significance letters were generated using RStudio (RStudio, 2020). A neighborhood-based clustering method was used to group the genotypes based on their means, and cluster plots were created using JASP software, version 0.11.1 (Love et al., 2019; Team, 2019).

3. Results and Discussion

This study aimed to investigate coleoptile length and some root traits of the three ploidy levels of wheat species. As a result of the study, all species combined, all the traits demonstrated the about normal distributions, which skewness and kurtosis values supports (Figure 1 and Table 2). A significant genetic diversity was observed for coleoptile length (COL), seminal root number (SRN), total seminal root length (TRL), longest root length (LRL), and root growth angle (RA) between genotypes combined (all species mixed) (Table 3). However, when analysing the related traits according to their ploidy levels individually, there was no statistically significant difference between the einkorn genotypes for all traits, whereas there was significant genetic diversity in durum landraces and durum cultivars for all traits, with significance levels ranging from $p<0.05$ to $p<0.001$, except for the SRN trait. By the way, bread wheat did not exhibit any significant genetic diversity for all traits, except for COL, which was significant at the $p<0.001$ level (Table 4). Tukey's HSD test also supports these results (Figure 2).
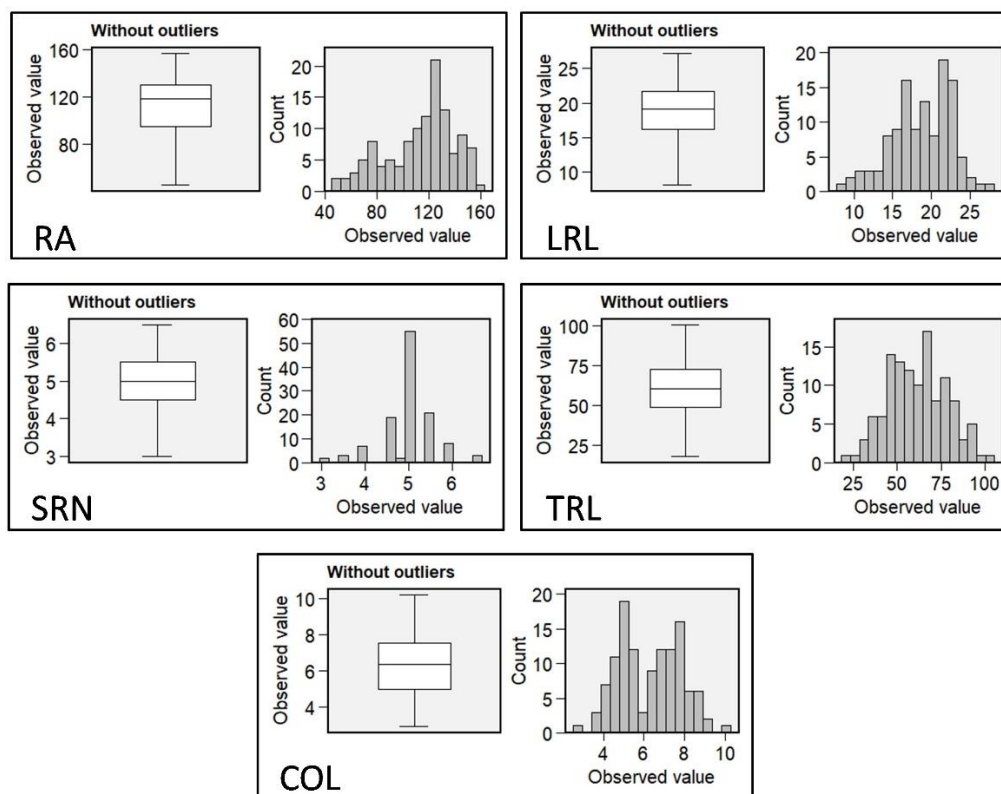
Figure 1. Box plots and distribution plots of investigated traits. Root growth angle (RA), Longest root length (LRL), Seminal root number (SRN), Total seminal root length (TRL), Coleoptile length (COL)

Table 2. Basic descriptive statistics of coleoptile length and root traits in three wheat species combined, using the average of both trials

Variable	Max	Mean	Min	Range	Skewness	Kurtosis	CV (%)	h^2
RA	156.69	113.14	45.78	110.91	-0.55	-0.46	10.02	0.93
LRL	27.23	18.56	6.16	21.07	-0.54	0.04	10.90	0.89
SRN	6.50	4.98	3.00	3.50	-0.42	1.48	10.39	0.53
TRL	100.71	61.24	18.29	82.41	0.01	-0.42	13.78	0.90
COL	10.24	6.25	2.93	7.30	0.08	-0.93	9.76	0.93

Root growth angle (RA), Longest root length (LRL), Seminal root number (SRN), Total seminal root length (TRL), Coleoptile length (COL), Coefficient of Variation (CV), Broad-sense Heritability (h^2)
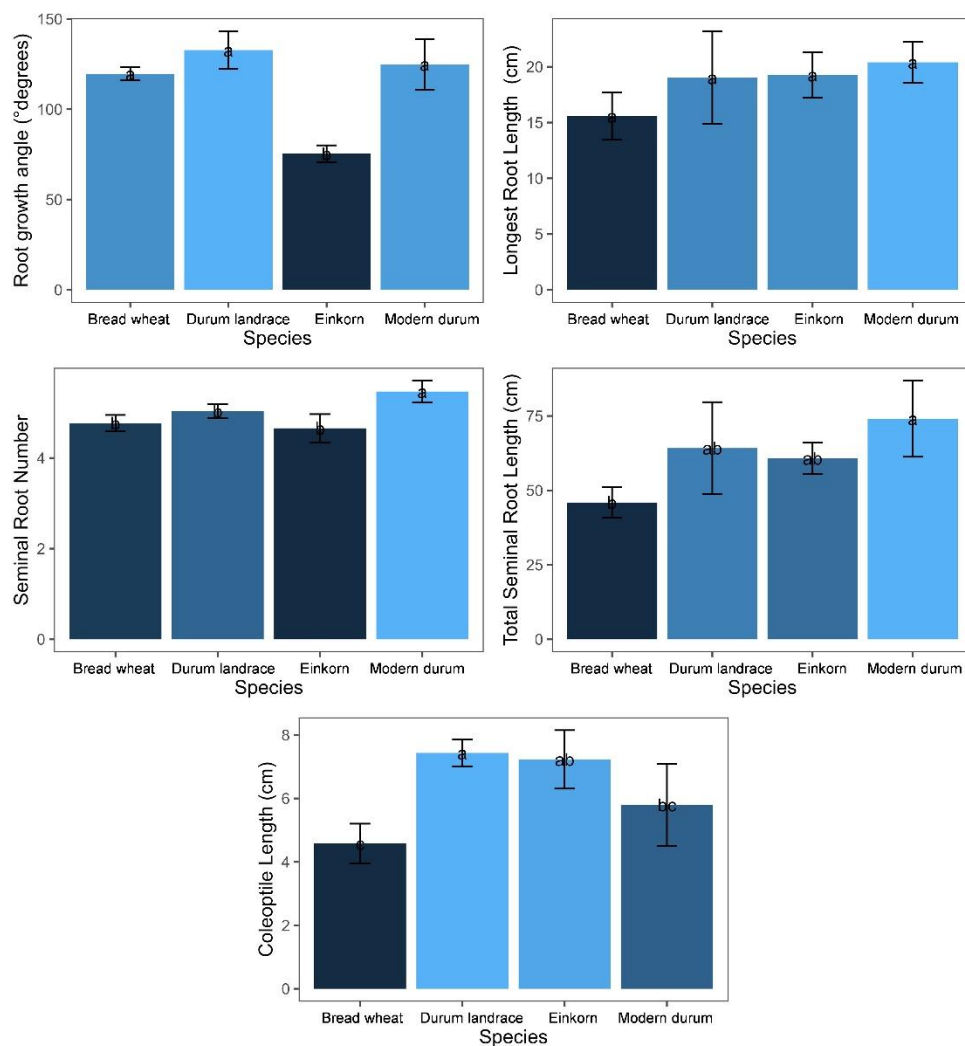
Figure 2. Tukey's HSD multiple comparison test plots of investigated traits. The error bars are standard deviations. Columns with the same letter are not significantly different at P < 0.05

Table 3. Combined ANOVA of coleoptile length and various root traits in all wheat genotypes, assessed across three species and two trials

Source of Variation	Df	Mean Square				
		RA	LRL	SRN	TRL	COL
Trial	1	108.266	16.919	0.011	156.494	0.817
Genotype	19	3564.161***	57.519***	0.883***	1218.406***	234.967***
Geno×Trial	19	201.987	20.639	0.348	221.371	11.126
Residuals	76	129.250	4.097	0.269	71.556	28.546

p<.001***, Root growth angle (RA), Longest root length (LRL), Seminal root number (SRN), Total seminal root length (TRL), Coleoptile length (COL)

Table 4. ANOVA of coleoptile length and root traits in all wheat genotypes, assessed across individual species and two trials

Species	Source of Variation	Df	Mean Square				
			RA	LRL	SRN	TRL	COL
Einkorn	Trial	1	7.560	0.003	0.494	0.937	0.245
	Genotype	4	128.562	24.819	0.587	166.209	5.063
	Geno×Trial	4	198.596	38.432	0.181	363.963	0.227
	Residuals	16	208.403	3.760	0.205	66.940	0.263
Durum Landrace	Trial	1	12.543	0.756	1.200	0.847	1.038
	Genotype	4	645.324*	179.144***	0.070	2043.379***	3.056**
	Geno×Trial	4	14.994	4.094	0.012	74.840	0.363
	Residuals	16	151.710	2.349	0.279	57.410	0.483
Modern Durum	Trial	1	6.822	1.158	0.033	100.203	0.286
	Genotype	4	1279.358***	18.280**	0.345	870.416***	9.311***
	Geno×Trial	4	339.535	1.683	0.179	30.910	0.168
	Residuals	16	40.813	3.282	0.162	84.518	0.900
Bread Wheat	Trial	1	37.675	2.575	0.533	4.570	0.006
	Genotype	4	95.103	7.798	0.195	105.989	2.236***
	Geno×Trial	4	66.089	2.684	0.387	38.719	0.168
	Residuals	16	190.551	3.947	0.229	36.077	0.178

$p<.05^{*}$, $p<.01^{**}$, $p<.001^{***}$, Root growth angle (RA), Longest root length (LRL), Seminal root number (SRN), Total seminal root length (TRL), Coleoptile length (COL)

When the measurements were evaluated combined, the average root angle (RA) ranged from 45.78° (einkorn) to 156.69° (durum landrace) (Table 2). The einkorn wheat had the narrowest angle, ranging from 45.78° to 104.62°, followed by durum landrace (103.22° to 156.69°), durum wheat (96.19° to 155.68°), and bread wheat (91.33° to 149.49°), with respective ranges of 58.84°, 53.47°, 59.49°, and 58.16° (Table 5). Average longest root length (LRL) ranged from 6.16 cm to 27.23 cm (Table 2). The longest root was found in durum landrace at 27.23 cm, followed by modern durum (25.86 cm), einkorn (24.39 cm), and bread wheat (21.54 cm) (Table 5). Seminal root number (SRN) averages ranged from 3.00 to 6.50 roots (Table 2). Durum landrace and modern cultivars both had the highest number, with 6.50 each. Einkorn had a maximum of 5.50 roots, while bread wheat reached 6.00 roots (Table 5). Average total seminal root length (TRL) varied from 18.29 cm to 100.71 cm (Table 2). Modern durum wheat had the highest TRL at 100.71 cm, followed by durum landrace (90.26 cm), einkorn (76.86 cm), and bread wheat (64.02 cm) (Table 5). Coleoptile length (COL), another important seedling stage characteristic, showed interesting results among the species. Average COL ranged from 2.93 cm to 10.24 cm (Table 2). The longest coleoptile was found in the diploid einkorn with 10.24 cm, followed by modern durum (8.96 cm), durum landrace (8.32 cm), and bread wheat (5.83 cm). However, on average, durum landraces had slightly longer coleoptiles (7.24 cm) than einkorn (7.22 cm) (Table 5).

The coefficient of variation values ranged from 9.76% (COL) to 13.78% (TRL) (Table 2), and the broad-sense heritability values varied between 0.53 (SRN) and 0.93 (RA, COL) (Table 2). Correlation analyses were conducted to determine potential relationships between the studied traits. As a result of these analyses, significant positive correlations were observed only between SRN and TRL (0.69) and TRL and LRL (0.87) (Table 6). Finally, neighborhood-based clustering analysis divided the genotypes into three populations, using all traits together. All einkorn genotypes were clustered in Group 1, which did not include any other species. All bread wheat cultivars clustered together in Group 3. This group also included one durum landrace and one durum modern cultivar. Contrary to expectations, durum wheat landraces and modern cultivars did not form distinct groups but instead clustered together within Group 4 (Figure 3).
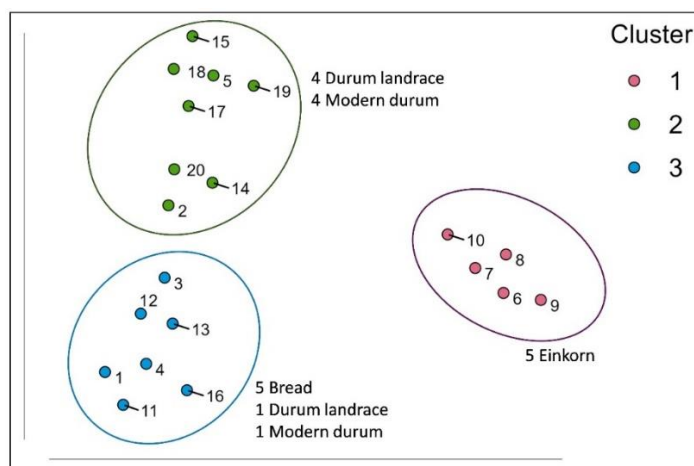
Figure 3. Neighborhood-based clustering results of species. The genotypes and groups are indicated with different colors

Table 5. Basic statistical parameters of coleoptile length and root traits in individual wheat species, using the average of both trials

Species	Variable	Max	Mean	Min	Range
Einkorn	RA	104.62	75.39	45.78	58.84
	LRL	24.39	19.26	10.22	14.17
	SRN	5.50	4.65	3.00	2.50
	TRL	76.86	60.76	31.41	45.45
	COL	10.24	7.22	5.89	4.34
Durum Landrace	RA	156.69	134.94	103.22	53.47
	LRL	27.23	18.87	9.65	17.58
	SRN	6.50	5.10	4.00	2.50
	TRL	90.26	64.74	30.41	59.85
	COL	8.32	7.24	5.15	3.16
Modern Durum	RA	155.68	127.62	96.19	59.49
	LRL	25.86	21.25	15.36	10.49
	SRN	6.50	5.46	4.50	2.00
	TRL	100.71	76.57	47.76	52.94
	COL	8.96	5.98	4.47	4.49
Bread Wheat	RA	149.49	119.20	91.33	58.16
	LRL	21.54	16.45	12.17	9.36
	SRN	6.00	4.77	3.50	2.50
	TRL	64.02	48.90	34.55	29.46
	COL	5.83	4.60	2.93	2.89

Root growth angle (RA), Longest root length (LRL), Seminal root number (SRN), Total seminal root length (TRL), Coleoptile length (COL)

Table 6. Statistical data of treatments with yield of plants

Pearson's Correlations of Coefficient			*r*
SRN	-	TRL	0.69**
SRN	-	LRL	0.38
SRN	-	RA	0.39
SRN	-	COL	-0.06
TRL	-	LRL	0.87***
TRL	-	RA	-0.04
TRL	-	COL	0.30
LRL	-	RA	-0.15
LRL	-	COL	0.34
RA	-	COL	-0.27

$p < .01$, ** $p < .001$***, Root growth angle (RA), Longest root length (LRL), Seminal root number (SRN), Total seminal root length (TRL), Coleoptile length (COL)

Wheat, a cornerstone of global food security, harbors a fascinating evolutionary history shaped by polyploidy, culminating in three distinct lineages: the ancient einkorn, the adaptable durum, and the versatile bread wheat. However, climate change threatens its yield with drought and heat stress. To combat this, researchers are focusing on two key traits: coleoptile length, aiding seedling emergence through scorching layers, and root architecture, optimizing water and nutrient uptake from deeper reserves. By studying these traits across einkorn, durum, and bread wheat, this research sought to unlock the effect of polyploidization on the coleoptile and root characteristics of wheat, paving the way for climate-resilient cultivars that guarantee abundant harvests for generations to come.

In the existing literature, many different characterization studies reported in diploid, tetraploid, and hexaploid wheat in terms of coleoptile length (Rebetzke et al., 2004; Rebetzke et al., 2007; Murphy et al., 2008; Md et al., 2014; Sesiz et al., 2024) and root traits within individual species (Bektas et al., 2016; Figueroa-Bustos et al., 2018; Bektas and Waines, 2020; Bektas et al., 2021; Bektas et al., 2023). However, there was limited information on coleoptile length and root traits comparison on the ploidy levels. Some of these reported findings were obtained from field, greenhouse, and/or laboratory conditions. Even though, this present study conducted under laboratory conditions, the obtained results have been compared to both control conditions and the field and greenhouse studies.

The findings of this research demonstrate that these species have some expected resemblance and interesting differences in terms of related traits. For example, in terms of RA, einkorn exhibits a dramatically lower value (75.39°) compared to the other two polyploid species. Tetraploid durum wheat, both landraces (134.94°) and cultivars (127.62°), and hexaploid bread wheat (119.20°) show higher values of RA with no statistical differences between them (Table 5, Figure 2). Hohn and Bektas (2020) report that

the root angle in bread wheat ranges from 28.18° to 111.74°, which closely aligns with our results. Here, durum landraces and cultivars exhibit wider angles than einkorn and bread wheat. This suggests that polyploidization positively affected the root angle in wheat.

For LRL, statistically no differences were observed among the species in this study (Table 5, Figure 2). However, the literature reports differing findings for this trait. For example, Akman et al. (2023) investigated different wheat species in terms of root traits and reported that tetraploid *T. turgidum* L. ssp. *turanicum* Jakubz. had the highest rooting depths, while *T. monococcum* had the lowest. Among ancient wheat species, *T. turgidum* L. ssp. *turanicum* Jakubz. accessions and *T. polonicum* displayed deeper rooting compared to *T. monococcum* accessions and *T. mirabile*. The current findings, together with previous reports, do not provide enough evidence for a precise interpretation of the effect of polyploidization on the LRL.

For SRN, only modern durum wheat differed from the other species, which agrees with findings from a few reports (Table 5, Figure 2). For example, a previous study reported that the seminal root number of *T. turgidum* ssp. *durum* differed significantly from einkorn wheat (Nakhforoosh et al., 2014). In another study, Akman et al. (2023) reported the highest seminal root numbers for *T. durum* among the other species. It seems that SRN may have been affected by polyploidization. Additionally, the B genome may have some superiority in terms of effecting SRN against the A and D genome.

For TRL, significant differences were observed among the species, where modern durum wheat explicitly differed (Table 5, Figure 2). Various findings have been reported for this trait. One study reported that einkorn wheat showed a significantly higher root length compared to the other polyploids (Nakhforoosh et al., 2012). Ayalew et al. (2015) found that the wild tetraploid forms (*T. turgidum* ssp. *dicoccoides* and *T. timopheevi*) showed the shortest roots, while *T.*

monococum exhibited a comparable root length to bread and durum wheat. Additionally, hexaploid wheat and other high polyploid artificial wheat hybrids displayed longer roots. Notably, the wild tetraploids, *T. turgidum* ssp. *dicoccoides* and *T. timopheevi*, showed significantly shorter roots than modern durum wheat and other wheat classes. Furthermore, diploid wheats (*Ae. Squarosa*, *T. monococcum*) genomes showed root lengths comparable to those of bread and durum wheats. Also, another report stated the superiority of *T. durum* against *T. polonicum* (Akman et al., 2023). While the present findings and previous reports show diverse findings among the species, einkorn wheat (A genome donor) consistently exhibits the longest roots compared to other polyploids.

The last traits, coleoptile length (COL), demonstrate significant differences among the species. Here, durum landraces and einkorn wheat exhibit the highest coleoptile length compared to both modern durum and bread wheat (Table 5, Figure 2). There is limited literature evaluating coleoptile length across different ploidy levels. Interestingly, Akman et al. (2023) reported that *T. polonicum* exhibited the longest coleoptile length, followed by *T. turanicum* with the second-highest, while *T. durum* showed the shortest among all species. Notably, in our study, *T. aestivum* had a short coleoptile (4.60 cm) compared to the other species. Einkorn also demonstrated an interesting result (7.22 cm on average), as this was not expected considering its small seed size compared to the larger durum and bread wheat seeds. The accumulation of dwarfing (Rht) genes in modern durum and bread wheat following the Green Revolution explains the expected shorter coleoptiles in these varieties, as Rht genes tend to reduce both plant height and coleoptile length (Sesiz et al., 2024). Consequently, einkorn and emmer wheats, lacking these genes, have unsurprisingly longer coleoptiles compared to modern wheats.

Correlation analysis shed light on the relationships between root traits in the present study. As expected, we found a positive correlation between two pairs of traits: SRN and TRL, and TRL with LRL. This confirms the interconnectedness of these key root architectural features. However, our hopes for a similar link between coleoptile length and the other root traits were not realized. No significant correlations were observed, suggesting that coleoptile length works independently in seedling stage. This independent role underscores the need for separate evaluation of coleoptile length when assessing root architecture and its impact on plant performance.

4. Conclusions

Polyploidization is a biological process that has played a major role in the evolution of flowering plants. However, the effect of polyploidy on coleoptile and root development remains an area for further investigation, especially in wheat. This study, focusing on coleoptile length and root system diversity, has investigated these traits within wheat genetic resources differing in ploidy levels. The results of the study demonstrated that domestication and selection pressures have altered wheat coleoptile length and some root system traits. It is concluded that polyploidization positively affected the root angle and seminal root numbers, negatively affected the total root length and coleoptile length, and had no significant effect on longest root length. We know that seminal root numbers, total root length, and root depth (longest root) facilitate access to soil moisture from deep layers, contributing to high yields in drought environments. In addition, root angle, depending on its wideness, can facilitate nutrient uptake from the upper layer of soil when wider and may protect the plant's root system from seasonal freezing occurring in the rhizosphere layer of the soil, when narrow. Coleoptile length is also important to allow deep sowing and utilizing moisture from deeper soil layers for seed germination with the least dependency on rainfall at the beginning of the season. This has led to the practice of sowing at standard times without any delay. Consequently, polyploidization might have had some beneficial effects on specific

morphological traits, essentially enhancing the wheat's robustness. However, this process also negatively impacted some other morphological traits. This implies that plant breeders still need to integrate wild relatives and modern wheat into their breeding programs to combine all the desirable traits that diverged during the evolutionary process.

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