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Root Trait Variability in Primary Synthetic Wheat Derived from *Aegilops tauschii* Across Diverse Soil Environments and Interactions with Root Endophytes

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Abstract: Modern wheat breeding has largely emphasized aboveground traits, often at the expense of belowground characteristics such as root biomass, architecture, and beneficial microbial associations. This has narrowed genetic diversity, impacting traits essential for stress resilience and efficient nutrient and water acquisition—factors expected to become increasingly critical under climate change. In this study, we evaluated 36 primary synthetic (PS) hexaploid wheat lines developed by crossing *Aegilops tauschii* with the durum wheat cultivar Langdon (LNG) and compared them with LNG and the hexaploid variety Norin 61 (N61). We observed significant variation in root length, biomass, and associations with fungal endophytes, including arbuscular mycorrhizal fungi (AMF), *Serendipita indica*, and *Alternaria*. Clustering analysis based on these traits identified three distinct PS groups: (1) lines with greater root length and biomass, high AMF and *S. indica* colonization, and low *Alternaria* infection; (2) lines with intermediate traits; and (3) lines with reduced root traits and high *Alternaria* susceptibility. Notably, these phenotypic patterns corresponded closely with the soil classification of the *Ae. tauschii* progenitors'' origin, such as Cambisols (supportive of root growth), Gleysols and Calcisols (restrictive of root growth). This highlights the soil microenvironment as a key determinant of belowground trait expression. Our findings demonstrate the potential of wild D-genome diversity, coupled with soil–environment interactions, to restore critical root traits in wheat. Incorporating PS lines with targeted soil and microbial considerations offers a promising strategy for breeding resilient cultivars with enhanced root systems.

Keywords: genetic diversity; root length; root biomass; Arbuscular Mycorrhizal Fungi (AMF); *Serendipita indica; Alternaria*; soil classification

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1. Introduction

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The need for sustainable wheat production has become increasingly urgent as climate change—manifesting as heat stress, drought, and waterlogging—continues to threaten global wheat yields and quality [1]. Addressing these challenges requires a transition toward integrated agricultural strategies that enhance resilience to both biotic and abiotic stresses while reducing environmental impacts [2]. Wheat's adaptability, global distribution, and high productivity have established it as a mainstay of global agriculture. However, domestication and modern breeding efforts have largely focused on aboveground traits such as grain yield and disease resistance, inadvertently narrowing genetic diversity, especially in belowground characteristics [3]. These root traits—including root length, diameter, biomass, and key plant–microbe interactions that support water and nutrient uptake—are essential for drought resilience and overall plant performance [4,5]. Every phase of root initiation, growth, and development is genetically regulated and shaped by diverse abiotic and biotic factors [6]. Additionally, root-associated microbial interactions, particularly with beneficial endophytes such as Arbuscular Mycorrhizal Fungi (AMF) and *Serendipita indica (S. indica*), play a pivotal role in enhancing stress tolerance, disease resistance, and plant growth [7,8]. The decline in these critical root-associated traits poses a growing concern for sustainable wheat production, especially under escalating climate pressures. Wild relatives of wheat, such as *Aegilops tauschii* (the D-genome donor of bread wheat), represent a valuable reservoir of genetic diversity, offering potential to improve wheat resilience and yield under both abiotic and biotic stresses. Studies have shown that the offspring of hexaploid wheat, including primary synthetic (PS) and multiple synthetic derivatives (MSD), exhibit enhanced stress tolerance and disease resistance compared to domesticated varieties [9–11]. However, research on root-associated traits in *Ae. tauschii* and its hybrids remains limited, especially relative to the extensive focus on aboveground traits. *Ae. tauschii* is predominantly distributed across, the Caspian Sea region, and parts of Central and South Asia—areas characterized by diverse soil types [12,13]. Soils in these regions, including Cambisols and Gleysols, exhibit distinct properties that influence both root development and microbial interactions [14]. Accessions of *Ae. tauschii* have demonstrated strong root systems, increased associations with AMF, and resistance to *Alternaria* infections [15]. Furthermore, our previous study [16] identified significant variation in root traits among *Ae. tauschii* accessions, with this diploid ancestor showing superior root traits (including enhanced AMF colonization) compared to cultivated tetraploid and hexaploid wheat. However, as *Ae. tauschii* is phenotypically distinct, these advantageous traits may not fully express in hexaploid wheat. To bridge this gap, we analyzed Primary Synthetic wheat (PS) lines derived from diverse *Ae. tauschii* accessions across diverse soil environmnts. These wild-derived root traits and their genetic regulation offer promising targets for improving belowground resilience and wheat performance [15,16]. By integrating root traits with microbial associations (AMF, S. indica) and aboveground selection [17,18], we aim to (1) quantify root trait variation in PS lines, (2) assess their interactions with beneficial (AMF/S. indica) and pathogenic (Alternaria) fungi relative to their *Ae. tauschii* soils classification, and (3) determine trait inheritance patterns to inform future breeding strategies.

2. Materials and Methods

2.1. Plant Materials and Experimental Conditions

We used 36 synthetic wheat lines (hexaploid, AABBDD) produced by crosses between a single durum wheat cultivar (*Triticum turgidum* var. *durum* cv. Langdon (LNG), tetraploid, AABB) and 36 accessions of *Ae. tauschii* (diploid, DD, wild species) originating from diverse regions with varied environmental conditions. The soil classification of collected *Ae. tauschii* accessions was determined by georeferencing coordinates against the Soil Atlas of Asia [13], with additional verification for Iranian accessions using FAO's national fertilizer report [17]. These accessions represented three distinct lineages of *Ae. tauschii* (TauL1, TauL2, and TauL3). LNG and a bread wheat cultivar (*Triticum aestivum* cv. Norin 61 (N61), hexaploid, AABBDD) were used as check cultivars (Table S1). Information related to the PS lines and their *Ae. tauschii* accession, including passport data source, origin, province, soil classification, reference for soil classification, and lineage, is presented in

Supplementary Table S1. The selected lines span a wide range of geographical regions, soil types, and climatic zones. Among the 36 PS lines, 14 belong to lineage 1 (TauL1), 18 to lineage 2 (TauL2), and 4 to lineage 3 (TauL3). Their diverse geographic origins and genetic backgrounds make these lines valuable material for this study.

The experiment was conducted in a greenhouse at the Arid Land Research Center (ALRC), Tottori University, Japan (35°32′04.5″N, 134°12′42.0″E), from 1 November 2023 to 6 March 2024. Temperature and relative humidity were recorded using a datalogger (SK-L754; Sato Keiryoki Mfg. Co. Ltd., Tokyo, Japan) (Figure S1). Daily temperatures ranged from 2°C to 26°C, with average daytime and nighttime temperatures of 16°C and 10°C, respectively. Relative humidity fluctuated between 40% and 90%, averaging 66% during the day and 60% at night.

2.2. Plant Growth and Experiment Setup

Seeds were sown in tall pots (60 cm high, 10 cm wide) designed for Glycyrrhiza cultivation, filled with approximately 4000 cm^3 of sandy soil sourced from the ALRC wheat field. Initial soil analysis revealed low fungal levels (10^3 CFU/g soil) and a low AMF spore count (5.1/g). To amend these limitations, we prepared a mixture of crude inoculum extracted from the same wheat field soil (containing 10^6 fungal CFU/ml) and a commercial AMF product, Mycogel (Agrocode Bioscience™, Roquetas de Mar, Almeria, Spain), containing *Rhizophagus irregularis* strain at 5×10^3 spores/ml [18]. Twelve days after germination, 1 ml of this mixture was inoculated into the rhizosphere to facilitate interaction and colonization of root cortical cells by AMF and root-associated endophytic fungi. The experiment was laid out in a randomized complete block design with six replications. Pots were irrigated every three days, and no fertilizers, insecticides, or fungicides were applied.

2.3. Harvest and Data Collection

After three months of growth, all plants were carefully removed from the pots. Shoots and root systems were separated, and roots were thoroughly rinsed with tap water. Several traits were assessed, including root, shoot, and biotic characteristics.

2.3.1. Shoot Traits

The shoot systems from six replicates were weighed and then dried at 68°C for two days in a constant-temperature drying oven to determine fresh and dry weights, as well as tissue water content.

2.3.2. Root Traits

As architectural traits of roots determine the spatial configuration of the root system; root length and weight were measured. Harvested roots were digitally scanned using a desktop scanner (Perfection V500 Photo; Epson, Japan) and analyzed with WinRhizo Pro 2008a software (Regent Instruments Inc., Quebec City, QC, Canada) to determine total root length. Roots were classified into two diameter categories: thin (0–0.5 mm) and thick (0.5–1.0 mm) for subsequent analysis (Figure S2). For biotic trait analysis, 60–65 root segments of 0.5 cm each (totaling 30 cm) were randomly selected from each wheat root. The remaining roots were processed similarly to shoots to assess fresh and dry weights and tissue water content.

2.3.3. Root Biotic Traits

Biotic traits, including colonization by AMF and *S. indica* and *Alternaria* infection, were evaluated following the method of [19]. Root fragments were disinfected with 70% ethanol, soaked in 10% KOH overnight, rinsed with distilled water, acidified in 5% HCl for 3 min, and stained overnight with trypan blue (500 mL glycerol, 475 mL distilled water, 25 mL acetic acid, 0.1 g trypan blue). After rinsing with acidified water, samples were observed under a digital microscope (VHX-7000; Keyence, Osaka, Japan). Colonization levels were quantified using the gridline intersect method [20]. The three fungi were identified based on their distinct morphological structures within the root (Table S6, Figure S5–S10).

2.4. Statistical Analysis

Data were tested for normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests. Welch's one-way analysis of variance was performed, followed by Tukey's honestly significant difference (HSD) test for mean comparisons. Pearson's correlation analysis was conducted to evaluate relationships among root traits. The statistical analyses were conducted using SPSS v.29 (IBM SPSS Statistics, 2024). Additionally, a hierarchical clustering heatmap and a biplot principal component analysis (PCA) were generated to classify PS lines based on trait similarities. Hierarchical clustering heatmaps and biplot PCA were created using R Statistical Software (v.4.4.1; R Core Team, 2024).

3. Results

3.1. Variation of Traits Among Wheat PS Lines

3.1.1. Root Traits

Total root length (cm), including both thin and thick root diameter classes, varied significantly across the wheat accessions studied ($p < 0.05$) Figure 1A. Thirty-one out of the 36 PS lines exhibited longer root lengths than LNG and N61, with PS63 showing the longest root system (6209 cm). Among the remaining lines, PS39 had a root length of 2944 cm, comparable to LNG (2906 cm), and both were significantly shorter than the first 31 PS lines. PS48 had a root length of 2679 cm, comparable to N61 (2609 cm). The shortest root lengths were recorded in PS30, PS29, and PS54 (2434 cm, 2430 cm, and 1949 cm, respectively) Figure 1A.

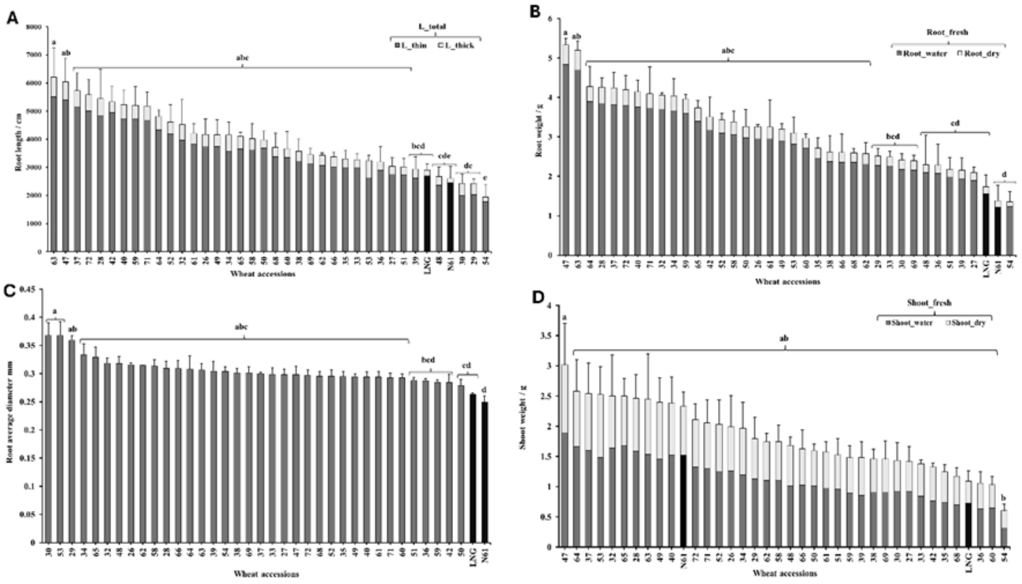


Figure 1. Root and shoot traits of 38 wheat accessions, including 36 primary synthetics (PS), Langdon, and Norin 61. (A) Total root length (cm), subdivided into thin and thick root diameter classes. (B) Root fresh weight (g), with subcategories of dry weight and water content. (C) Average root diameter (mm). (D) Shoot fresh weight (g), with subcategories of dry weight and water content (g). L_total, total root length (cm); L_thin, thin roots (0 > 0.5 mm diameter); L_thick, thick roots (0.5-1 mm diameter). Bars sharing the same letter are not significantly different (Tukey's Honestly Significant Difference [HSD] test, *p*< 0.01).

Root dry weight and water content also varied significantly among the studied wheat lines ($p < 0.05$) Figure 1B. Twenty-six PS lines exhibited significantly higher root weights than the remaining 10 lines, and LNG and N61. Among them PS47 and PS63 had the highest root dry weights of 0.498 g and 0.506 g, and water contents of 4.83 g and 4.688 g, respectively.

Regarding average root diameter, all PS lines showed significantly greater diameters than LNG, except PS 51, 36, 59, 42, and 50, which had values comparable to LNG Figure 1C. N61 displayed the smallest average root diameter among all accessions.

3.1.2. Shoot Traits

Shoot dry weight and water content were comparable across all PS lines, N61, and LNG. PS47 recorded the highest shoot dry weight (1.13 g) and water content (1.89 g). Only PS54 showed significantly lower shoot weight and water content compared to PS47, with values of 0.29 g and 0.31 g, respectively Figure 1D.

3.1.3. Biotic Traits

Biotic traits, including root colonization by beneficial fungi (AMF and *S. indica*) and pathogenic fungi (*Alternaria*), varied significantly among all wheat accessions (Figure 2). AMF colonization exhibited notable variation and allowed classification of the accessions into 11 distinct groups based on colonization levels. All PS lines demonstrated significantly higher AMF colonization than LNG and N61, which had the lowest colonization rates at 25% and 18%, respectively Figure 2A. Among the 36 PS lines, PS37, PS63, and PS42 exhibited the highest AMF colonization rates (>60%). In contrast, colonization levels gradually declined below 30% in N61 and LNG, marking them as the least colonized accessions.

S. indica colonization was lower than that of AMF but showed significant variation among all wheat accessions. The highest *S. indica* colonization was observed in three PS lines (63, 37, and 72), with colonization rates exceeding 25%. Similar to AMF, *S. indica* colonization gradually declined across the PS lines, reaching the lowest levels in PS 50 and LNG (12.5% and 11.6%, respectively). N61 exhibited an intermediate colonization rate of 16% Figure 2B.

N61 exhibited the highest infection rate of *Alternaria* (32%) among all 38 wheat accessions, while LNG had the lowest (11%). Among the PS lines, 19 lines (PS51, PS54, PS48, PS36, PS68, PS69, PS27, PS50, PS30, PS39, PS60, PS66, PS29, PS38, PS33, PS35, PS49, PS52, and PS59) showed higher infection rates, ranging from 24% to 17%. Infection levels gradually declined among the remaining PS lines (16%–12%), with the lowest infection among PS lines observed in PS42 (11.75%) Figure 2C.

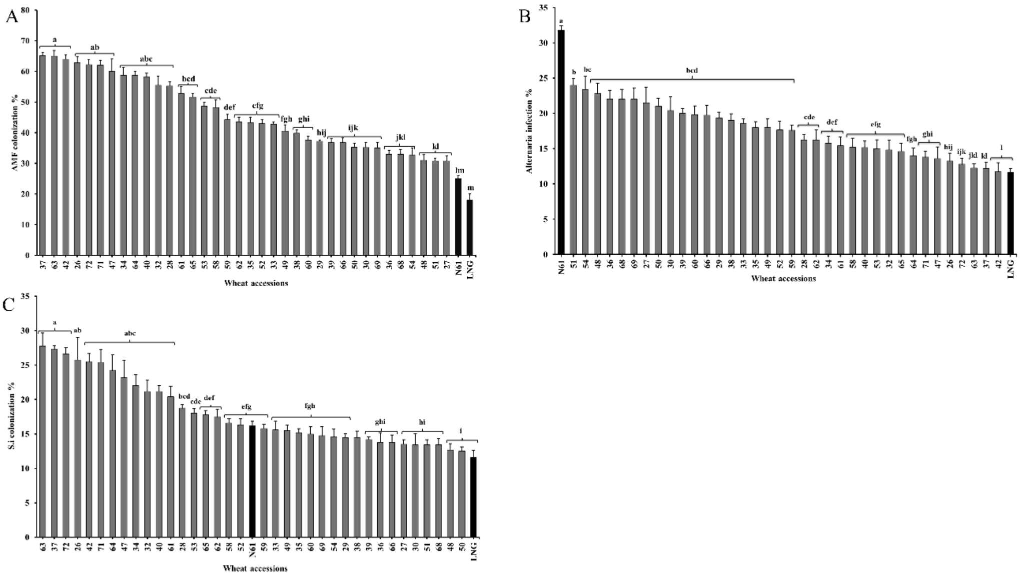
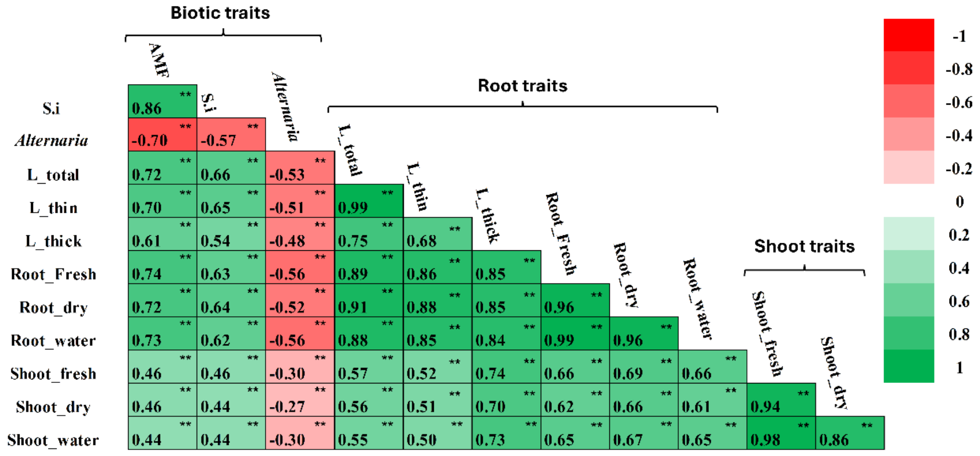


Figure 2. Biotic traits of 38 wheat accessions, including 36 primary synthetics (PS), Langdon, and Norin 61. (A) Arbuscular mycorrhizal fungi (AMF) colonization % (B) Serendipita indica (S.i) colonization %. (C) Alternaria infection (%). Bars sharing the same letter are not significantly different (Tukey's honestly significant difference (HSD) test, $p < 0.01$).

3.1.4. Association Among Wheat Accession Traits

All wheat accession traits (root, shoot, and biotic) exhibited highly significant positive correlations with one another, except for Alternaria infection, which showed a highly significant negative correlation with all other traits, including beneficial endophytic fungi (AMF and S. indica) Figure 3. Shoot fresh and dry weights were most strongly correlated with thick root length, which plays a crucial role in water uptake for the shoot system.



	AMF	S.i	*Alternaria*	L_total	L_thin	L_thick	Root_Fresh	Root_dry	Root_water	Shoot_fresh	Shoot_dry
S.i	0.86 **										
Alternaria	-0.70 **	-0.57 **									
L_total	0.72 **	0.66 **	-0.53 **								
L_thin	0.70 **	0.65 **	-0.51 **	0.99 **							
L_thick	0.61 **	0.54 **	-0.48 **	0.75 **	0.68 **						
Root_Fresh	0.74 **	0.63 **	-0.56 **	0.89 **	0.86 **	0.85 **					
Root_dry	0.72 **	0.64 **	-0.52 **	0.91 **	0.88 **	0.85 **	0.96 **				
Root_water	0.73 **	0.62 **	-0.56 **	0.88 **	0.85 **	0.84 **	0.99 **	0.96 **			
Shoot_fresh	0.46 **	0.46 **	-0.30 **	0.57 **	0.52 **	0.74 **	0.66 **	0.69 **	0.66 **		
Shoot_dry	0.46 **	0.44 **	-0.27 **	0.56 **	0.51 **	0.70 **	0.62 **	0.66 **	0.61 **	0.94 **	
Shoot_water	0.44 **	0.44 **	-0.30 **	0.55 **	0.50 **	0.73 **	0.65 **	0.67 **	0.65 **	0.98 **	0.86 **

Figure 3. Pearson's correlation coefficients among root traits, shoot traits, and biotic traits (endophytic fungi colonization). Significance levels: $^*p < 0.05$, $^{**}p < 0.01$. AMF: arbuscular mycorrhizal fungi colonization (%); S.i: Serendipita indica colonization (%); Alternaria: Alternaria colonization (%), L_total: total root length (cm), L_thin: thin root (0 - 0.5 mm diameter) length (cm), L_thick: thick root (0.5-1.0 mm diameter) length (cm).

3.2. Wheat Accession Grouping and Traits Association

Hierarchical clustering heatmap of root traits revealed four distinct groups among the wheat accessions Figure 4. (group 1) comprised six PS lines exhibiting the highest root and shoot traits, the strongest AMF colonization, and the lowest *Alternaria* infection. Notably, all group 1 PS lines were derived from *Ae. tauschii* accessions originating exclusively from Cambisols. (group 2) included 13 PS lines with intermediate trait values. Their *Ae. tauschii* progenitor accessions were derived from diverse soil classes: three from Gleysols; two each from Cambisols, Calcisols, and Lithosols; and one each from Xerosols and Vertisols, along with two accessions from unclassified (NA) soils. (group 3) consisted of 17 PS lines along with their parent LNG. This group was characterized by lower root and shoot trait values and the highest *Alternaria* infection among PS lines. Their *Ae. tauschii* progenitors soil classification showed six accessions from Gleysols, five from Calcisols, two from Cambisols, and one each from Xerosols and Fluvisols, with three unclassified (NA) accessions. (group 4) included only accession N61, which displayed the most extreme phenotypic divergence. It showed minimal root development, sustained shoot growth, and the highest level of *Alternaria* infection observed in the study Figure 4.

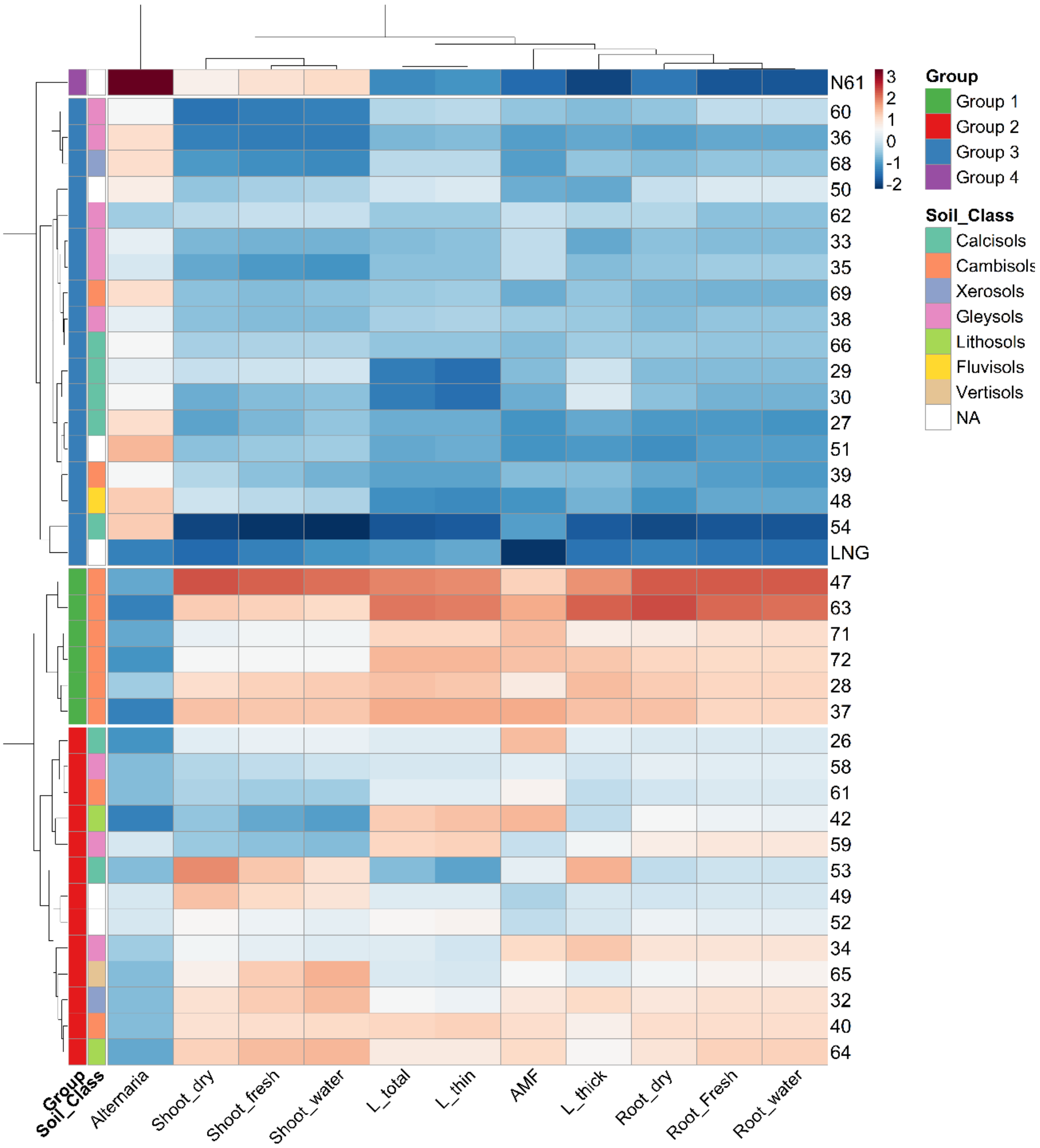


Figure 4. Hierarchical clustering heatmap based on 11 standardized traits values across 38 wheat accessions, including 36 primary synthetics (PS), Langdon and Norin 61. Group shows the four accession groups classified by the clustering analysis. Soil Class shows the soil classes determined from GPS coordination of Ae. tauschii progenitor's origin (NA: unknown soil class because GPS data was not found). AMF: arbuscular mycorrhizal fungi colonization (%), Alternari: Alternaria, colonization (%), L_total: total root length (cm), L_thin: thin root (0- 0.5 mm) length (cm), L_thick: thick root (0.5-1.0 mm) length (cm), Root_Fresh: root fresh weight (g), Root_Dry: root dry weight (g), Root_water: root water content (g), Shoot_fresh: shoot fresh weight (g), Shoot_dry: shoot dry weight (g), Shoot_water: shoot water content (g).

PCA biplot explained 91% of the total variance (PC1: 78.97%, PC2: 12.1%) Figure 5. The result showed similar classification into groups with hierarchical clustering heatmap shown in Figure 4. PS lines were separated into three groups, with their progenitor LNG. N61 formed the fourth group. Notably, some overlap was observed between Groups 1 and 2, primarily due to similarities in root traits. PS54, although classified within Group 3 in Figure 4, deviated even more than its LNG progenitor, from group 3, driven by its exceptionally low root and shoot traits and high *Alternaria* infection Figure 5.

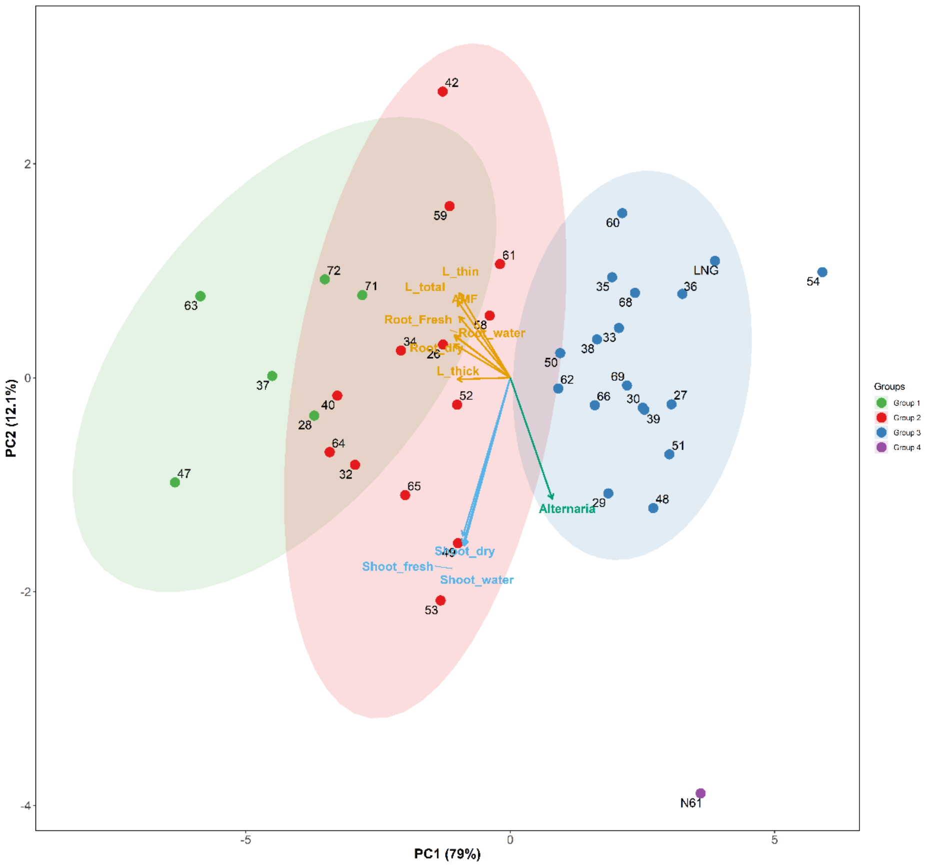


Figure 5. Biplot principal component analysis (PCA) based on the same dataset of heatmap in Figure 4. Showing four accessions groups found in Figure 4. Arrows show the correlations of each root trait with PC1 and PC2.

4. Discussion

Root phenomics is crucial in crop breeding; among key root traits, root length and biomass are vital for water and nutrient uptake [21]. Root traits such as increased root length, variation in thin and thick root diameters, and greater biomass enhance wheat performance, particularly under stress, by improving water uptake, boosting nutrient acquisition, and fostering beneficial microbial interactions [4,5,22]. Breeding strategies that target these traits can enhance drought resilience and nutrient efficiency, ultimately increasing yields in challenging environments [6,23,24]. This study identified significant variation in root traits-length, diameter, and biomass—as well as in biotic interactions with endophytic fungi (AMF, *S. indica*, and *Alternaria*) among 36 PS wheat lines derived from 36 *Ae. tauschii* accessions and LNG. The considerable variability in wheat root traits observed in this study, especially among the 36 PS lines (Figure 1A, 1B–and 1C), underscores the influential role of the wild D-genome from *Ae. tauschii* in shaping root architecture. The improved root traits in certain PS lines are likely inherited from *Ae. tauschii* [16]. Conversely, reduced root traits in some PS lines may reflect the influence of the AB genome from LNG, which has undergone domestication-related selection focused primarily on aboveground traits [25]. Previous research has highlighted the critical role of the D-genome in root trait development. For instance, [15] identified *TaLBD16-D* as a key gene regulating lateral root number, with the D-genome making notable contributions in both diploid and hexaploid wheat compared to the tetraploid AB genome. In our earlier study [16], *Ae. tauschii* accessions KU-2109, KU-2074, KU-2136, IG 126387, AT76, and IG 47259 consistently demonstrated superior root traits compared to LNG and N61. In the current study, their corresponding PS lines PS64, PS33, PS66, PS29, PS51, and PS54 retained many of these advantages, though they did not include the top-performing PS lines overall. Notably, all lines except PS29 and PS54 showed greater root length than LNG and N61, and only line PS54 exhibited a root dry weight similar to N61 and lower than LNG. Average root diameter was consistently higher across all six lines relative to LNG and N61. These findings illustrate the inheritance of beneficial root traits from *Ae. tauschii* progenitors and highlight their valuable contribution to root trait enhancement in synthetic hexaploid wheat.

Biotic traits variability among PS lines: AMF, *S. indica*, and *Alternaria* resistance

Beneficial AMF and *S. indica* have been shown to enhance wheat performance by improving nutrient uptake, promoting root growth, increasing drought tolerance, and strengthening resistance to biotic stresses such as fungal pathogens [26–28]. In contrast, *Alternaria* represents a major biotic stressor in wheat, contributing to root rot and post-harvest spoilage [7]. Investigating these root endophytic fungi holds significant promise for advancing sustainable wheat production, particularly under biotic and abiotic stress conditions. In this study, as with root traits, the biotic traits, including both beneficial and pathogenic root endophytic fungi, varied significantly among wheat accessions and across the 36 PS lines. All PS lines exhibited significantly higher colonization by AMF compared to LNG and N61, supporting the same hypothesis as for root traits: that the wild D-genome of *Ae. tauschii* may contribute to promoting beneficial fungal associations. [29] proposed that AMF symbiosis-related genes in wild ancestors can be transmitted without major disruption from AB genome introgression.

Resistance to *Alternaria* colonization also varied across the PS lines. While some PS lines retained strong resistance, others exhibited increased susceptibility; nevertheless, all PS lines showed lower *Alternaria* colonization than the domesticated hexaploid N61, closely resembling their progenitor *Ae. Tauschii,* as reported in our previous study [16]. At the same time, all PS lines exhibited significantly higher *Alternaria* colonization than their domesticated progenitor LNG, which appeared to suppress both beneficial and pathogenic fungal associations, a finding consistent with our earlier observations in *Ae. tauschii* [16]. In our prior study, *Ae. tauschii* accessions KU-2109, KU-2074, KU-2136, IG 126387, AT76, and IG 47259 consistently demonstrated higher AMF colonization and lower Alternaria infection compared to LNG and N61. In the current study, we assessed their derived PS lines-PS64, PS33, PS66, PS29, PS51, and PS54—which displayed intermediate biotic traits. Although not the top-performing PS lines overall, they still outperformed LNG and N61 by exhibiting higher AMF colonization and reduced *Alternaria* infection, reflecting the trends seen in their wild progenitors.

Root-biotic trait associations

The strong positive correlation observed between root traits and beneficial biotic traits (AMF and *S. indica*) (Figure 3) underscores the interconnected nature of plant–microbe interactions. Enhanced root length and biomass likely facilitated the recruitment of beneficial fungi, reinforcing symbiotic associations [30] and contributing to the suppression of *Alternaria*. This is further supported by the negative correlation between *Alternaria* and both root traits and beneficial fungi, aligning with our previous study [16], which focused exclusively on the two progenitors of the PS lines (*Ae. tauschii* and LNG).

Soil classification affects wheat root traits grouping

Significant variability in root traits, AMF colonization, and *Alternaria* susceptibility among wheat accessions and PS lines was captured through hierarchical clustering and PCA, consistently resolving four distinct groups (Figures 4 and 5). Group 1, comprising six PS lines, exhibited superior root and shoot traits, stronger AMF colonization, and reduced *Alternaria* infection, marking them as valuable germplasm for improving belowground traits. PS47 and PS63, derived from *Ae. tauschii* accessions KU-2159 and KU-2103 (Iran), were among the top performers.

Remarkably, Group 1 PS lines originated from all three *Ae. tauschii* lineages and diverse geographic regions but shared association with Cambisols—well-drained, moderately weathered soils favorable for root growth [13,17,31]. This convergence suggests that soil type, rather than lineage or climatic region, exerts a dominant influence on belowground phenotypes. Unlike climate, soil acts as the direct interface for belowground development, which helps explain the variation observed among PS lines originating from similar regions but differing soil types, contrasting to [12] who found aboveground traits more influenced by climatic region and lineages.

In contrast, PS lines in Groups 2 and 3—despite similar lineage and regional origins—were primarily linked to Gleysols and Calcisols. These soils are known to restrict root development due to waterlogging and high $CaCO_3$ content, respectively. Gleysols reduce root elongation [14], while Calcisols form cemented pans and chemically suppress root hair and AMF development [32–34]. These edaphic limitations were reflected in reduced root biomass and higher *Alternaria* susceptibility.

Altogether, these findings highlight soil type—particularly Cambisols—as a critical and underappreciated factor influencing root performance. Incorporating soil origin into *Ae. tauschii* donor selection may enhance breeding strategies targeting belowground resilience.

Impact of domesticated LNG on PS lines

Beyond soil and genetic diversity within *Ae. tauschii* lineages, the hybridization effect from the second progenitor, the domesticated LNG, likely contributes to trait variability among PS lines. This is supported by the consistently lower root traits and weaker fungal associations observed in both the current and previous study [16], highlighting the presence of admixed PS lines.

A key distinction between the hexaploid PS lines and the domesticated hexaploid N61 lies in the origin of their D-genome. While N61's domesticated DD genome has undergone selection pressures that may have reduced investment in belowground traits, the PS lines retain a wild DD genome, preserving the genetic potential for enhanced root development and beneficial biotic interactions [29,35,36]. This highlights the critical importance of conserving wild germplasm to improve wheat resilience to both abiotic and biotic stresses.

Limitations and future directions

A primary limitation of this study is the restricted number of PS wheat lines derived from *Ae. tauschii.* Although these lines represent various geographical regions and taxonomical lineages, they cover only a small fraction of the extensive *Ae. tauschii* germplasm available. Additionally, all PS lines were developed using the AB genome background from LNG, which may not fully capture the diversity of the A and B genomes found in wheat. Future directions include utilizing Multiple synthetic derivatives (MSDs), created by crossing PS lines with elite hexaploid wheat, as a powerful platform for tracking trait inheritance in breeding populations. The application of Genome-Wide Association Study could further identify genes linked to beneficial root traits. Lastly, exploring how soil types and microbial communities influence root development can inform the strategic deployment of cultivars across diverse global environments.

5. Conclusions

This study highlights substantial variation in root traits and biotic interactions among 36 PS wheat lines, shaped by the genomic interplay between their two progenitors: the wild *Ae. tauschii* and the domesticated LNG. The presence of enhanced root traits and beneficial fungal associations in several PS lines indicates a strong influence from the *Ae. tauschii* genome, whereas reduced traits in other lines reflect domestication-related effects inherited from LNG. Trait expression was further modulated by soil type, with Cambisols supporting superior root development and microbial colonization compared to Gleysols. These findings underscore the complex genetic and environmental interactions that govern belowground traits in wheat. The clustering of PS lines into three distinct phenotypic groups offers a practical framework for selecting genotypes based on root traits and endophytic fungal associations. From Group 1, six PS lines—47, 63, 71, 72, 28, and 37—are recommended for future exploration in wheat breeding programs due to their superior performance. Leveraging wild genetic diversity through the targeted use of PS lines, while accounting for soil environments and microbial interactions, presents a promising path toward developing resilient wheat cultivars optimized for root performance and stress tolerance.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org; 1- Mohammedali et al., Supplementary tables excel sheet containing: **Table S1:** Information of Aegilops tauschii accessions used for production of 36 primary syntetic (PS) wheat lines. **Table S2:** Root , shoot and biotic traits; and their statistical significance of differences among the 38 wheat accessions

(36 P.S lines, LNG and N61). **Table S3:** Eigenvalues for the screeplot Fig S2 and Biplot PCA Fig 5. **Table S4:** Wheat accessions scores for Biplot PCA Fig 5. **Table S5:** Traits loading for Biplot PCA Fig 5. 2- Mohammedali et al., Supplementary materials PDF containing: **Figure S1:** Daily average temperature and relative humidity in greenhouse during the experiment (March 30th to June 30th, 2023). **Figure S2:** Screeplot for the percentage of explained variance for the dimensions of the biplot PCA in figure 5. **Figure S3:** scanned Images with Epson scanner for WinRhizo analysis of wheat accessions root system from both studies demonstrates the variation of the root systems among P.S accessions, and both their parents Aegilops tauschii and Langdon compared to the control Norin61. **Figure S4:** Image of scanned root fragment illustrating the subcategory classes of the root according to the diameter: Thin root 0 > 0.5 mm and thick root 0.5-1.0 mm. **Figure S5:** Arbuscules of Mycorrhizal Fungi structure inside wheat root cortical cells: Visualized by Keyence VHX digital microscope. **Figure S6:** Spores of Arbuscular Mycorrhizal Fungi structure inside wheat root cortical cells: Visualized by Keyence VHX digital microscope. **Figure S7:** Vesicles of Arbuscular Mycorrhizal Fungi structure inside wheat root cortical cells: Visualized by Keyence VHX digital microscope. **Figure S8:** Inter and intracellular Hyphae and Vesicles of *Serendipita indica* Fungi structure inside wheat root cortical cells: Visualized by Keyence VHX digital microscope. **Figure S9:** Spores of *Serendipita indica* Fungi structure inside wheat root cortical cells: Visualized by Keyence VHX digital microscope. **Figure S10:** *Alternaria spp.* Fungi structure inside wheat root cortical cells: Visualized by Keyence VHX digital microscope. **Table S6:** features and structures of root endophytic fungi: AMF (*Rhizophagus irregularis*), *Serendipita indica* and *Alternaria sp.* inside wheat root cells.

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