#### Root Architectural Diversity in Wheat Cultivars and Its Role in Response to Terminal Water Stress

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Afshin Zamani<sup>1</sup>, Yahya Emam<sup>1\*</sup>, and Aref Nowrouzi<sup>1</sup>

#### 2 Abstract

3 Root architecture plays a key role in optimizing water uptake and sustaining yield under drought stress. The aim of this study was to investigate the role of vertical root distribution in determining 4 the performance of three irrigated wheat cultivars under terminal water stress (TWS). A field 5 experiment was conducted during the 2021-2022 growing season at Shiraz University (School of 6 7 Agriculture), evaluating three bread wheat cultivars: Pishgam, Torabi, and Sirvan. The low-cost "pasta strainer" method, originally developed for evaluating rice root systems using plastic baskets, 8 was adapted and employed to assess wheat mature root architecture in this context. To assess 9 vertical root distribution, roots protruding through the side perforations of strainers were counted 10 at three defined depth intervals: shallow (2-8 cm; SRN), middle (8-10 cm; MRN), and deep (10-11 13 cm; DRN). The results revealed significant differences in root architecture among cultivars. 12 Compared to Sirvan and Torabi, Pishgam exhibited a more uniform root distribution across SRN, 13 MRN, and particularly DRN. A significant interaction between irrigation and cultivar was 14 observed for root weight, root volume, shoot biomass, and grain yield (GY). Pishgam appeared to 15 be superior in most of measured traits. GY showed a positive association with MRN and DRN. 16 which was further supported by principal component analysis aligning GY with root weight, root 17 volume, MRN, and DRN. Despite the advantages and limitations of the pasta strainer method, our 18 19 results demonstrated that cultivar with deeper rooting type, exhibited better drought performance. 20 Keywords: Pasta strainer, Root phenotyping, Root architecture, Mature root, Vertical root 21 distribution.

#### 1 Introduction

Bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is the most important cereal crop with significant increase in contribution of human food particularly in providing both calories and protein (Amini et al., 2023; Arzani & Ashraf, 2017). With widespread cultivation across more than

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<sup>&</sup>lt;sup>1</sup> Department of Plant Production and Genetics, School of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.

<sup>\*</sup> Corresponding author; e-mail: yaemam@shirazu.ac.ir or yaemam@gmail.com

219 million hectares, wheat contributes significantly to global agricultural production. Annually, 28 wheat production exceeds 800 million tons of grain (FAO, 2024), making it a critical staple crop 29 for sustaining populations worldwide. Climate change, particularly drought, reduces wheat 30 production by limiting water availability, impairing growth, and decreasing yield potential. Rising 31 temperatures and erratic rainfall further stress crops, leading to shorter growing seasons, lower 32 grain quality, and reduced productivity (Pirnajmedin et al., 2024).

Drought, one of the foremost abiotic stresses, poses a considerable challenge to crop production, 33 34 including wheat, and is further exacerbated by the depletion of water resources and climate change 35 (Haghpanah et al., 2024). The severity, duration, and timing of drought stress occurrence during the growth stages of wheat can significantly reduce productivity, with potential losses ranging 36 37 from 40% to 92% (Mahdavi et al., 2023; Senapati et al., 2019; Zamani et al., 2024; Zampieri et al., 2017). The primary effects of drought stress include dehydration, osmotic imbalance, 38 dysfunction of endosomes and plasma membranes, loss of cellular turgor, inhibition of metabolite 39 synthesis, depletion of cellular energy, oxidative stress, nutrient imbalance, reducing starch and 40 41 protein content, and impaired photosynthesis (Haghpanah et al., 2024; Mokhtari et al., 2025). Water deficits manifest after the flowering stage, a phenomenon known as terminal drought, 42 characterized by a decrease in precipitation and an increase in evaporation rates (Turner & Nicolas, 43 1987). However, the development of a profound water deficit can be mitigated by the presence of 44 a deeper root system, enabling the wheat plants to access water stored at deeper soil layers, even 45 during terminal drought stress (Figueroa-bustos et al., 2020). Khodaee et al. (2021) reported that 46 47 the most root characteristics are under genetic control and significant associations were found for the deep rooting component. They identified candidate loci having significant effect on different 48 aspects of the studied population particularly the deep rooting system and the root development 49 50 under water-stress environment.

51 While both above and belowground traits play critical roles in plant responses to drought, most 52 studies have primarily focused on aboveground traits, neglecting the importance of underground 53 characteristics (Asefa et al., 2022). This oversight persists because root studies are inherently 54 challenging due to the hidden nature of roots, soil heterogeneity, difficulties in non-destructive 55 sampling, and the complex interactions between roots and their surrounding environment 56 (Pirnajmedin et al., 2015, 2016). Therefore, it is crucial to shift attention towards investigating and 57 comprehending the belowground characteristics of wheat plants.

Most studies on terminal drought tolerance in wheat have focused on evaluating morphological 58 59 and physiological traits (Haghshenas et al., 2021; M. Mohammadi et al., 2021; Mokhtari et al., 2024, 2025; Raherison et al., 2020; Zamani et al., 2024). While these traits serve as important 60 indicators for drought selection, a holistic understanding requires deeper exploration of root-61 62 system dynamics. By elucidating the intricate interplay between above- and belowground traits, we can unravel the plant's full adaptive mechanisms to drought stress. This integrated approach 63 will enable the development of more effective breeding strategies to enhance drought resistance in 64 wheat cultivars. Roots, as one of the primary organs of plants, play a pivotal role in detecting soil 65 66 dehydration cues, highlighting the critical importance of elucidating optimal root system architecture, particularly with respect to its capacity for deep soil penetration and moisture 67 68 extraction (X. Li et al., 2019). The responsiveness of the subterranean organ, especially under 69 conditions of limited soil moisture, is fundamental for efficient water uptake and sustained 70 physiological activity (Ranjan et al., 2022). Modeling studies have demonstrated that for each 71 additional millimeter of soil water extracted by wheat roots after anthesis, grain yield may increase 72 by approximately 55 kg per hectare (Manschadi et al., 2006), underscoring the substantial contribution of root traits to yield formation, particularly in environments where evaporative 73 demand often exceeds precipitation during grain filling. These observations emphasize the need 74 75 for comprehensive investigations into the functional relationships between root architectural traits and drought tolerance mechanisms. 76

The direct selection of root traits in breeding programs remains challenging, primarily due to the 77 high costs, labor intensity, and time-consuming nature of root system studies. To overcome these 78 79 limitations, several studies have explored root system architecture using diverse phenotyping 80 approaches. These include small-scale controlled-environment studies (Basirat et al., 2019; Chen 81 et al., 2020; Gioia et al., 2017; Hosseini et al., 2024; Khodaee et al., 2021; Nagel et al., 2020; 82 Richard et al., 2015), field-based root phenotyping (Crocker et al., 2003; Fradgley et al., 2020; Maccaferri et al., 2016; Mehrabi et al., 2021; A. Wasson et al., 2016), as well as indirect root trait 83 84 assessment through remote sensing technologies (Deery et al., 2019; Gutierrez et al., 2010; X. Li et al., 2019; Pinto & Reynolds, 2015). Additionally, many of the existing root phenotyping 85 86 techniques lack sufficient accuracy and reproducibility (El Hassouni et al., 2018; Ober et al., 2021; A. P. Wasson et al., 2012). To address these limitations, we employed the "pasta strainer (basket)" 87 method in our field experiment. This technique has previously been validated as a robust and 88

89 practical approach for capturing genotypic variation in root system architecture, as demonstrated 90 in both rice (Uga et al., 2009) and durum wheat under controlled conditions (El Hassouni et al., 91 2018), with significant implications for agronomic performance. The pasta strainer method offers 92 multiple advantages, including operational simplicity, cost-effectiveness, and ease of deployment 93 in field settings. The aim of this study was to gain insights into how root traits could contribute to 94 the performance of three Iranian wheat cultivars under terminal water stress conditions.

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#### 96 2 Materials and Methods

#### 97 2.1 Growth conditions

98 During the 2021-2022 growing season, a field experiment was carried out at the research field of 99 the School of Agriculture, Shiraz University, located at 29°43' N latitude and 52°35' E longitude 100 in Iran. The experimental site had an elevation of 1788 m above sea level. The study involved three irrigated winter wheat cultivars, which were manually sown into pasta strainer baskets on 101 November 9, 2021. Following sowing, drip irrigation was immediately initiated, with individual 102 103 drip tapes installed along each row to ensure uniform water delivery. Standard integrated pest, weed, and disease management protocols were implemented throughout the growing season to 104 105 maintain optimal crop health. The physico-chemical characteristics of the experimental soil are provided in Table 1. Based on soil test recommendations, nitrogen fertilizer was applied at a total 106 rate of 150 kg N ha<sup>-1</sup> in the form of urea, distributed equally in three split applications at sowing, 107 108 early tillering, and anthesis stages. Detailed meteorological data were recorded throughout the 109 cropping season (Table 2).

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**Table 1**. Physico-chemical characterization of the soil (0-30 cm depth) at the experimental site during the 2021–2022 growing season.

experimental site during the 2021 2022 growing season.									
Electerical	Clay	Silt	Sand	Total	Organic	Phosphorus	Potassium		
conductivity	(%)	(%)	(%)	Nitrogen	matter	(mg.kg <sup>-1</sup> )	(mg.kg <sup>-1</sup> )		
$(dS.m^{-1})$				(%)	(%)				
0.97	39	40	21	0.12	0.50	21.5	561		

**Table 2**. Climatic data of the experimental site at School of Agriculture during the 2020–2021 cropping season.

Month	Tmax (°c)	Tmin (°c)	Tavg (°c)	RHavg (%)	Pavg (mm)	SD (h/day)	Evap (mm)	Wind (m/s)
November	17.7	2.3	10	51.8	2.2	6.6	2.08	0.75
December	12.7	-1.3	5.7	55.3	2.0	5.9	1.0	0.92
January	13.8	-7.1	3.4	45.3	0.0	8.0	1.5	1.24
February	16.9	-2.7	7.1	52.2	1.7	8.4	8.3	1.75
March	21.2	2.7	12.0	46.0	0.3	8.3	9.3	1.76
April	26.4	4.7	15.5	46.0	0.2	8.7	11.9	1.45
May	29.9	9.0	19.5	41.4	0.0	10.3	13.7	1.65
June	36.6	13.6	25.1	25.9	0.0	12.0	20.3	1.1

Abbreviation. Tmax: Maximum Temperature; Tmin: Minimum Temperature; Tavg: Average Temperature; RHavg: Average Relative Humidity; Pavg: Precipitation Average; SD: Sunshine Duration; Evap: Evaporation.

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#### 117 2.2 Experimental design and treatments

The experiment was conducted following a split-plot design based on a randomized complete 118 119 block design with three replicates. The main plots were assigned to two irrigation regimes: well-120 watered (WW) and terminal water stress (TWS). The subplots consisted of three irrigated winter 121 wheat cultivars i. e. Pishgam, Sirvan, and Torabi (Table 3). In the WW treatment, irrigation 122 continued until maturity, ensuring non-limiting soil moisture conditions. In contrast, the TWS treatment involved withholding irrigation from the 50% anthesis stage onwards to simulate 123 124 terminal drought stress conditions representative of semi-arid environments such as the Persian 125 Plateau (Najafi & Alizadeh, 2023).

Table 3. Characteristics of bread wheat cultivars evaluated in the experiment.									
Wheat cultivars	Release year	Growth period	Climate	Growth habit	Pedigree	Origin	References		
Pishgam	2002	Middle- ripening	Cold regions with drought- stressed conditions	Spring	Bkt/90-Zhong87	Iran	(Mahfoozi et al., 2009)		
Sirvan	2011	Relative ly early- ripening	Temperate regions with drought- stressed conditions	Spring	PRL/2*PASTOR	Mexico (CIMMYT)	(Najafian et al., 2012)		
Torabi	2019	Early- ripening	Temperate regions with drought- stressed conditions	Spring	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92/ 4/BERKUT	Mexico (CIMMYT)	(Bakhtiar et al., 2021)		

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#### 135 **2.3** Evaluation of mature root system characteristics

136 The pasta strainer method employed in this study was adapted from the methodology described by 137 Uga et al. (2009), which was originally developed for assessing rice root systems using plastic baskets. This approach was subsequently modified for durum wheat under controlled conditions 138 139 by El hassouni et al. (2018). In the present study, we further refined and applied this method under 140 field conditions to evaluate root traits in irrigated bread wheat cultivars (Fig. 1). The plastic strainers used measured 13 cm in height and 25 cm in diameter. Each strainer was filled with field 141 soil and buried to a depth of 13 cm to ensure alignment with the surrounding soil profile. To allow 142 for adequate plant spacing and unrestricted three-dimensional root development, strainers were 143 144 placed 50 cm apart, resulting in a final plant spacing of 75 cm between individual plants (Fig. 1a). 145 Five seeds were sown at the center of each basket, and seedlings were later thinned to one plant 146 per basket at the early tillering stage (Fig. 2b) (Zadoks et al., 1974). At physiological maturity, the 147 aboveground plant parts were harvested at the soil surface, and shoot biomass (SB) was determined 148 after oven-drying. Spikes were subsequently harvested and threshed to quantify grain yield (GY) 149 using a precision balance. Following the harvest of aboveground parts, the plastic strainers 150 containing the root systems were carefully extracted from the soil through manual excavation with 151 a spade to allow for detailed root trait evaluation. Each strainer was then marked and sectioned 152 into three vertical soil layers: shallow (2-8 cm), intermediate (8-10 cm), and deep (10-13 cm),

corresponding to angular sectors of 0–30°, 30–60°, and 60–90° from the vertical axis, respectively
(A schematic illustration is shown in Fig. 2c). For each section, the number of roots protruding
through the perforations in the side walls of the containers was counted (SRN, MRN, and DRN,
respectively), enabling quantitative evaluation of root distribution across soil depths.

Following root counting, all visible roots protruding from the container walls were carefully clipped to isolate a fixed soil-root volume equivalent to that of the pasta strainer. The baskets were then submerged in buckets of water to facilitate gradual disintegration of the soil matrix while minimizing mechanical damage to the root structures. The root samples were subsequently rinsed thoroughly with clean water to remove any adhering soil particles. Thereafter, the cleaned roots were oven-dried at 40 °C for 48 hours to a constant weight, and the resulting dry mass was recorded as root weight (RW). 

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**Fig. 1**. Application of the pasta strainer technique to evaluate root system architecture in three irrigated bread wheat cultivars under terminal water stress in field conditions. (a) Bed preparation for basket burial and seed sowing at the center of each basket. (b) Wheat plants grown in the pasta strainers. (c) Schematic illustration of a pasta strainer removed from the soil, showing depth-based segmentation and root counting. The strainer was divided into three depth intervals: 2–8 cm (shallow root number, SRN), 8–10 cm (middle root number, MRN), and 10–13 cm (deep root number, DRN). Roots protruding through the side perforations were counted at each depth to quantify vertical root distribution.

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5 2.4 Statistical analyses

177 The collected data were subjected to statistical analyses using SAS software (version 9.3; SAS 178 Institute, Cary, NC, USA) to assess the significance of treatment effects. The Shapiro–Wilk test 179 was employed to evaluate the normality assumption of the residuals, while Bartlett's test was used 180 to verify the homogeneity of variances. Analysis of variance (ANOVA) was performed to detect

181 significant differences among treatment groups, and mean comparisons were conducted using the 182 least significant difference (LSD) test at a significance level of p < 0.01. In addition, Pearson 183 correlation coefficients were calculated using SRplot software (Tang et al., 2023) to examine the 184 associations among measured traits. Principal component analysis (PCA) was carried out in R 185 software (version 4.4.2; R Core Team, Vienna, Austria) utilizing the *ggplot2* and *factoextra* 186 packages for visualization and multivariate data interpretation.

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#### 188 **3 Results & discussion**

189 The ANOVA revealed significant main effects of irrigation on RW, and RV, SB, and GY. In

190 contrast, the cultivar factor had a significant effect on all measured traits, including root number

191 in the shallow, middle, and deeper soil layers (SRN, MRN, and DRN, respectively), total root

192 number (TRN), RW, RV, SB, and GY. Moreover, the interaction between irrigation and cultivar

193 was also significant for RW, RV, SB, and GY (Table 4).

**Table 4.** Analysis of variance (ANOVA) for above- and belowground traits in three wheat cultivars under two irrigation regimes including well-watered and terminal water stress conditions.

<u> </u>	df	SRN	MRN	DRN	TRN	RW	RV	SB	GY
Block	2	7.39	20.22	33.50	142.17	3.73	37.50	45.37	2.55
Irrigation	1	20.05	26.88	9.388	162	78.33***	272.22**	3411.65*	555**
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Irrigation *Block	2	2.06	6.99	22.72	33.17	4.561	26.39	62.81	3.77
Cultivar	2	48.39**	49.55**	236.17*	852.66**	20.11***	304.16**	745.50**	136.28
					*		*	*	**
Irrigation*Cultivar	2	0.72	2.89	35.72	26	19.97***	134.72*	149.42**	50.48*
-								*	
Error	8	5.88	6.72	48.36	80.75	2.25	21.52	15.81	10.50
CV		9.19	9.22	9.50	7.04	21.31	1.44	7.24	14.27

\*,\*\*,\*\*\* Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Abbreviations: WW, well-watered; TWS, terminal water stress; SRN, shallow layer root number; MRN, middle layer root number; DRN, deeper layer root number; TRN, total root number; RW, root weight; RV, root volume; SB, shoot biomass; GY, grain yield.

#### 195 **3.1 Variability in rooting pattern**

The cultivars exhibited distinct variability in root distribution patterns (Fig. 2). In the shallow soil layer (Fig. 2a), Pishgam had the highest SRN (29.33), followed by Sirvan (26.17) and Torabi (23.67). Similarly, in the intermediate layer (Fig. 2b), Pishgam and Sirvan displayed higher MRN (30.67 and 28.67, respectively) compared to Torabi (25.00). In the deepest layer (Fig. 2c), Pishgam again showed the highest DRN (78.67), followed by Sirvan (74.50), while Torabi had the lowest DRN (66.33). Overall, Pishgam consistently exhibited a higher TRN across soil depths (Fig. 2d),

202 particularly in the deeper layers, suggests that Pishgam may possess a more extensive and
 203 developed root system, potentially enabling greater access to deeper soil moisture.

204 Moreover, significant variation among cultivars in the assessed belowground traits highlights the 205 utility of the pasta basket method as a valuable tool for genetic studies on root distribution. El 206 Hassouni et al. (2018) stated that genotypes that allocate a larger proportion of their root at depth 207 demonstrated a significant increase in GY, ranging from 28 to 42% on average. Similarly, Severini et al. (2020) found a positive correlation between deeper root systems and yield advantages. A 208 209 narrow root system architecture facilitates deeper soil penetration and enhances the plant's ability 210 to extract moisture reserves during the critical grain-filling phase (Botwright Acuña & Wade, 211 2012; Ober et al., 2021). In bread wheat (X. Li et al., 2019), barley (Robinson et al., 2018), durum 212 wheat (El Hassouni et al., 2018), triticale (Severini et al., 2020), deeper root have been linked to 213 improved yields. The orientation of root growth plays a crucial role in shaping root system 214 architecture, influencing how roots are distributed within the soil profile (Borrell et al., 2014; 215 Boudiar et al., 2021). In climates where terminal water stress often coincides with critical growth 216 stages, deeper root growth becomes essential, as it allows plants to access water from deeper soil 217 layers and sustain growth despite surface water scarcity. Conversely, a wider root angle promotes exploration of the upper soil layers, which can be advantageous for capturing moisture from the 218 219 surface and from frequent, low-intensity rainfall events (Alahmad et al., 2019; Nakhforoosh et al., 220 2021; Ober et al., 2021).

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**Fig. 2.** Comparison of mean root numbers for three irrigated bread wheat cultivars across soil layers assessed using the pasta strainer technique. Root numbers were recorded at (a) shallow (2-8 cm), (b) middle (8-10 cm), (c) deep (10-13 cm) soil depths, and (d) total root number. The same letters in each column suggest a 5% probability of non-significance for the least significant difference (LSD) test.

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**Fig. 3.** Comparison of mean (a) root weight and (b) root volume for three irrigated bread wheat cultivars assessed using the pasta strainer technique under contrasting irrigation treatments: well-watered (WW) and terminal water stress (TWS). The same letters in each column suggest a 5% probability of non-significance for the least significant difference (LSD) test.

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232 According to Fig. 3, Sirvan displayed a significantly higher RW and RV under WW conditions than other cultivars (13.31 g, 38.33 mm<sup>3</sup>, respectively). The elevated RW observed in Sirvan may 233 234 be attributed to its greater RV, which is determined by multiplying root diameter by root length. 235 In wheat, RW is influenced by various components, including root number, length, and diameter (Ehdaie et al., 2012). Therefore, the increase in RW in Sirvan could be the result of an increase in 236 one or a combination of these root system traits. As the number of mature roots is typically 237 238 established prior to the onset of TWS, the higher RW in Sirvan is likely due to thicker roots, which 239 aligns with observations made during the experiment. Although Sirvan exhibited higher RW than 240 Pishgam under WW conditions, it is noteworthy that Sirvan produced lower GY than Pishgam. This disparity suggests that factors beyond RW and RV may play a critical role in determining the 241 242 overall performance of these cultivars.

#### 3.2 Above-ground features influenced by irrigation and cultivar interaction

Under WW conditions, the Pishgam had significantly higher GY (35.08 g plant<sup>-1</sup>) compared to the other cultivars (Fig. 4a). Sirvan followed with a mean GY of 28.65 g plant<sup>-1</sup>, which, although slightly lower than Pishgam, was still significantly different. In contrast, Torabi showed a mean GY of 21.07 g plant<sup>-1</sup>, significantly lower than Sirvan under WW conditions. These results indicate

that Pishgam possesses a strong ability to perform under optimal water availability, underscoringits potential as a high-yielding cultivar.

However, the situation changed markedly under TWS conditions (Fig. 4a). Pishgam, which had 251 demonstrated exceptional performance under WW conditions, experienced a significant reduction 252 253 in GY, with a mean of 21.00 g plant<sup>-1</sup>. Similarly, Sirvan showed a notable decrease in GY under TWS, recording a mean value of 16.65 g plant<sup>-1</sup>. There was no significant difference between 254 255 Pishgam and Sirvan under stress conditions. Torabi also experienced a reduction in GY under TWS, with a mean of 13.84 g plant<sup>-1</sup>, reflecting a significant decline compared to its performance 256 257 under WW conditions. TWS led to a yield reduction of 40% in Pishgam, 42% in Sirvan, and 34% 258 in Torabi. Despite the reductions, Pishgam consistently maintained the highest GY under both 259 WW and TWS conditions, outperforming both Torabi and Sirvan. Nonetheless, Torabi 260 demonstrated greater yield stability under stress compared to the other two cultivars.

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**Fig. 4.** Comparison of mean (a) grain yield and (b) shoot biomass for three irrigated bread wheat cultivars assessed using the pasta strainer technique under contrasting irrigation treatments: well-watered (WW) and terminal water stress (TWS). The same letters in each column suggest a 5% probability of non-significance for the least significant difference (LSD) test.

A similar trend was observed for SB among the cultivars (Fig. 4b). The imposition of TWS can substantially impair photosynthetic capacity, primarily through the disruption of carbon assimilation processes and the subsequent translocation of photoassimilates to developing grains. Such impairments compromise the grain filling process, ultimately resulting in decreased grain

size and GY (Senapati et al., 2019; Xie et al., 2015). Consistent with these observations, Zamani 267 268 et al. (2024) using the same cultivars, reported that plants exposed to TWS exhibited a reduction 269 in grain size accompanied by a more rapid depletion of grain moisture content relative to those 270 grown under WW conditions. Extracting each millimeter of water from the soil throughout the 271 crucial grain filling phase could lead to an increase in wheat yield by around 55 kg per hectare 272 (Manschadi et al., 2006, 2008). Physiological responses to water deficit are largely governed by the functionality and efficiency of the root system. Nevertheless, under certain conditions, these 273 274 adverse effects can be mitigated by root systems possessing enhanced capacity for water foraging, 275 even under restricted soil moisture availability (Awad et al., 2018; Shazadi et al., 2024).

276 As shown in Fig. 5, strong and statistically significant positive correlations were observed between 277 GY, SB, MRN, and DRN. In contrast, the correlation between GY and SRN was weaker and not 278 statistically significant. Also, the principal component analysis (PCA) biplot (Fig. 6) illustrates the 279 multivariate relationships among traits, functional attributes, and cultivar responses under 280 contrasting water regimes. The first two principal components (Dim1 and Dim2) explained jointly 281 92.1% of the total variance, with Dim1 accounting for 71.6% and Dim2 for 20.5%, thereby 282 capturing the predominant patterns within the dataset. Dim1, which explained the majority of the 283 variation, was predominantly driven by root system architecture traits, including TRN, MRN, and 284 DRN, as indicated by their high squared cosine values ( $\cos^2 > 0.9$ ) and extended vector lengths. 285 This highlights the substantial contribution of these root traits to the overall variability observed 286 among treatments. Similarly, RV and RW also exhibited strong associations with Dim1, further 287 emphasizing the integral role of root biomass in differentiating cultivar performance under varying 288 water availability. GY and SB were associated with both Dim1 and Dim2, reflecting the combined 289 influence of root architectural traits and environmental conditions on these productivity 290 parameters. The alignment of GY and SB vectors with those of RV, RW, and deeper root 291 distribution traits such as DRN and MRN suggests a positive association between increased root 292 biomass, enhanced root penetration into deeper soil layers, and aboveground productivity. This 293 reinforces the pivotal role of belowground resource capture in driving SB accumulation and yield 294 formation. These findings suggest that selecting for a narrower root distribution (i.e., higher MRN 295 and DRN) may represent a more effective breeding strategy for enhancing yield under water-296 limited conditions. From a physiological standpoint, the development of a deep root system 297 enables plants to exploit water reserves located in deeper soil horizons, particularly during critical

reproductive phases such as grain filling, when transpirational demand is heightened. This trait 298 299 confers a substantial adaptive advantage under terminal drought conditions, which are typified by the rapid depletion of surface soil moisture (C. Li et al., 2021; Palta et al., 2011; Ranjan et al., 300 301 2022; Shazadi et al., 2024). Deep rooting thus enhances drought avoidance mechanisms and 302 contributes to yield stability under water-limited environments. In contrast, while shallow root systems may facilitate early-season nutrient acquisition, their limited penetration depth restricts 303 304 water uptake during prolonged soil moisture deficits, ultimately compromising yield sustainability (R. Mohammadi, 2024; Vadez, 2014). Clearly, each root architectural configuration exhibits 305 306 differential adaptive suitability depending on specific environmental conditions.



**Fig. 5.** Correlation coefficient for above- and belowground traits in bread wheat cultivars under irrigation regimes. The size of each circle indicates the correlation intensity between two features. \*, \*\* Significant at the 0.05 and 0.01 probability levels, respectively. Abbreviations: TRN, total root number; DRN, deeper layer root number; MRN, middle layer root number; SRN, shallow layer root number; RW, root weight; RV, root volume; SB, shoot biomass; GY, grain yield.

Recent studies on wheat root have primarily focused on characterizing the root system during the 308 seedling stage, which may not provide a complete understanding of the root system in adult plants. 309 Additionally, these studies have often overlooked examining of the relationship between root and 310 311 shoot traits, including the crucial trait of GY. In this study, we sought to address these limitations by investigating the root system and its behavior at plant maturity. By doing so, we aimed to gain 312 a more comprehensive understanding of the adult plant root system and its association with GY 313 under varying conditions. Our approach allowed us to explore the intricate connections between 314 315 root distribution in different soil layers and GY. Notably, root distribution across the evaluated 316 depths remained unaffected under TWS condition, further supporting the notion of strong genetic 317 control over these traits. However, it should be acknowledged that the absence of significant effects 318 observed in this study does not preclude the possibility that alternative water regimes could exert 319 more pronounced influences on rooting patterns. By adopting this approach, the efficiency, 320 consistency, and scalability of root trait evaluation can be substantially improved while minimizing 321 resource and time requirements. However as root features are challenging to enhance through 322 conventional selection strategies, and therefore, molecular approaches such as genome-wide association studies (GWAS) may help overcome some of the limitations (Khodaee et al., 2021; 323 324 Pirnajmedin et al., 2016).

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Fig. 6. Principal component analysis (PCA) biplot analysis between above and underground, functional traits treatments. RW, root weight; RV, root volume; SRN, shallow root number; MRN, middle root number; DRN, deeper root number; TRN, total root number; SB, shoot biomass; GY, grain yield; WWP, well-watered Pishgam; WWS, well-watered Sirvan; WWT, well-watered Torabi; TWSP, terminal water stress Pishgam; TWSS, terminal water stress Sirvan; TWST, terminal water stress Torabi.

#### 328 4 Conclusions

329 Phenotyping root traits in wheat has long been constrained by inadequate methodologies. Despite 330 the advantages and limitations of the pasta strainer method, our results demonstrated that cultivar 331 with deeper rooting type, exhibited better drought performance. Based on the PCA results and the significant positive correlations observed between GY with both MRN and DRN underscore the 332 333 critical contribution of root system architecture to crop productivity. These findings suggest that 334 selecting for enhanced root traits can aid in identifying high-yielding cultivars under both TWS 335 and WW conditions. As climate variability intensifies, especially in regions reliant on stored soil moisture, the promotion of vertical root development will be essential for improving wheat 336

- resilience. The present study provides usable knowledge to inform breeding programs toward more
   adaptive, drought-resilient cultivars especially in semi-arid and arid regions.
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