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Assessment and Comparison of Root Architecture and Morpho-Anatomy of Quinoa (*Chenopodium quinoa* Willd.) Cultivars under Arid and Semi-arid Climate

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Plants in arid and semi-arid climates face limitations in accessing water resources. In addition, roots have a significant role in water uptake, nutrient absorption, hormone regulation, and mechanical anchoring. Therefore, we conducted a randomized complete block design study with four replications to investigate the root systems of four Quinoa cultivars (Rosada, Black, Titicaca, and Multi-hued Bulk) in arid and semi-arid climates. This research aimed to identify the most efficient cultivars based on their root length, root width, root fresh and dry weight, root density, root surface area, root volume, and shoot length in response to environmental stresses. No significant difference was observed between the Black and Rosada cultivars, but they outperformed the Titicaca and Multi-hued Bulk cultivars regarding root development, showing a better balance of dry matter allocation between the roots and aerial parts. The wavy and large root surface areas were observed in the Rosada cultivar, leading to improved yield. The Rosada and Black cultivars were more efficient ($p<0.01$) in water absorption compared to the other two studied cultivars based on various critical factors, including root-to-shoot weight ratio (0.09, 0.07), root diameter (0.66, 0.46mm), specific root length (18.6, 32.2cm.g^{-1}), root surface area (42.6, 34.6cm^3), root length (16.2, 17.3cm), root penetration and expansion into deeper soil layers (with root width and volume, 13.9, 15.3cm, and 8.3, 5.3cm^3, respectively), and root surface area density (32.18, 30.16cm^2). Therefore, Rosada and Black cultivars are well-suited for breeding programs in regions with moisture limitations.

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1. Introduction

Diverse production of agricultural products is required to ensure food security due to the increase in worldwide population (Chen and Gong, 2021). This challenge calls for developing highly adaptable cultivars to mitigate non-advantageous stresses such as drought, salinity, and heat, which are significant challenges in arid regions (Akram *et al.*, 2021); Plants in arid and semi-arid climates face limitations in accessing water resources since their water availability depends on environmental conditions, root system size/shape, and root competition (Zurita Silva *et al.,* 2015); Studies have also expressed that soil moisture level is among the limiting factors for plant root system size and shape (Tumber-Davila *et al.*, 2022); Jalal *et al.*, 2023).

Many plant species increase the proportion of photosynthetic materials in the roots, leading to enhanced root growth and a higher root-to-shoot ratio and allowing them to utilize available water more efficiently under moisture scarcity or stress (Emadi *et al.*, 2022); Tumber-Dávila, 2022). Additionally, root epidermal cells' growth and differentiation result in root hair formation, significantly increasing the root's surface area in contact with the soil and enhancing water and mineral absorption (Warren *et al.*, 2023). Another adaptive strategy of plants in arid conditions is increasing the specific root length, which promotes elongation and subsequently improves absorption capacity by increasing the dry matter content in the roots. Genetic factors primarily control the diversity of root systems in different plant cultivars. Therefore, plants with greater flexibility in adapting to different climates have a higher chance of survival (Fan *et al.*, 2017). Identifying the genetic diversity of plant roots under water-deficient conditions can serve as a basis for breeding programs to improve seedling establishment in saline and arid soils and enhance overall plant yield (Shirinpour *et al.*, 2023).

Quinoa (*Chenopodium quinoa* Willd.), an annual dicotyledonous plant from the Amaranthaceae family (Ghous *et al.*, 2022), is a facultative halophytic (Rakhmankulova *et al.*, 2023) and pseudocereal native to the Andean mountains in Chile, Bolivia, and Peru. In addition, Quinoa exhibits wide genetic diversity and is applied for animal and human feed due to its essential amino acids (Alvarado *et al.*, 2022). Quinoa roots can reach up to 30cm in length under normal conditions and exhibit deep growth (Jancurová *et al.*, 2009). Limited research has been conducted on the morphological and anatomical characteristics of Quinoa roots in response to drought, and the deep roots have been found to contribute to the plant's resistance. The presence of secondary roots, which adapt to soil conditions, allows Quinoa roots to extend up to 1.5m under such conditions (Alvarez-Flores, 2012). The extensive genetic diversity of Quinoa is believed to enable its tolerance to harsh environmental conditions, including temperature stress, salinity, and drought (Issa Ali *et al.*, 2019); (Hussain *et al.*, 2021).

Root development under water scarcity conditions is a critical indicator of drought tolerance, as roots are the first plant part to sense fluctuations in moisture. Previous studies have highlighted the significance of root growth parameters, such as length, fresh and dry weight, diameter, surface area, and root fineness, in Quinoa's drought tolerance (Gámez *et al.*, 2019); (Nguyen *et al.*, 2022); Therefore, due to the significant reduction in rainfall over the past few years, the increasing consumption of water resources, and the environmental challenges resulting from climate change, which has led to the desertification of agricultural lands, this research aimed to investigate and compare the root systems of four Quinoa cultivars in an arid and semi-arid climate (Yazd Province in Iran). In other words, superior cultivars are sought to be identified based on their growth and anatomical root characteristics that respond well to environmental stresses in these regions.

2. Materials and Methods

2.1. Study area

In this study, specific anatomical and growth indices of the root system of four Quinoa cultivars, including Rosada, Black, Titicaca, and Multi-hued Bulk, were investigated under arid and semi-arid climatic conditions in Yazd, Iran (Table 1).

Table 1. Quinoa cultivars evaluated in this study

Cultivar Name	Origin	Seed Sources	Other research(es)
Rosada	Junín, Peru	Seed and Plant Improvement Institute of Karaj, Iran	Ludena Urquizo *et al.*, 2017; Bagheri *et al.*, 2020
Black	Bolivia (South America)	Seed and Plant Improvement Institute of Karaj, Iran	Melini and Melini *et al.*, 2021
Titicaca	Peru and Bolivia	Seed and Plant Improvement Institute of Karaj, Iran	Bagheri *et al.*, 2020; Jafari *et al.*, 2023
Multi-hued Bulk	British Colombia, Canada	Seed and Plant Improvement Institute of Karaj, Iran	Maliro *et al.*, 2017

To this end, an experiment was conducted in the Research field of the School of Natural Resources and Desert Studies, Yazd University, Iran, from mid-August to early January of the agricultural year 2019-2020 (Fig 1, Table 2). The field had the following geographical coordinates: 35°21'54" N latitude, 40°49'31" E longitude, and an altitude of 1261 meters above sea level, with an average annual rainfall of approximately 56mm (Rezaei *et al.*, 2021).

The root samples were collected from a depth of 30cm (Akcay *et al.*, 2021); (Jancurová *et al.*, 2009) after sowing the cultivars in the soil during the vegetative growth stage and before flowering.

Fig 1. The research field of Yazd University (the model field of arid and semi-arid climate)

Table 2. Soil properties of Yazd University research field

Soil depth	EC	pH	Total N	CaO	Organic carbon	K	P	Soil texture
cm	dSm^{-1}		%			Available (ppm)		
0-30	6.53	8.13	0.026	31.5	0.51	225	3.41	Loamy sand

2.2. Methodology

The number of branches of each plant was counted after collecting plant samples. Later, the root length (RL; cm), root width (RW; cm), and shoot length (ShL; cm) of the four Quinoa cultivars (Rosada, Black, Titicaca, and Multi-hued Bulk) were measured using a measuring tape. The root volume (RV; cm^3) was determined by measuring the difference in volume before and after placing the root in a graduated cylinder with a capacity of 50mm. The root fresh weight (RFW; g) and shoot fresh weight (ShFW; g) were recorded. The root dry weight (RDW) and shoot dry weight (ShDW) were measured using a digital balance (0.001g) after 10 days of air drying. The root surface area (RSA; cm^2) was also calculated using the Atkinson equation (Atkinson, 2000):

$$RSA = 2(RV\ (RL * 3.14))^{0.5} \quad (1)$$

The root diameter (RD) was calculated using Equation 2 (Hajabbasi, 2001):

$$RD = (4*RFW/RL*3.14)^{0.5} \quad (2)$$

The root surface area density (RSAD) was obtained using Equation 3 (Hajabbasi, 2001):

$$RSAD = RL \times RD \times 3.14 \quad (3)$$

The root density (RDE) was achieved using Equation 4 (Hajabbasi, 2001):

$$RDE = RDW/RV \quad (4)$$

The specific root length (SRL) was determined using Equation 5 (Mahanta *et al.*, 2014):

$$SRL = RL/RDW \quad (5)$$

The root fineness (RF) was calculated using Equation 6 (Hajabbasi, 2001):

$$RF = RL/\ RFW \quad (6)$$

2.3. Statistical analysis

The means were compared using a randomized complete block design (RCBD) with four replications via SPSS 26 confirming the data normality using the Kolmogorov-Smirnov test and conducting Bartlett's/Levene's tests for homogeneity of variances. The Duncan's test was also run to compare means at a significance level of 5% ($p<0.05$). The graphs show the arithmetic means and standard errors of the recorded data.

3. Results

Based on the analysis of variance (ANOVA), no significant differences were observed among the four studied Quinoa cultivars regarding root density, root branches, shoot length, and shoot weight. However, there were significant differences in other traits ($p<0.01$) (Table 3).

3.1. Root Length and Width

The highest root length (17.3cm) and width (15.3cm) were observed in the Black cultivar. In comparison, the lowest root length (12.6cm) and width (11.2cm) were attributed to the Multi-hued Bulk cultivar (Fig. 2A). However, no significant difference was observed between the Rosada and Black cultivars regarding dry root weight.

3.2. Root Diameter

A comparison of the cultivars' root diameters clarifies that Rosada had the largest root diameter (0.66mm) among the investigated cultivars, while the Titicaca cultivar showed the smallest (0.38mm) root diameter (Fig. 2B).

Table 3 ANOVA for the growth and anatomical characteristics of the studied Quinoa cultivars in the arid and semi-arid climate of Yazd

Sources of variance	df	Mean squares								
		Root length	Root width	Root diameter	Number of sub-roots	Root fresh weight	Root dry weight	Root surface area	Root volume	Root fineness (Vigor)
Cultivar	3	162.80**	147.50**	0.03**	1.14^{ns}	12.40**	8.30**	2397.50**	146.70**	8.60*
Block	3	1.040^{ns}	7.40^{ns}	0.05^{ns}	0.50^{ns}	0.70^{ns}	0.70^{ns}	33.30^{ns}	4.50*	0.90^{ns}
Error	9	2.90	3.20	0.03	0.90	1.60	0.50	16.00	1.50	2.30

Sources of variance	df	Mean squares							
		Root density	Root surface area density	Specific root length	Shoot fresh weight	Shoot length	RDW to ShDW ratio	RFW to ShFW ratio	RL to ShL ratio
Cultivar	3	0.040^{ns}	479.558*	2606.500**	65.400^{ns}	3.200^{ns}	0.005**	0.010**	0.090**
Block	3	0.020^{ns}	227.400^{ns}	46.100^{ns}	201.900*	1.900^{ns}	0.004^{ns}	0.008^{ns}	0.040^{ns}
Error	9	0.030	7100	502.500	37.700	3.700	0.001	0.002	0.001

df, RDW, ShDW, RFW, ShFW, RL, and ShL are degrees of freedom, Root dry weight, Shoot dry weight, Root fresh weight, Shoot fresh weight, Root length, and Shoot length, respectively. ^{ns} Non-significant, * Significant at α=5%; ** Significant at α=1%.

3.3. Weight of Fresh and Dry Roots

Comparing the mean weight of fresh and dry roots indicated that the Rosada and Titicaca cultivars manifested the highest and lowest weight of fresh (5.08 and 2.2g, respectively) and dry (2.1 and 0.9g, respectively) roots (Fig. 2C).

3.4. Root Surface Area

Although the highest root length and width were attributed to the Black cultivar, Rosada and Titicaca cultivars had the highest (41.43cm^3) and lowest (19.2cm^3) root surface, respectively (Fig. 2D).

3.5. Root Volume

Rosada and Titicaca cultivars had the largest (8.3cm^3) and smallest root volumes (2.6cm^3), respectively (Fig. 2E).

3.6. Root Fineness (Vigor)

The Black cultivar exhibited the highest (4.9cm.g^{-1}), while the Multi-hued Bulk cultivar presented the lowest (3.04cm.g^{-1}) root fineness, respectively (Fig. 3A).

3.7. Specific Root Length

Given the mean specific root length index, the Black cultivar had the highest (33.2cmg^{-1}), but the Multi-hued Bulk cultivar had the lowest (11.3cmg^{-1}) specific root length. Although the Rosada and Multi-hued Bulk cultivars differed significantly, the Multi-hued Bulk cultivar showed the lowest specific root length (Fig. 3B).

3.8. Root Surface Area Density

The Rosada cultivar had the highest root surface density (32.2cm^2), while the Titicaca cultivar had the lowest (18.4cm^2) root surface density (Fig. 3C).

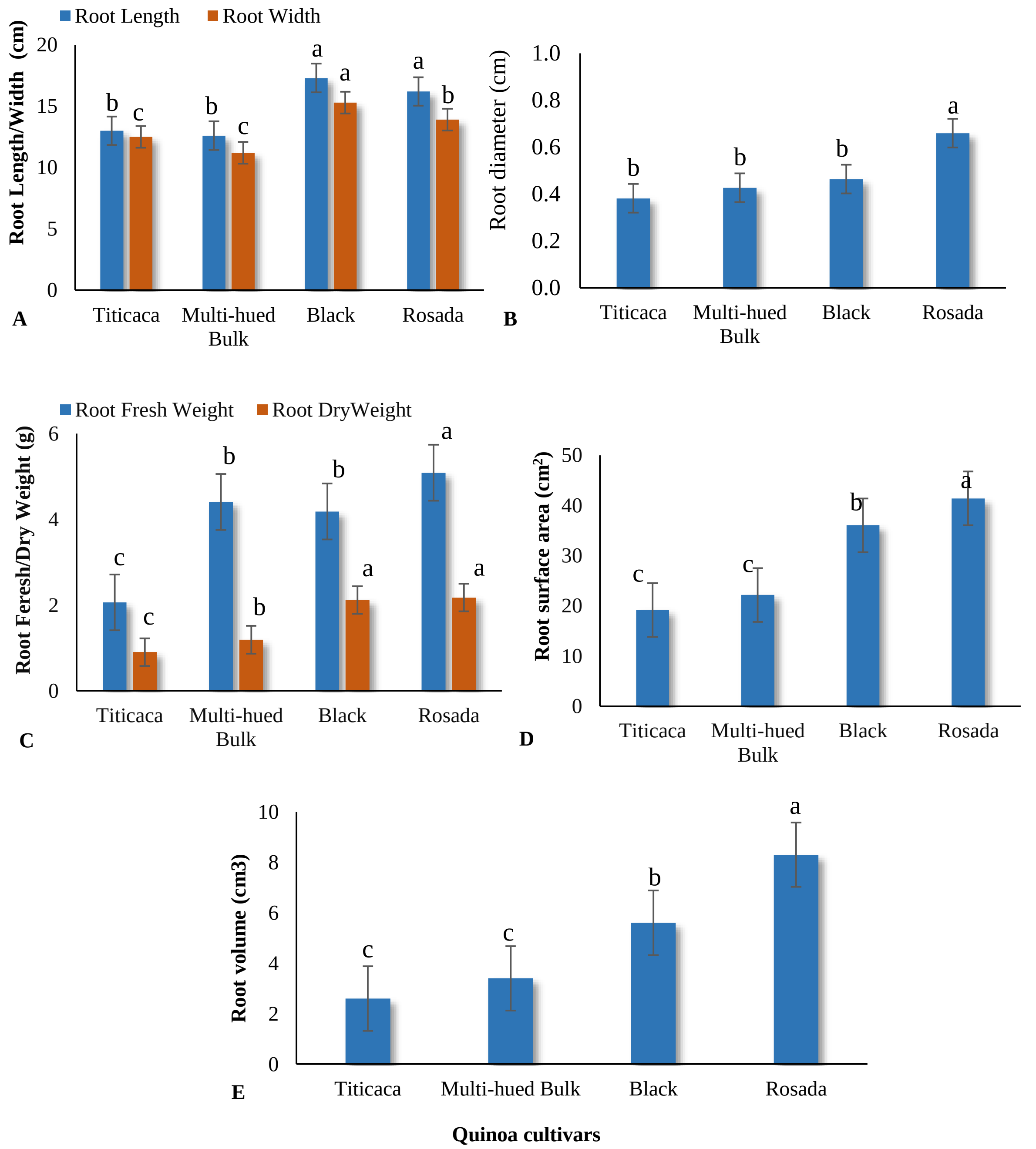

Fig 2. The effect of arid and semi-arid climate on A) root length and root width, B) root diameter, C) root fresh weight and root fry weight, D) root surface area, and E) root volume of the studied Quinoa cultivars
Based on Duncan's test, different letters above each column indicate a significant difference ($p<0.05$).

3.9. Root to Shoot Ratios

Although the Rosada, Multi-hued Bulk, Black, and Titicaca cultivars did not show a significant difference regarding their root-to-shoot length ratio, the Black cultivar exhibited the most significant difference compared to the other three cultivars (0.46) (Fig. 3D). Regarding the root-to-shoot fresh weight ratio, the Rosada cultivar (0.09) had the highest value but was not significantly different from Black and Multi-hued Bulk cultivars (Fig. 3D). The highest value of the root-to-shoot dry weight ratio belonged to the Black (0.06) cultivar but the lowest value

(0.02) was related to the Titicaca cultivar (Fig. 3D).

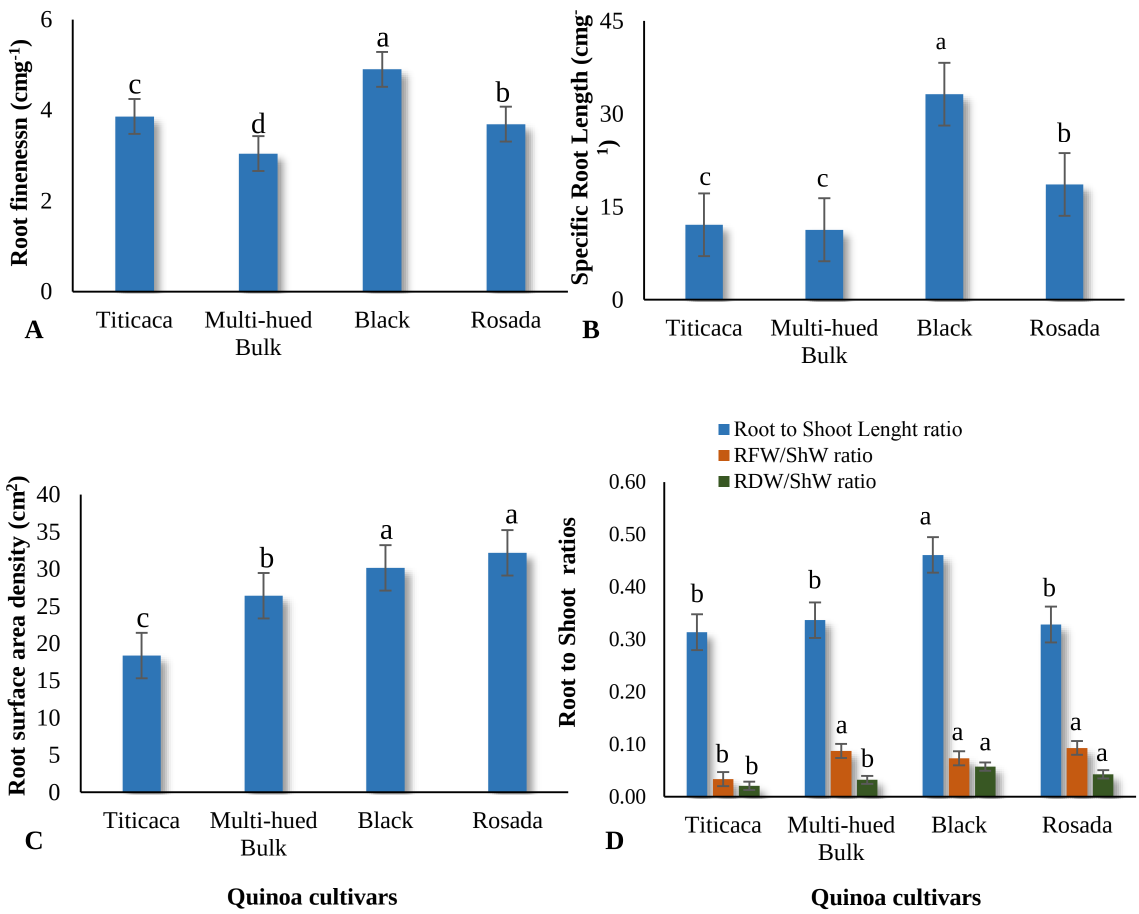

Fig 3. The effect of arid and semi-arid climate on A) root fineness, B) specific root length, C) root surface area density, and D) root-to-shoot ratios of the studied Quinoa cultivars
Based on Duncan's test, different letters above each column indicated a significant difference ($p<0.05$).

3.10. Root Architecture of Quinoa

The observations on root architecture confirmed a herringbone shape in Quinoa roots. Although all the examined cultivars have root branches, this structure was more clearly visible in Rosada and Black cultivars (Fig. 4).

4. Discussion

Consistent with the present results, (Liu *et al*., 2020) explained that plants tend to expand their root systems both horizontally and vertically in deeper soil layers to enhance adaptability in arid and semi-arid climates since moisture declines rapidly in the surface layers of soil (Liu *et al*., 2020). In other words, a decrease in water availability in soil reduces root growth and weight. In such conditions, drought-tolerant Quinoa cultivars accumulate a large portion of their photosynthetically derived products in their roots rather than in their stems and aerial parts. This accumulation of dry matter in the roots leads to an increase in root length and width in deeper soil depths (Miranda-Apodaca *et al*., 2018); (Li *et al*., 2021), root volume (Mirzaee-nodoushan *et al*., 2015), and specific root length (Vadez *et al*., 2013). Additionally, an increase in the root surface area (Nguyen *et al*., 2022) enhances the plant's ability to absorb more water and nutrients from the soil (Liu *et al*., 2020).

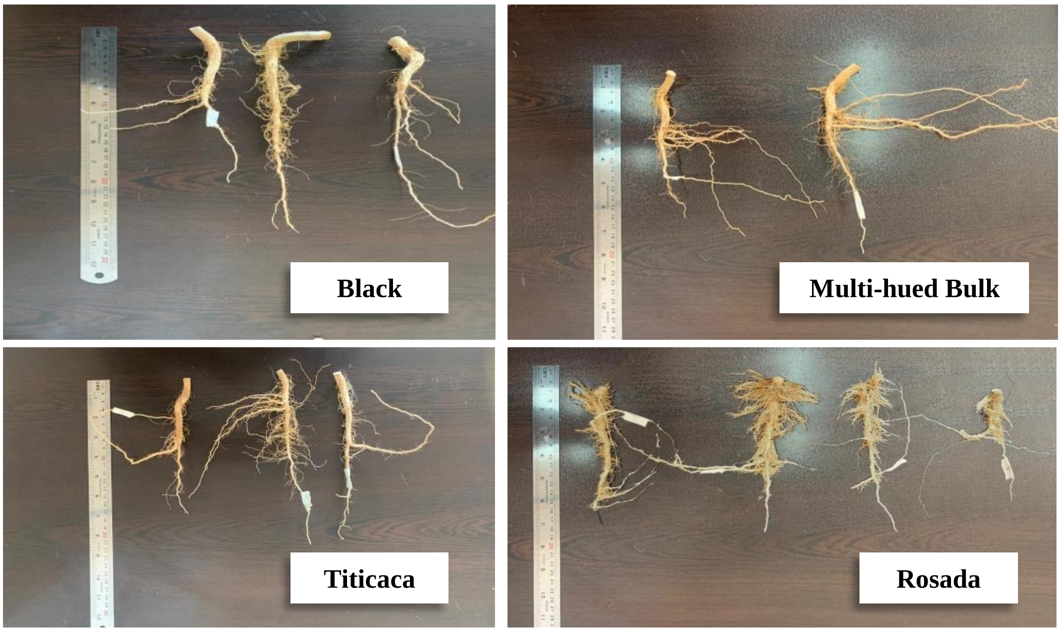

Fig 4. The root architecture of the four studied Quinoa cultivars in the arid and semi-arid climate of Yazd**.**

Similarly, an investigation on wheat plants indicated that the average root surface area was significantly higher in drought-tolerant cultivars than in drought-sensitive cultivars (Hosseinalipour *et al*., 2020). Another study on seven Triticeae species showed that the root surface area increased in drought-tolerant genotypes, such as TR39477 and IG32864 while decreasing in the sensitive genotypes (Ullah *et al*., 2017). In the present study, Rosada and Black cultivars exhibited significantly higher root surface areas than the other cultivars, which can be attributed to the more significant number and fineness of root hairs.

The literature shows that root fineness correlates directly with root diameter, indicating that finer roots absorb water and nutrients more efficiently (Hajabbasi, 2001). In other words, fine roots with numerous channels created in the soil increase soil porosity, affect soil structure, and improve water infiltration (Comas *et al*., 2013). Therefore, roots with smaller diameters and longer lengths have better water and nutrient absorption. Research on the root system of different barley cultivars under drought conditions has demonstrated that cultivars with higher root vitality exhibited better water and nutrient absorption (Naseri *et al*., 2021). In the same vein, the Quinoa Atlas and Want-2 cultivars under drought stress responded significantly by reducing or maintaining root diameter and allocating more dry matter for the longitudinal growth of the root (Nguyen *et al*., 2022).

In this study, all examined cultivars of Quinoa exhibited a herringbone-shaped root structure, but this pattern was more distinct in the Rosada and Black cultivars. Given the critical role of root architecture in the plant's adaptation to water-limited climates (Liu *et al*., 2019); Quinoa root architecture resembles a herringbone pattern. The herringbone pattern illustrates that secondary roots branch out at various depths as the primary roots extend and may even reach the surface, depending on the cultivar and environmental conditions (Figure 4). This structural adaptation enables Quinoa roots to explore a larger volume of soil, enhancing the roots' ability to access water and nutrients in challenging conditions (Zurita-Silva *et al*., 2015); (Singh *et al*., 2021). A larger and stronger root system, primarily contributing to root biomass, allows plants to access a greater soil volume and extract more nutrients from different soil depths (Xiong *et al*., 2021).

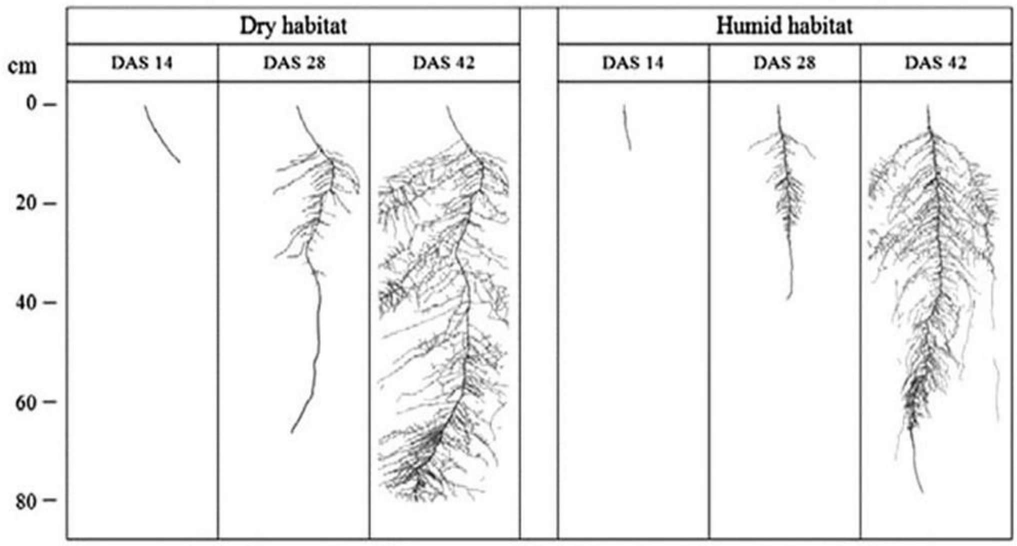

Fig 5. Root architecture of Quinoa at different time intervals in dry and humid habitats (Zurita-Silva *et al*., 2015).

5. Conclusion

The results highlighted the superior yield of the Black and Rosada among the studied cultivars in arid and semi-arid climate conditions across various traits. Black and Rosada cultivars outperformed the Titicaca and Multi-hued Bulk cultivars regarding root development and dry matter allocation between the roots and aerial parts. The Rosada cultivar demonstrated a wavier and larger root surface area, improving yield across the measured traits. The root-to-shoot weight ratio, root diameter and fineness, root surface area, root depth, root penetration and expansion into deeper soil layers, as well as lateral root dispersion for efficient water absorption, should be considered to select cultivars with higher yield and growth potential in arid and semi-arid climates. Given that aerial organs and seed yield traits in arid and semi-arid climates were not evaluated in the present study, further field evaluations are required on the Rosada and Black cultivars.

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