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Root System Traits and their Association with the Yield of Safflower under Different Irrigation Regimes

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ABSTRACT

Root system is recognized to play a crucial role in enhancing plant tolerance and stability during drought conditions. Therefore, this study aimed to assess safflower (*Carthamus tinctorius* L.) genotypes under various irrigation regimes and explore the relationship between root traits and yield. A two-year factorial experiment was conducted with three spring safflower genotypes, namely Parnian, Goldasht, and Kazak under two irrigation regimes, including full and deficit. Irrigation treatments maintained residual moisture levels at 60% and 20% of available water. Root traits such as biomass (RB), length (RL), diameter (RD), dry weight (RDW), and root length density (RLD) were measured. Additionally, seed yield (SY), yield components, and oil content (OC) were assessed in all treatments. The results showed that drought stress reduced safflower RB and RL, but Kazak genotype had a significant increase in RDW (48%) and RL (12%) under deficit irrigation. Root biomass distribution and RLD varied among genotypes across soil layers. Parnian and Goldasht genotypes had the highest root biomass at 30 cm depth with full irrigation, while Kazak showed the highest values at 60-90 cm depth under deficit irrigation. Drought stress significantly reduced safflower SY by 71% and Kazak genotype showed the highest SY under deficit irrigation, suggesting better performance stability. SY had a strong positive correlation with RDW (0.57) and RD (0.84), indicating a significant relationship. Furthermore, SY was significantly correlated with RLD at depths of 60-90cm (0.72-0.68). These results suggested that root traits such as RDW, RD, and RLD in deeper soil layers were crucial for breeding programs aiming to develop drought-tolerant genotypes.

Keywords: Biomass distribution; correlation; root dry weight; root length density; seed yield

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a drought-tolerant plant cultivated as an oilseed crop in arid and semi-arid areas. The seeds contain 30-40% of high nutritional value oil due to the presence of 85-90% of oleic and linoleic unsaturated fatty acids (Rahmani et al., 2019; Singh et al., 2016). As a drought-resistant deep-rooted crop, safflower can be considered for developing climate-smart agricultural programs and ensuring food security in areas affected by water shortage. The plant root system plays numerous vital roles, including anchoring plants in the soil, absorbing water and nutrients, building biological interactions, as well as adapting to various biotic and abiotic stresses (Wasaya et al., 2018). A well-developed root system

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allows crops to uptake water in deeper soil layers, and improving the system architecture helps prevent drought in crops (Uga et al., 2013). Therefore, morphological and physiological traits of plant roots have a crucial role in supporting shoot growth and overall yield (Ghosh & Xu, 2014). Although root phenotype and morphology are generally genetically controlled, environmental factors and plant growth conditions strongly affect root traits (Hamedani et al., 2020). Root structure and functions are affected by soil conditions, and drought stress increases root density in the deeper soil layers (Kim et al., 2020).

The volume of available water to the plant is influenced by the structure, distribution, and characteristics of the plant root system. Therefore, to develop drought-tolerant genotypes and maintain production sustainability in drought conditions, improving root traits is a remarkable strategy (Ahmed et al., 2019; Comas et al., 2013). Determining root response to drought stress and identifying key root traits in improving plant adaptation to drought conditions is essential to pursue this strategy. Root traits are related to plant productivity under stress conditions and recently various studies have been conducted to determine this relationship and improve stress tolerance in crops (Li et al., 2015; Paez-Garcia et al., 2015). Among root traits, biomass and density, length and diameter as well as root-to-shoot ratio (RtoSH) are key and effective in increasing plant adaptation to environmental factors. The correlation of root density and length with crop yield under stress conditions has well been documented (Fang et al., 2017; Hamedani et al., 2020).

Genetic improvement programs to increase plant adaptation to drought stress can be short-term and cost-effective. However, the existence of different drought tolerance mechanisms makes it difficult to fully understand the details of maintaining crop productivity in water deficit conditions (Ramamoorthy et al., 2017). The breeding approach based on the key traits provides better output in developing stress-compatible genotypes and maintaining yield stability (Comas et al., 2013). A thorough investigation of the plant root system, including distribution in various soil layers and the relationship between traits and yield components under normal and drought conditions, is essential for understanding tolerance mechanisms and achieving desired outcomes (Bishopp & Lynch, 2015; Ramamoorthy et al., 2017). However, studies on root system of drought-tolerant crops such as safflower are limited. This knowledge is critical for identifying and breeding tolerant genotypes with favorable characteristics. This study aimed to evaluate the response of safflower genotypes to different irrigation regimes as well as to investigate the correlation between root traits and yield to determine key traits and desired genotypes for future breeding programs.

METHODS

Site Description

Evaluation of root and shoot characteristics of safflower genotypes was carried out in the experimental field of Seed and Plant Improvement Institute of Karaj, Iran (35° 47′ N, 51° 8′ E and 1328 m), during the growing seasons of 2019-2020 and 2020-2021. The experimental area has a semi-arid climate with hot and dry summers and cold rainy winters. The mean annual temperature and rainfall were 13 °C and 240 mm, respectively. Figure 1 shows the average monthly temperature and rainfall data for the Karaj meteorological station during the crop years 2019-2020 and 2020-2021. The physicochemical characteristics of the experimental soil are shown in Table 1. Field soil samples were randomly collected and combined to create a composite. The physical and chemical properties of the soil were then analysed using standard soil analysis methods.

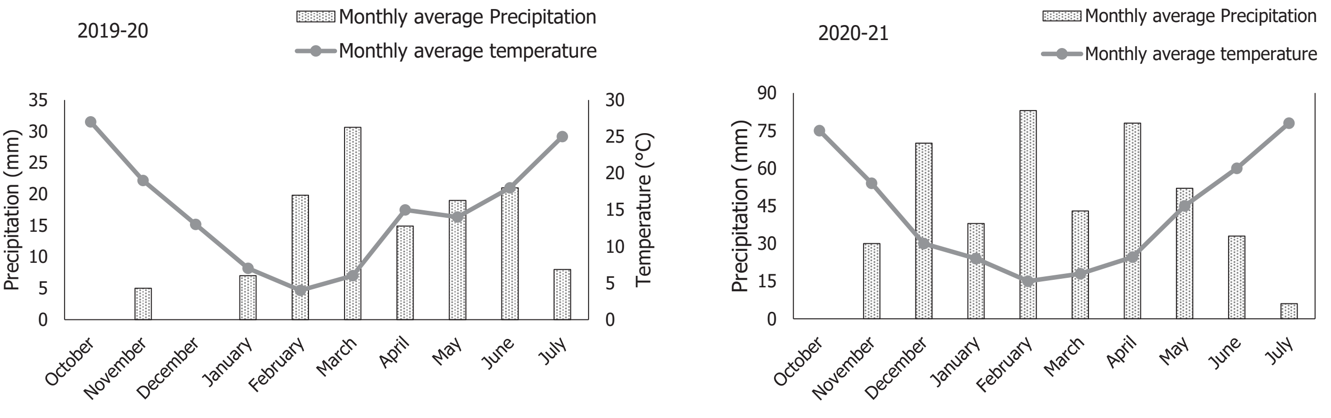


Figure 1. Average monthly temperature and rainfall at the meteorological station in the Karaj Region

Table 1. The physicochemical characteristics of the experimental soil

pH	OM (%)	EC (dS/m)	N (%)	*AP (ppm)	*AK (ppm)	FC (%)	PWP (%)	Sand (%)	Silt (%)	Clay (%)	Texture
7.2	0.23	1.5	0.06	12.6	256	24	9	49	19	32	Sandy Clay Loam

*AP: available phosphorus, AK: available potassium

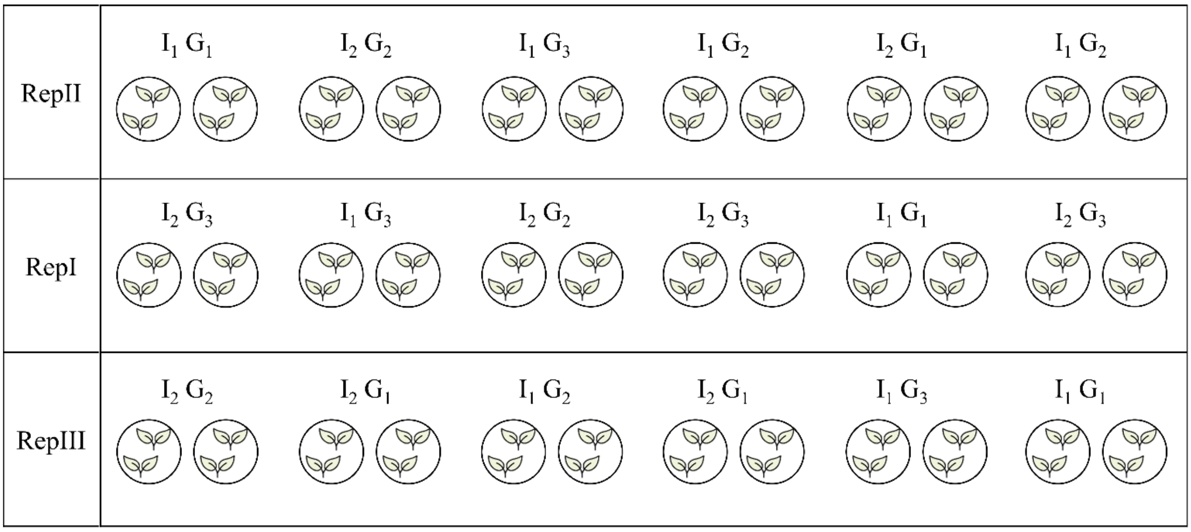


Figure 2. An illustration of plot design. I: irrigation regimes (I_1: full-irrigation, I_2: deficit-irrigation) and G: genotypes (G_1: Parnian, G_2: Goldasht, and G_3: Kazak)

Root Evaluation Columns

For planting, soil columns with a height of 150 cm and a diameter of 23 cm were used. Initially, 6-meter branches of polyethylene pipes (U-PVC) were cut into 1.5-meter lengths. These lengths were then divided longitudinally into halves for easier evaluation and connected using adhesive tape. After preparing the pipes, 40 cm deep pits were dug in the field using a dibble, and the pipes were placed vertically inside the pits approximately 1 meter above ground level, and filled with the soil mixture. Planting took place in March, with each experimental unit consisting of two pipes totalling 36, each containing three disinfected seeds.

Experimental Design and Procedure

The response of safflower genotypes to different irrigation regimes was evaluated as factorial experiments in a completely randomized design with three replications. The crop seasons spanned March 5 to July 26 in 2019-20 and from March 9 to July 25 in 2020-21. Experimental treatments included two irrigation regimes (full-irrigation and deficit-irrigation) and three spring safflower genotypes (Parnian, Goldasht, and Kazak). Four disinfected safflower seeds were sown in rhizo-tubes in October and thinned at the two-leaf stage to reach two plants per column (Figure 2). After seed sowing, all the rhizo-tubes were irrigated to field capacity and different irrigation regimes were applied at the stem-elongation stage. Irrigation regimes were applied based on the residual water content at root development zone (Hamedani et al., 2020). To achieve this, changes in soil moisture were monitored daily while watering was applied for full and deficit irrigation treatments at residual moisture of 60 and 20% of available water, respectively. The field capacity moisture content and permanent wilting point of the experimental soil were determined as 24% and 9% (w), respectively, using pressure plates. The moisture content for 60% and 20% of the residual available water was calculated as 18% and 12% (w), respectively. Daily soil moisture monitoring was performed by placing five Time Domain Reflectometry probes (30 cm intervals) in each soil column (6050X1, Trase System 1, Soil moisture Equipment Corp. Santa Barbara, California, USA). Moreover, a drip watering system was used to have exact and even irrigation in all treatments.

Sampling and Measurements

Plant sampling was carried out at the end of the experiment during the plant maturity stage in July (2020 and 2021). To achieve this, plants in each soil column were cut off from the ground level and shoot height and branch number (BrN) were recorded. Soil columns were opened and after shaking the bulk soil, the plant roots were carefully separated using water flow to measure the traits. After drying roots with a paper towel, traits such as length, diameter, dry weight (oven-dried at 70˚C) and length density (total length of roots per unit of soil volume) were measured. RtoSH was also calculated in all treatments (Hamedani et al., 2020). Seed yield (SY) of safflower genotypes and components such as BrN, number of seeds, and weight of thousand seeds were measured in all treatments. Furthermore, oil content (OC) of safflower seed in different treatments was determined by nuclear magnetic resonance (NMR) spectroscopy method (Colnago et al., 2011). This method entails inducing magnetic resonance in a strong external magnetic field with pulsed radio waves. By placing 3 grams of the sample in the magnetic field, the energy levels of protons in the sample, which were previously degenerate, were separated and excited from low to high energy levels by absorbing energy from radio waves. The absorption of radio frequency energy was then measured and correlated with OC of the sample.

Statistical Analysis

Before the combined analysis of variance (ANOVA), the homogeneity of the variance of data obtained from 2 years was verified with Levene's test. Finally, data were subjected to the combined ANOVA by GLM procedure in SPSS statistical analysis software version 24 (SPSS Inc., Chicago, IL, USA). Means of data were compared based on Duncan's multiple range test at the 5% level. Microsoft Office Excel (Microsoft, NY, USA) was used to draw the histograms. Pearson correlation plots were also provided by R v. 3.5.1 software using the interface RStudio.

RESULTS AND DISCUSSION

ANOVA results showed that irrigation regime had a significant effect on safflower root system traits but no significant effect was observed on root dry weight (RDW). Under deficit irrigation, root length (RL) decreased by 6.2 cm and plant height (PH) was also 5.8 cm shorter than in full irrigation. In contrast, root diameter (RD) increased by 7% under deficit irrigation. RtoSH and BrN in different irrigation regimes showed no significant difference (Table 2).

Different genotypes had a significant effect on safflower root and shoot traits. The highest RDW was observed in Goldasht genotype by 8.07g. However, the highest RL and RD values were obtained in Kazak genotype by 97.9 cm and 9.27 mm, respectively. The lowest related value was also observed in Goldasht genotype. Kazak genotype showed the highest PH by 73.7 cm, while Parnian and Goldasht genotypes had no significant difference. The highest RtoSH and BrN were recorded in Kazak genotype (Table 2).

ANOVA results showed a significant interaction effect of irrigation×genotype on safflower RDW and RL.

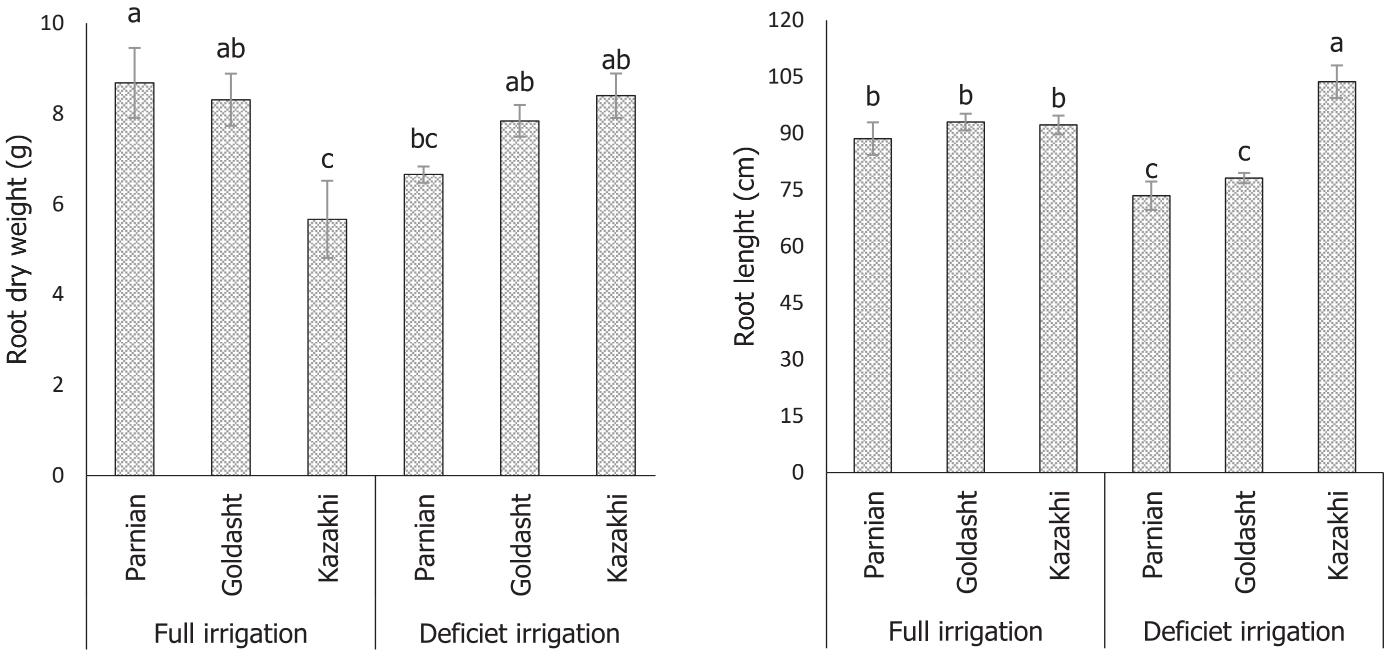
Table 2. Effect of different irrigation regimes and varieties on safflower root system and shoot traits

Variability	Root dry weight (g)	Root length (cm)	Root diameter (mm)	Shoot height (cm)	Root/shoot (cm/cm)	Branch number
Year						
2019-20	7.41^{a}	89.7^{a}	8.55^{a}	71.9^{a}	1.26^{a}	4.33^{a}
2020-21	7.77^{a}	86.6^{a}	8.31^{a}	70.4^{a}	1.23^{a}	4.06^{a}
Irrigation regime						
Full	7.55^{a}	91.2^{a}	8.14^{b}	74.1^{a}	1.24^{a}	4.22^{a}
Deficit	7.63^{a}	85.0^{b}	8.71^{a}	68.3^{b}	1.25^{a}	4.17^{a}
Genotype						
Parnian	7.67^{ab}	81.0^{b}	7.76^{b}	71.2^{ab}	1.14^{b}	3.50^{c}
Goldasht	8.07^{a}	85.5^{b}	8.25^{b}	68.5^{b}	1.25^{ab}	4.08^{b}
Kazak	7.03^{b}	97.9^{a}	9.27^{a}	73.7^{a}	1.34^{a}	5.00^{a}

Values are expressed as mean (n=3). Values with the same letter indicate no significant difference according to Duncan's multiple range test (p=0.05)

Under full irrigation treatment, the highest and lowest RDW were observed in Parnian and Kazak genotypes by 8.6 and 5.6 g, respectively. Application of drought stress decreased safflower root biomass in Parnian and Goldasht genotypes. Drought stress affects many vital plant growth and development processes, such as cell elongation and cell division, photosynthesis rate and root structure, and consequently reduces crop growth and yield (Hodge et al., 2009; Li et al., 2015). The decreased root and shoot biomass as well as seed and oil yield of different genotypes under deficit irrigation stress have previously been reported (Joshan et al., 2019; Rahmani et al., 2019). RDW of Kazak genotype showed a significant increase under deficit irrigation. However, no significant difference was observed between different genotypes (Figure 3a). RL of Kazak genotype also increased significantly compared to normal conditions. In Parnian and Goldasht genotypes, RL decreased significantly under deficit irrigation. Root is the first plant organ to face drought stress, playing a key role in absorbing water and nutrients (Ramamoorthy et al., 2017). Plant root biomass production and expansion are affected by drought stress (He et al., 2017). However, there was no significant difference between different genotypes under stress-free conditions (Fig. 3b). The highest RtoSH ratio was observed in Kazak genotype under deficit irrigation, while other genotypes showed no significant differences in various irrigation treatments (Fig. 4a). In this study, the measurement of root-related traits showed decreased RDW and RL for different safflower genotypes under deficit irrigation. Kazak genotype had the greatest root biomass under deficit irrigation and showed the highest RtoSH ratio. Water shortage restricts the growth of shoots more than roots and increasing RtoSH ratio responds to drought stress in plants as a mechanism to increase tolerance to water shortage conditions. Under drought conditions, deeper soil layers contain a considerable amount of available moisture for plants. Therefore, increasing the plant RL and expansion to the subsoil is a required trait in drought-tolerant genotypes (Figueroa-Bustos et al., 2018; Ramamoorthy et al., 2017).

The results showed that irrigation regime had a significant effect on safflower SY and yield components. In the deficit irrigation regime, seed number per plant (SN) decreased by 74%. Safflower thousand seeds weight (TSW) was also decreased by 1.7g under stress conditions. Diminished components under deficit irrigation resulted in a 70% reduction in SY. However, seed OC showed no significant difference in different irrigation regimes (Table 3).



(a) (b)

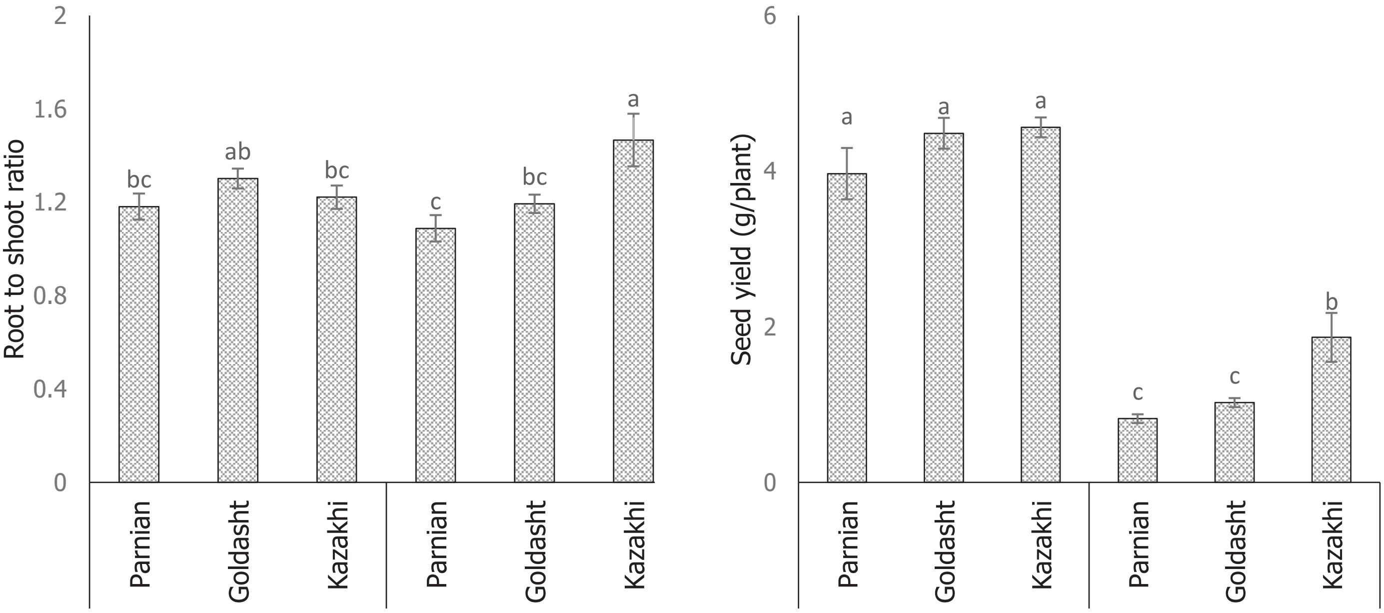
Values expressed as means, bars indicate the standard deviation of three replicates. Columns marked by the same letter indicate means not differing significantly from one another according to Duncan's multiple range test (*p*=0.05)

Figure 3. Interaction effect of irrigation regime and genotype on safflower RDW (a) and RL (b)

Table 3. Effect of different irrigation regimes and varieties on safflower SY and OC

Variability	Bull number (per plant)	Seed number (per plant)	Thousand seeds weight (g)	Seed yield (g/plant)	Oil content (%)
Year					
2019-20	4.22^{a}	91^{a}	39.9^{a}	2.91^{a}	28.2^{a}
2020-21	3.83^{a}	80.3^{b}	39.3^{a}	2.67^{a}	27.4^{b}
Irrigation regime					
Full	4.18^{a}	136^{a}	43.2^{a}	4.34^{a}	27.9^{a}
Deficit	3.88^{a}	35.3^{b}	36.1^{b}	1.24^{b}	27.8^{a}
Genotype					
Parnian	3.78^{a}	73.9^{b}	40.8^{a}	2.39^{c}	27.0^{c}
Goldasht	3.77^{a}	82.5^{b}	41.2^{a}	2.76^{b}	27.8^{b}
Kazak	4.54^{a}	100^{a}	36.9^{b}	3.21^{a}	28.7^{a}

Values are expressed as mean (n=3). Values with the same letter indicate no significant difference according to Duncan's multiple range test (p=0.05)



(a) (b)

Values expressed as means, bars indicate the standard deviation of three replicates. Columns marked by the same letter indicate means not differing significantly from one another according to Duncan's multiple range test (*p*=0.05)

Figure 4. Interaction effect of irrigation regime and genotype on safflower root/shoot ratio (a), and SY (b)

Safflower genotypes showed significant differences in SY and other components. Parnian and Goldasht genotypes had the highest TSW by 40.8 and 41.2g, respectively. However, the highest number of seeds per plant and the highest SY were recorded in Kazak genotype. The highest OC was observed in Kazak genotype by 28.7% (Table 3). SY was also significantly affected by irrigation×genotype interaction. Under normal conditions, there was no significant difference in SY of genotypes. Drought stress during pollination and seed filling can reduce the effective inoculation rate, presumably due to the dryness of pollen grains and

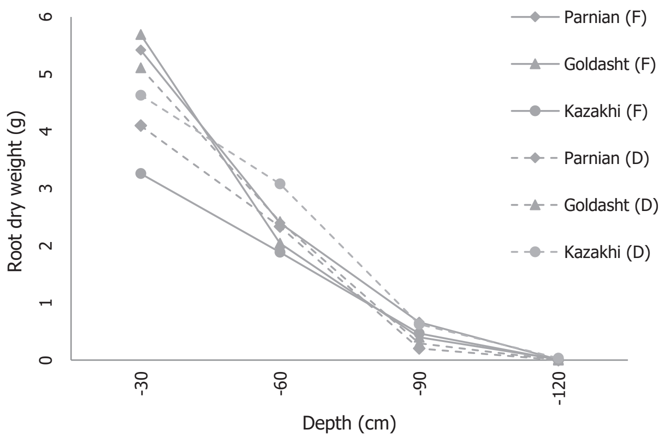


Figure 5. Alteration of RDW of safflower genotypes in the full (solid line) and deficiet (dashed line) irrigation regimes and depths

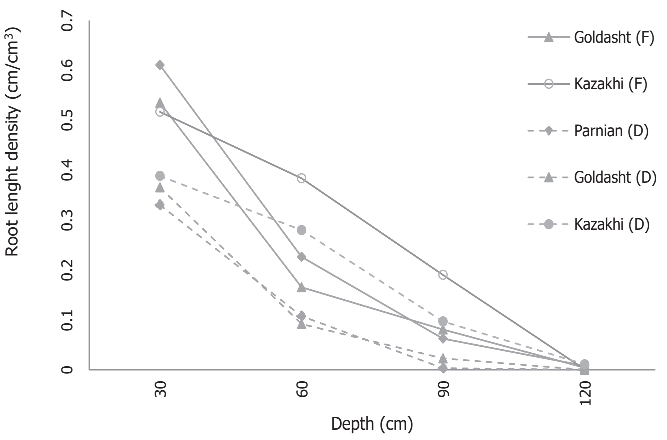


Figure 6. Alteration of RLD of safflower genotypes in the full (solid line) and deficiet (dashed line) irrigation regimes and depths

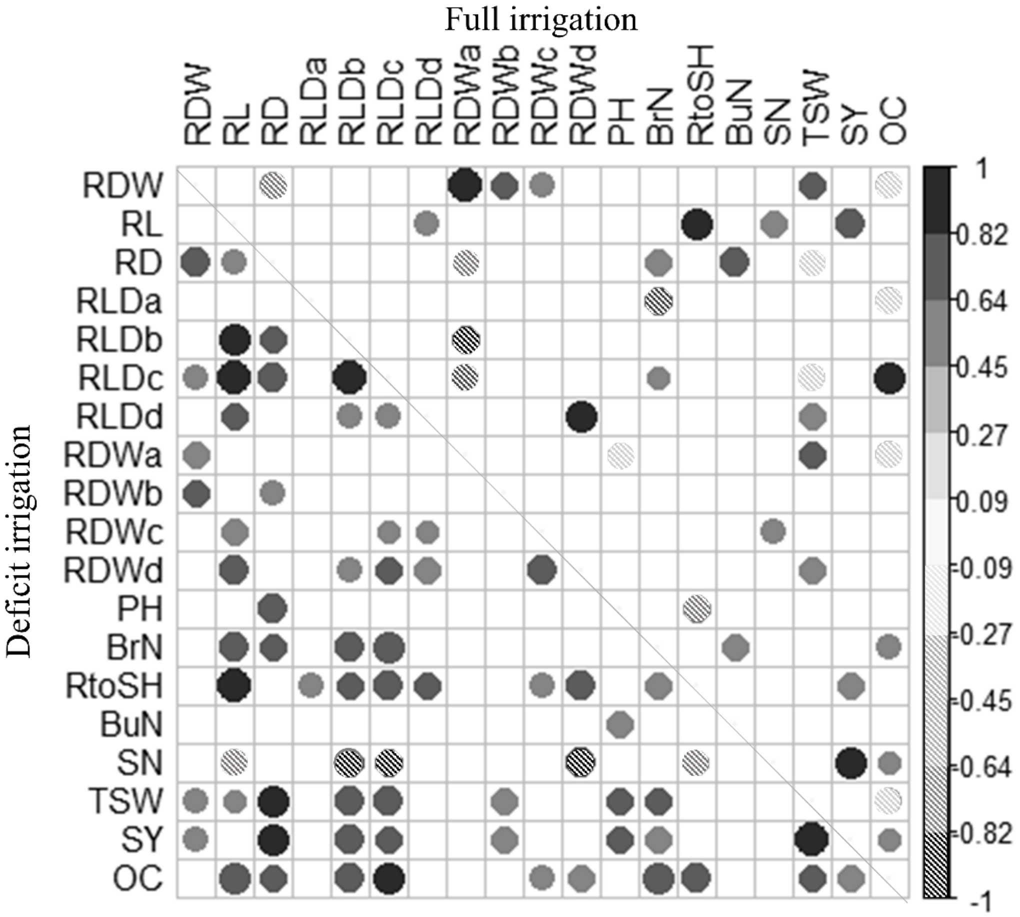
pistil stigma, as well as the weakening of seed filling. These factors lead to a decrease in the number and weight of a thousand seeds, ultimately resulting in a significant decrease in grain yield. However, Kazak had a significantly higher performance than other genotypes in deficit irrigation (Fig. 4b). Crop yield stability in different environmental conditions is a priority trait for breeding programs. Drought stress severely reduces SY and yield components of oilseed crops (El Sabagh et al., 2019). A robust and well-developed root system should contribute to maintaining SY stability under drought-stress conditions (Polania, Poschenrieder, et al., 2017). Therefore, a parallel evaluation of root traits and SY in normal and stress conditions is needed to determine the relationship between root traits and SY under drought. The measured yield of different safflower genotypes showed a dramatic reduction in SY under deficit irrigation. Under normal conditions, there was no significant difference in SY of genotypes while Kazak had a significantly higher yield than the other genotypes under deficit irrigation. According to a previous study, a deep root system leads to better SY by improving water uptake from soil deeper layers (Li et al., 2015).

The changes in safflower root biomass under different irrigation regimes at varying depths are summarized in Figure 5. Under the full irrigation regime, the highest root biomass at a depth of 30 cm was observed in Goldasht and Parnian genotypes, while the lowest related value was recorded in Kazak. Increasing the soil depth caused a significant decrease in safflower root biomass, and no significant difference was observed between genotypes at the depth of 60-120 cm. Under deficit irrigation, Goldasht and Kazak genotypes showed the highest root biomass at the depth of 30 cm. The decrease in root biomass with increasing depth was less steep in Kazak genotype, hence, the highest RDW at the depth of 60-90 cm was observed in this genotype.

Under normal conditions, Parnian genotype had the highest root length density (RLD) at a depth of 30 cm. By increasing the depth up to 60 cm, RLD in Parnian and Goldasht genotypes decreased by more than 60%, while the reduction rate was only 25% for Kazak genotype. Similarly, RLD in Kazak was significantly higher than other genotypes at the depth of 90 cm. A similar trend in different genotypes was observed under deficit irrigation. Kazak genotype showed the highest RLD at a depth of 30-90 cm. However, there was no significant difference in RLD of different genotypes at a depth of 120 cm (Figure 6).

The expansion and distribution of plant roots in different soil layers is more important than increasing length and biomass (Zhang et al., 2019). Further distribution of plant roots in deeper soil layers is more effective in increasing drought tolerance. In other words, the genotype with higher RLD in deeper soil layers will be more tolerant to drought stress (Thangthong et al., 2018). Under normal conditions, Parnian genotype had the highest RLD at a depth of 0-30 cm. However, in the deeper layers (30-90 cm), the highest value was observed in Kazak genotype. Under deficit irrigation, the highest RLD was recorded in Kazak genotype at all depths. Increasing root penetration into deeper soil layers as well as reducing RLD in topsoil and raising the level in deeper layers is the primary mechanism of plants to avoid drought stress (Ramamoorthy et al., 2017; Thorup-Kristensen et al., 2020).

In this study, the correlation of root system traits with safflower yield and other components under different irrigation regimes was evaluated (Figure 7). Under normal conditions, a positive significant



*RDW: root dry weight, RL: root length, RD: root diameter, RLDa: root length density 30cm, RLDb: root length density 60 cm, RLDc: root length density 90 cm, RLDd: root length density 120 cm, RDWa: root dry weight 30 cm, RDWb: root dry weight 60 cm, RDWc: root dry weight 90 cm, RDWd: root dry weight 120 cm, PH: plant height, BrN: branch number, RtoSH: root-to-shoot ratio, BuN: bull number, SN: seed number, TSW: thousand seed weight, SY: seed yield, OC: oil content

Figure 7. Pearson correlation coefficients of the measured traits of safflower genotypes in the full irrigation (upper triangle) and deficit irrigation (lower triangle) regimes*

correlation was observed between RDW with TSW (0.68) as well as RL with SN and SY (0.59, 0.72). However, no significant correlation was obtained between root biomass distribution and RLD with SY. There was a negative correlation between RDWa and RLDa (30 cm) with OC (upper triangle). In the deficit irrigation, a higher correlation was recorded between root system traits and yield components. TSW and SY also had a positive significant correlation with RDW (0.58 and 0.57) and RD (0.83 and 0.84). SY significantly correlated with RLD at 60-90 cm depth (0.72 and 0.68). In addition, OC showed a positive significant correlation with RLD at the lower triangle.

Determining the correlation between root traits and SY requires further study to identify the traits associated with increased drought tolerance. RLD, total root biomass, and RtoSH are critical traits to improve plant tolerance to drought stress and associated with SY under drought conditions (Ramamoorthy et al., 2017). Li et al. demonstrated that total RL and surface area play a crucial role in drought tolerance and are positively correlated with SY under water shortage conditions. Therefore, these can be considered crucial traits in selecting superior genotypes in drought tolerance (Li et al., 2015). The results showed a positive significant correlation of SY with RDW and RD. Moreover, under drought conditions, SY significantly correlated with RLD at 60-90 cm depth. These observations show that a vigour root system is crucial for better performance even in normal conditions. Aside from a robust root system, penetration into deeper soil layers is essential to increase stress tolerance and maintain production stability under drought conditions (Polania, Rao, et al., 2017).

CONCLUSION

In conclusion, a 2-year field trial showed that genotypes with vigour and deeper root systems were generally more drought tolerant. Under the deficit-irrigation regime, the highest SY was recorded in Kazak genotype with a vigorous and deep root system. In other words, the production sustainability under stress conditions was higher in this genotype. Under deficit irrigation, RDL in the deep soil layer showed a positive correlation with SY. This underscored the role of a robust root system, contributing to better water uptake from deep soil layers and maintaining performance stability. The results showed that among the measured root traits, RDW, RD, and RLD in deeper soil layers should be a priority for breeding programs to develop drought-tolerant genotypes. However, the selection of superior genotypes based only on root traits and without combination with profitable yield traits would not achieve the desired result.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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