RESEARCH ARTICLE Legume Research- An International Journal, Volume 44 Issue 3: 308-314 (March 2021)

Root-based Responses of Well-watered and Water-stressed Chickpea (*Cicer arietinum* L.) Genotypes Varying for Drought Tolerance and Biomass

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10.18805/LR-593

ABSTRACT

Background: Chickpea is a pivotal grain legume crop and is grown in rain-fed conditions where its production has been challenged by drought.

Methods: To understand precisely the root-based responses to well-watered (WW) and water-stressed (WS) treatments, 14 chickpea (*Cicer arietinum* L.) genotypes differing in drought tolerance and biomass were studied in 100-cm cylinders under glasshouse conditions.

Result: The genotypes exhibited significant variations in rooting depths ranging from 84.5 to 100.3 cm and 78.7 to 121 cm in WW and WS treatments, respectively and root biomasses varied from 0.23 to 1.01 g and 0.38 to 0.91 g. The average root biomass of drought-tolerant genotypes was 61.3% in WS treatment and 64.4% in WW treatment higher than that of drought-sensitive genotypes. Moreover, genotype with high biomass revealed greater root biomass and deeper rooting than the genotype with low biomass in both treatments. The root biomass in the deeper soil profile differed between drought-tolerant and drought-sensitive genotypes and was generally greater in WS compared to WW treatment. Overall, screening the variability in root features of chickpea genotypes with varying levels of drought tolerance and biomass contributes to new insights for understanding drought adaptation mechanisms and the improvement of new cultivars with superior root traits in breeding programs.

Key words: Biomass, Chickpea, Drought tolerance, Root biomass, Rooting depth, Water stress.

INTRODUCTION

Chickpea is the second most important pulse crop after the common bean (*Phaseolus vulgaris* L.) and is grown on area of about 17.8 million ha globally with a total annual production of 17.2 million t in the world (FAOSTAT 2018). It is a nutritionally important resource in the human diet, serving as a good source of protein, vitamins, essential amino acids, carbohydrate, crude fiber, linoleic acid and oleic acid, with protein quality, as regarded to be better than other pulses (Jukanti *et al.* 2012). Globally, chickpea is mainly grown in arid and semi-arid regions where rain-fed conditions may induce drought and cause significant yield losses (Kashiwagi *et al*., 2006). Chickpea production losses in under drought have been predicted worldwide to be about 33%; however, 19% of these losses can be reduced through enhanced tolerance improvement (Kashiwagi *et al.* 2015). Hence, adaptive crop improvement with better drought tolerance may have the advantage of minimizing or preventing yield losses.

Drought stress has effects on several physiological, morphological, phenological and biochemical traits of crops in both the shoot and root systems (Bhaskarla *et al.* 2020). In the root system architecture, rooting depth and root biomass have been linked to drought avoidance and tolerance in drought-prone regions (Saxena *et al.* 1993; Serraj *et al.* 2004; Kashiwagi *et al.* 2015). Viewing importance in drought avoidance, the deep-rooted genotypes have been proposed in rain-fed conditions where crop growth relies on seasonal rainfall to maintain yield improvement since deep rooting would be beneficial for water exploitation in deep soil profile under water stress (Kashiwagi *et al.* 2005). It was suggested that yield and yield components can be enhanced through superior root traits (Karadavut and Sözen, 2017). However, root system architecture has been largely neglected by researchers, that have primarily focused on above-ground agronomic traits (Canales *et al.* 2019; Fikre *et al.* 2019). The main reasons have been the technical difficulties of simultaneously and accurately measuring whole root system growth in a large set and the requirement of much time and intensive labor, particularly for field and greenhouse-based root phenotyping methods (Akman *et al.* 2012; Bontpart *et al.* 2020).

The main objective of this work is to investigate variations in the root traits of chickpea genotypes varying for drought tolerance and biomass under water-stressed and well-watered conditions and furthermore to eventually obtain

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How to cite this article: Akman, H. (2021). Root-based Responses of Well-watered and Water-stressed Chickpea (*Cicer arietinum* L.) Genotypes Varying for Drought Tolerance and Biomass. Legume Research. 44(3): 308-314. DOI: 10.18805/LR-593.

Submitted: 19-10-2020 **Accepted:** 25-12-2020 **Online:** 09-03-2021

a view of the drought adaptation mechanisms underlying chickpea root growth.

MATERIALS AND METHODS

This study was carried out to elucidate the root and shoot characteristics in chickpea genotypes and the relationship between root features under water-stressed (WS) and well-watered (WW) treatments in 2019 crop season at Sarayönü Vocational School, Selçuk University, Konya, Turkey. It was established in completely randomized plot design as three replicates. A set of 14 chickpea genotypes varying for drought tolerance/sensitivity and high/low biomass was chosen as the material to represent a wide range of diversity in the experiment (Table 1). Non-Turkish originated genotypes were sourced from the US Department of Agriculture Germplasm Resource Unit in the USA (https://www.ars-grin.gov). Three adaptive Turkish genotypes were also selected for the study.

The climate of the glasshouse during plant growth (39 DAS) was measured with a datalogger (T and D Corporation/TR-74Ui) recording data in five-minute intervals. The average temperature was 24.5°C day/13.1°C night, respectively. The mean relative humidity was approximately 37.4% day/ 67.7% night depending on the season with a photosynthetic photon flux density of 240.9 µmol $m^{-2}s^{-1}$ during the day length.

The soil used in the experiment, taken from the field (0-40 cm), was clay-loam with low organic matter (1.67%) and high levels of $CaCO_3$ (23.7%) and Ca (5451 mg/kg). EC was 0.6 mmhos/cm. Soil pH was 7.7 and no salinity problems (0.02%) were observed. Levels of P_2O_5 (45.8 kg/ha), Zn (0.67 mg/kg) and Mn (8.13 mg/kg) were low. In addition, K_2O (1265 kg/ha), Mg (464.4 mg/kg), Fe (5.1 mg/kg) and Cu (3.1 mg/kg) were found as adequate.

The study was conducted in glasshouse conditions from April 24 to June 1. Seeds were sown into long PVC cylinders (100 cm in depth and 12 cm in diameter), which were filled by field soil (Fig 1a). Drought stress was initiated on May 13. The plants were harvested in early bloom when one open flower had appeared on the chickpea plant (R1). The whole root system was washed and cleaned and then measured for maximum root length as an estimate of rooting depth (Fig 1b). The root system was then sliced into three sections of 0-30 cm, 30-60 cm and +60 cm to calculate root biomass distribution (RBD) at each of the depths of the root system. Root biomass was recorded after drying at 80°C for three days. The RBD in each depth layer was obtained as a percentage by dividing the whole root biomass by the biomass of each section. For above-ground morphological characteristics, shoot height was measured with a ruler and branch number was manually counted.

Effects of treatments on root and shoot traits were analyzed by analysis of variance (General Linear Model procedure) and Tukey's pairwise comparison test using Minitab Version 16 (Minitab Inc., State College, PA, USA). Regression analyses were performed in Microsoft Excel (Excel version in Microsoft Office 2016 for Windows) for significantly correlated traits.

RESULTS AND DISCUSSION

Significant variations were found between treatments and cultivars in terms of root and shoot traits per plant such as root biomass (RB), rooting depth (RD), root biomass distribution (RBD) in different depths, shoot height (SH) and branch number (BN) ($P<0.05$ and $P<0.01$) (Table 2).

Rooting pattern

Rooting depths of the genotypes varied from 84.5 to 100.3 cm in the WW and 78.7 to 121 cm in the WS treatment, indicating lower values in control plants compared to the WS treatment (Fig 2a). Similarly, previous researchers observed significant variation among chickpea genotypes for rooting depth (Kumar *et al.* 2012) and blackgram genotypes (Parakash *et al.* 2018). This study showed that the magnitude of variation in average rooting depth under WS treatment was higher than that in the case of WW treatment. An earlier study reported a similar range of rooting

Table 1: Accession number, trait, name and origin of the studied chickpea genotypes.

Inventory	Trait	Name	Origin
PI 451656	Drought-tolerant	RPIP 12-100-04092	Morocco
PI 451161	Drought-tolerant	RPIP 12-071-03831	Iran
PI 451005	Drought-tolerant	RPIP 12-071-03493	Iran
PI 450908	Drought-tolerant	RPIP 12-071-01992	Iran
PI 450806	Drought-tolerant	RPIP 12-069-01293	India
PI 451500	Drought-tolerant	RPIP 12-071-06928	Iran
PI 451287	Drought-tolerant	RPIP 12-071-04002	Iran
PI 193482	Drought-sensitive	-	Ethiopia
PI 360599	Drought-sensitive	RPIP 12-071-06359	Iran
W6 26286	High biomass	ICARDA 140101	Tajikistan
W6 25917	Low biomass	ICC 1422	India
Sarı-98	Adaptive-	Cultivar	Turkey
Azkan	Adaptive-	Cultivar	Turkey
Seçkin	Adaptive-	Cultivar	Turkey

Table 2: Analysis of variance for root and shoot traits of chickpea genotypes.

Sources	DF	Mean sequare						
		Shoot height	Branch number	Rooting depth	Root biomass	RBD (0-30 cm)	RBD (30-60 cm)	RBD +60 cm
Genotype	13	52.29**	3.09**	233.2**	0.255**	221.5**	168.4**	40.0*
Treatment	1	646.3**	10.7**	429.8**	0.546**	2866.2**	835.8**	593.3**
G×T	13	15.61**	0.61**	149.1**	0.052**	173.6**	99.1*	40.9*
Error	56	5.440	0.18	19.9	0.022	67.4	48.2	18.6
Total	83							

**P< 0.01, *P< 0.05.

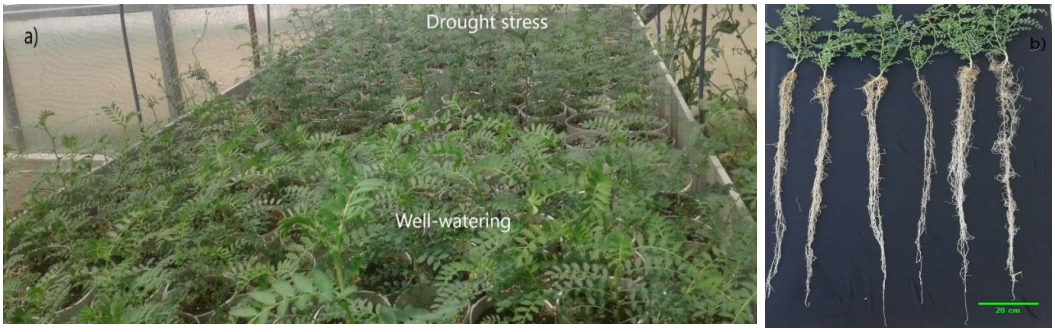


Fig 1: (a) is the image for a representative of well-watering (WW) and drought stress (WS) treatments withheld water for 19 d under glasshouse conditions. Genotypes were grown in 100-cm PVC cylinders. Image **(b)** indicates the washed and cleaned whole root system architecture of various chickpea genotypes.

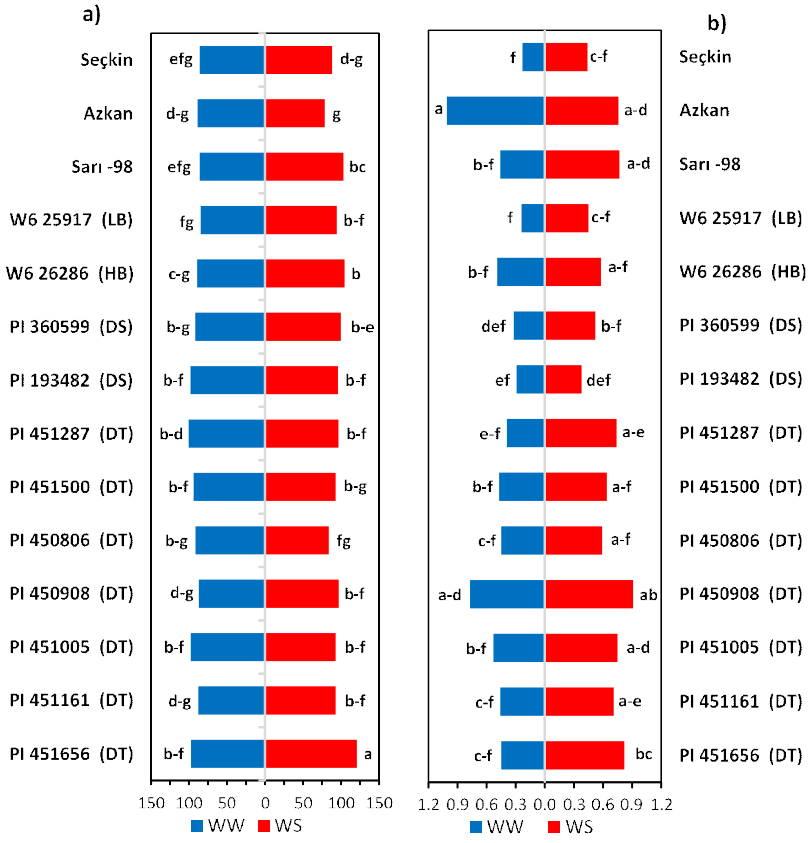


Fig 2: (a) is representative of rooting depth and **(b)** is representative of root biomass of chickpea genotypes in WW and WS treatments. Note: Means that do not share a letter are significantly different (P< 0.01). P indicates level of significance. Abbreviations: LB- Low biomass; HB- High biomass; DT- Drought-tolerant; DS- Drought-sensitive.

depth as in this study under WS treatment, finding that the roots reached depths of 92 to 122 cm at full bloom stage under rain-fed conditions with those plants being deeper than irrigated chickpeas (Kumar *et al.* 2012).

This study also found that drought-sensitive genotypes did not have shallower rooting depths compared to drought-tolerant genotypes; however, genotype W6 26286 with high biomass had relatively higher rooting depth in both treatments than W6 25917 with low biomass. The importance of deep rooting on yield has been shown in different crops. For example, yield of a deep-rooted sorghum genotype increased up to 20% under water-deficit conditions (Jordan *et al.* 1983). Furthermore, deep rooting in chickpea has been reported to confer yield advantages under rain-fed conditions (Sinclair 1994; Soltani *et al.* 1999; Kashiwagi *et al.* 2015). In the study, the drought-tolerant genotype PI 451656 had the deepest rooting among all genotypes in the WS treatment. Moreover, Azkan had a shallow root system and Sarı-98 had a deep

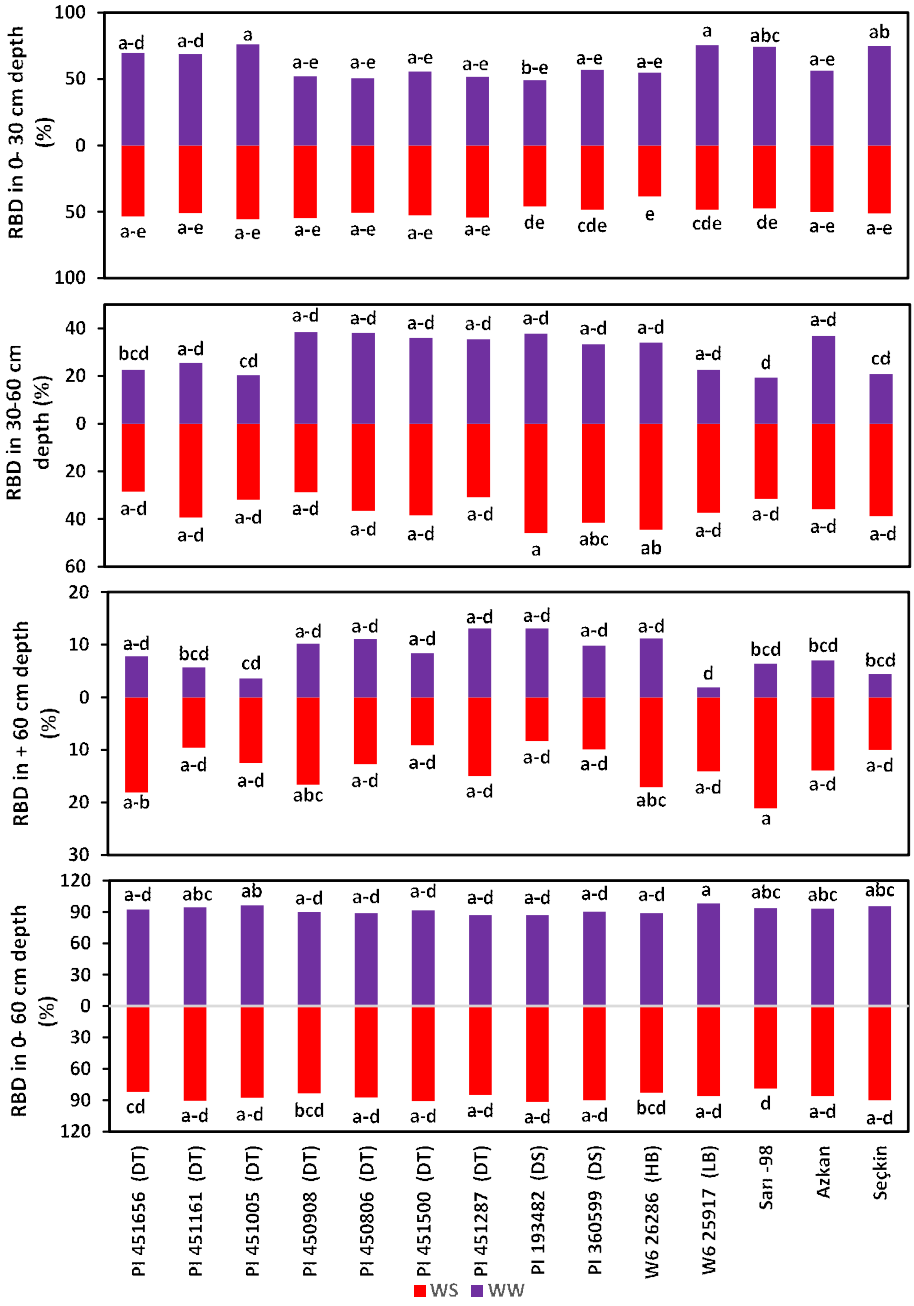


Fig 3: Root biomass distribution (RBD in 0-30 cm (P<0.01), 30-60 cm (P<0.05), +60 cm (P<0.05) and 0-60 cm (P<0.05) rooting depth of chickpea genoytpes in WW and WS treatments. Note: Means that do not share a letter are significantly different (P< 0.01). P indicates level of significance. Abbreviations: LB- Low biomass; HB- High biomass; DT- Drought-tolerant; DS- Drought-sensitive.

root system among the cultivars under WS treatment. This genotype may therefore be beneficial in terms of adaptive potential under drought; however, future studies are needed to more fully elucidate the understanding clearly of this event.

Root biomass

Considerable variation in root biomass was observed among chickpea genotypes in both WW and WS treatments, varying from 0.23 to 1.01 g and 0.38 to 0.91 g, respectively (Fig 2b). The average root biomass of genotypes was increased in WS compared to WW treatment (Figs 2 and 5). Earlier studies showed that drought stress during the vegetative growth stage increased root development but reduced growth rate (Ludlow and Muchow 1990; Kashiwagi *et al.* 2005). The findings of Kumar *et al.* (2012) were in agreement with the results of this study. They revealed that root biomass in five chickpea genotypes was higher in drought-prone areas than in irrigated areas. A previous study showed that a drought-resistant chickpea genotype possessed 30% higher root biomass than a drought-sensitive genotype under drought stress (Saxena *et al.* 1993). The result was in conformity with the findings of the present study. The average root biomass of drought-tolerant genotypes was 61.3% and 64.4% higher than that of drought-sensitive genotypes under WS and WW treatments, respectively (Fig 2b and 5). The highest root biomass in the WS treatment was observed in drought-tolerant genotype PI 450908, which also maintained a relatively high value in the WW treatment. This genotype may be evaluated for the improvement of new cultivars with superior roots in breeding programs. Comparing the root biomass values of cultivars, Seçkin had similar values to those of drought-sensitive genotypes, while the root biomass of Azkan and Sarı-98 was similar to that of drought-tolerant genotypes. Genotypes with high biomass revealed greater root biomass than genotypes with low biomass in both treatments. This could have resulted from the investment of more photosynthetic assimilates into the roots in genotypes with high shoot biomass.

Root biomass distribution

The results of this study further suggested that there was significant variation among genotypes in terms of RBD in rooting depths of 0-30 cm, 30-60 cm, +60 cm and 0-60 cm. Significant rates of root biomass accumulated at 0-60 cm soil profile as 91.1% in WW and 86.6% in WS (Fig 3). The RBD at 0-30 cm varied from 49.2% to 76.2% in WW and 38.4% to 55.6% in WS (Fig 3), proving 11.6% lower in WS plants compared to plants undergoing WW treatment. Zhou *et al.* (2020) indicated that about 73.2%-82.3% of mung bean root were in 0-20 cm soil layer.

Also, drought-sensitive plants had lower RBD values than drought-tolerant genotypes, particularly in the WS treatment. This finding was altered in rooting depths of 30-60 cm, with higher values in drought-sensitive genotypes that accumulated higher biomass than drought-tolerant

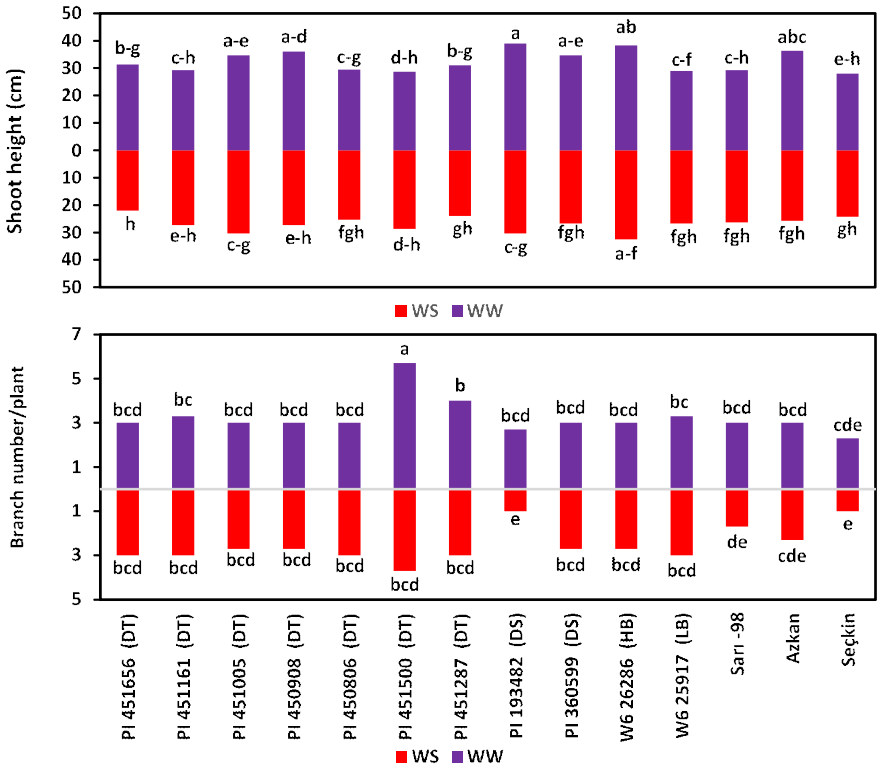


Fig 4: Shoot height and number of branches of chickpea genoytpes in WW and WS treatments. Note: Means that do not share a letter are significantly different (P< 0.01). P indicates level of significance. Abbreviations: LB- Low biomass; HB- High biomass; DT- Drought-tolerant; DS- Drought-sensitive.

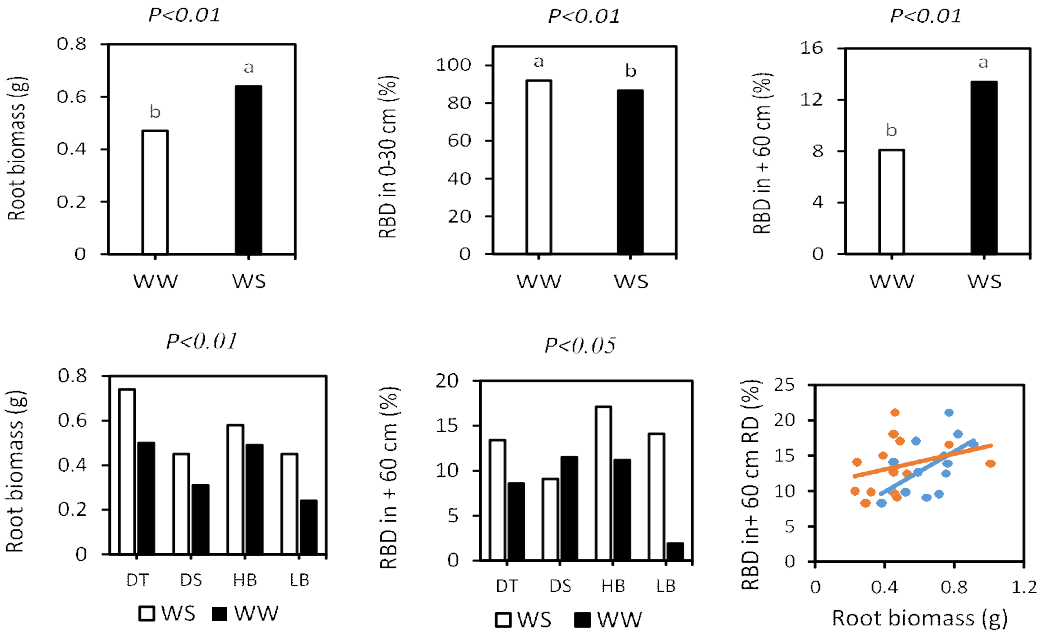


Fig 5: Root traits in response to WW and WS in contrasting chickpea genotypes.

Note: Means that do not share a letter are significantly different. P indicates level of significance.

Abbreviations: LB- low biomass; HB- high biomass; DT- drought-tolerant; DS- drought-sensitive.

A regression analysis of red line in WW (y = 5.5547x + 10.826; r^2 = 0.09) and blue line in WS (y = 14.275x + 4.1906; r^2 = 0.35).

genotypes. Moreover, RBD in this rooting depth was higher in WS plants than plants in WW treatment. This trend aligned with RBD in the +60 cm rooting depth, revealing a high RBD in WS plants compared to WW. These results clearly showed that almost all genotypes under WS treatment accumulated higher root biomass in the +60 cm soil profile to access more water in the deep soil under drought conditions. Water uptake in deep soil (90-120 cm) was reported to ensure better drought adaptation (Ramamoorthy *et al.* 2017). Previous researchers also indicated that root biomass in deep soil had a positive effect on chickpea yield in water-deficit conditions (Kashiwagi *et al.* 2006; Ramamoorthy *et al.* 2017). In the present study, drought-sensitive genotypes had lower root biomass in rooting depths of +60 cm than drought-tolerant genotypes under WS treatment. This study further showed a stronger relationship between whole root biomass and RBD at +60 cm under WS treatment (r^2=0.35) than WW treatment (r^2=0.09). Genotypes such as Sarı-98, PI 451656 (drought-tolerant), PI 450908 (drought-tolerant), PI 451287 (drought-tolerant) and W6 26256 (high biomass), having higher root biomass in deep soil, may be advantageous for sustaining more drought adaptation under drought conditions.

Shoot height and branch number

The present study showed that PI 451005 (drought-tolerant), PI 193482 (drought-sensitive) and W6 26286 (high biomass) had taller shoot heights than other genotypes in both treatments, while Azkan and PI 450908 had high values only in the WW treatment (Fig 4). It was stated that plant height and number of branches per plant were positively correlated with seed yield (Singh *et al.* 1990; Shamsi *et al.* 2010). Maximum number of branches was generally found in PI 450806 (drought-tolerant) and PI 451287 (drought-tolerant) in both treatments, while minimum values were obtained from PI 193482 (drought-sensitive) and Seçkin. Drought treatment has been shown to lead to severe reductions in shoot height and number of branches. A previous study revealed that chickpea yield was severely decreased by a greater reduction in the number of pods and branches per plant under water stress (Yadav *et al.* 2006). Similarly, the average results of this study were influenced in WW treatment compared to WS treatment with values respectively ranging from 32.5 to 27.0 cm for shoot height and 3.2 to 2.5 for branch number.

CONCLUSION

Chickpea utilizes various mechanisms via both morpho-physiological and biochemical adjustments to reduce the harmful effects of drought stress. A comparison of different treatments in chickpea genotypes varying for drought tolerance/sensitivity and biomass is therefore significant in perceiving the mechanisms used by crops to deal with adverse cases of water stress. The present study shows that chickpea genotypes that differ concerning drought tolerance and biomass demonstrate notable and different responses to water stress. For future studies, we propose that these different genotypes should be molecularly assessed to obtain new views regarding the genetic possibility of increasing drought tolerance for chickpea improvement in breeding programs.

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