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Original Paper

Roots and shoot traits contributing to drought tolerance from germination to maturity stages for bread wheat

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Drought is the major bottleneck for food security worldwide. Identifying reliable selection traits that sustain plant growth and productivity under water-deficient conditions is essential for releasing resilient drought tolerant wheat genotypes. Herein, in order to identify the best combination of drought tolerance related-traits, this study was carried out to assess the effect of 21 shoot and 17 root secondary traits on grain yield of 40 bread wheat genotypes from germination to maturity stages under laboratory, greenhouse and field conditions. In this research, germination rate (GR), mean germination time (MGT), roots weight (RW), roots number (RN) and Root : Shoot ratio (R/S) were relevant for discriminating drought tolerant genotypes, expressing negative moderate correlation ($r \sim -0.5$) and explaining a significant part of yield variation (8 to 30%). Grain number (GN) was the most important agronomic trait (r ~0.70–0.94) which explained up to 88% of yield variation. This latter effect is reinforced by the indirect effects of productive spikes number (FSN), thousand kernel weight (TKW) and grains number per fertile spike (GNFS). The biomass (BY), ground cover (GC), spike (SL) and pedoncule lengths (PL) were also relevant, especially under severe stress ($r = 0.4$–0.6; $\beta = 0.33$–0.40); while the canopy temperature depression (CTD) was determinant under moderate stress ($r = -0.45$; $\beta = -0.46$). Combined selection for these traits will be effective to improve the process of developing high-yielding bread wheat varieties adapted to drought prone areas.

Keywords: bread wheat, drought tolerance, combined selection, roots traits, shoots traits

1 Introduction

Bread wheat (*Triticum aestivum* L.) is among key staple crops widely grown for sustainable food security, providing one-third of the burgeoning world population with calories, proteins, in addition to fiber, fats, vitamin B, zinc, calcium, and iron. In the last decades, wheat yield is increasing at a low rate of 1.1% p.a., insufficient to meet the increasing demand projected to feed 9.1 billion people in 2050. This gap represents a serious challenge for future food security and is emphasized by the current climatic context, especially drought (Nakhforoosh et al., 2014). More than ever, drought stress continues to be the most prevalent threatening environmental stress for agriculture production, causing up to 90% of wheat yield losses. Climate change forecasts prevent more severe drought episodes in terms of intensity and occurrence. Simulation studies have reported an increase in yield loss risk by 9% for wheat by the end of 21st century (Leng and Hall, 2019). Therefore, future wheat demand will need to be fulfilled through the development of resilient varieties to drought, against a background of dwindling arable lands and water resources (Mwaszingeni et al., 2016).

However, drought tolerance is a complex quantitative trait, depending on various interacting physiological, biochemical and morphological processes occurring through the crop cycle, within a large size of wheat genome. Breeding for drought tolerance is further complicated by the unpredictability of drought

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regime in its timing, interlude, severity, the genotype × environment interaction of related traits and the growth stage of the plant (Panda et al., 2021).

Screening germplasm for drought resistance has been conducted earlier in various corners of the world. However, only a few drought-tolerant varieties are yet recognized resulting from unsuitable screening methods and selection criteria (Panda et al., 2021). Many traits have been reported to improve resistance to drought ranging from yield components, shoot and root system architecture to physiological metrics related to water status. Taking advantage from high throughput sequencing technologies and marker techniques advances, a large number of QTLs has been identified for drought tolerance related-traits mainly on the chromosomes 2B, 3A, 4A, 4B, 7A and 7B; especially for physiological traits, agronomic components and root traits (Gupta et al., 2017). Yet, no single trait was identified for its unique and dominant contribution to drought resistance (Monneveux et al., 2012; Mwadzingeni et al., 2016). Many drought-responsive genes are involved differing in expression at different growth stages and generally making a minor contribution to the trait (Sallam et al., 2019). Moreover, efficient traits identified in a given environment are not equally useful in other water-limited environments. Consequently, breeding for drought tolerance requires the adoption of integrated multi-traits selection to improve grain yield and encounter the environment specific nature of drought. The success of secondary trait-based breeding for drought tolerance will depend on the extent of genetic variability and heritability of the trait and its significant correlation with final yield. Moreover, the main challenge in the process will be the development of rapid, accurate, cost-effective and preferably non-destructive selection methods which can be applied for high-throughput phenotyping on the field (Nakhforoosh et al., 2014; Mwadzingeni et al., 2016).

In this perspective, the present study aims to identify the best combination of agro-morphological and physiological shoot and root traits associated with drought tolerance at different growth stages and which contributes to improve the process of designing bread wheat genotypes adapted to drought. The main objectives of this study were to:

1. understand the mechanism and response of plants in water deficient conditions under different drought profiles (intensity, duration, occurrence) and stages of plant growth;
2. identify efficient agro-morphological and physiological shoot and root traits contributing to improved drought resistance under a Mediterranean regime,
3. determine the best combination of selection criteria in breeding bread wheat for drought resistance.

2 Material and methods

2.1 Plant material

Forty bread wheat genotypes (Table 1), supplied from different breeding programs (ICARDA, CIMMYT, Australia and Morocco), were evaluated for their response to drought stress in laboratory, glasshouse and under field conditions over three seasons from 2013 to 2015.

2.2 Laboratory experiment

Experimental design: The experiment was conducted in the laboratory of wheat breeding, National Institute of Agricultural Research in Morocco (INRA Morocco). Drought stress was induced by polyethylene glycol (PEG-6000), considering two PEG-6000 concentrations (15% and 25% corresponding to -3 MPa and -7 MPa respectively) and distilled water as control. The seeds were first sterilized with 5% sodium hypochlorite then washed with the distilled water. Under dark conditions, sterilized seeds were grown for 12 days in petri-dishes (diameter 9 cm) between two sheets of filter paper. The temperature was around 25 ±2 °C (day) and 21 ±1.2 °C (night). Each 2 days, seeds were watered with 5ml of the respective solution depending on the treatment. A seed was considered as germinated when the emerging radicle elongated to 1–2 mm.

Data collection: After seed germination, several parameters were calculated for each genotype to analyse the germination process in terms of capacity, time and rate under different drought treatments. The number of germinated seeds was recorded every 24 h for 12 days to calculate the germination capacity (GC) as a percentage of germinated seeds. Mean germination time (MGT) was calculated as the mean weight of the germination time. The number of seeds geminated in the intervals of time established for data collection is used as weight. The germination value (GV) is expressed as the product of peak value of germination and mean daily germination. Germination rate (GR) was calculated as the sum of the number of germinated seeds divided by days to first count. Roots number (RN), root and coleoptile lengths (*RL* and CL) and their respective fresh and dry weights were measured on the twelfth day. The water content of coleoptile and roots (RWC and CWC) was then calculated as the ratio of the amount of water at organ sampling to that present when dried. Germination vigor index (VI) was calculated by multiplying the sum of the root and shoot lengths by the germination percentage (Ranal & De Santana, 2006).

Table 1 List of tested genotypes for drought tolerance

Code	Name	Origin	Code	Name	Origin
1	NEJMAH-11	ICARDA	21	SB062	CIMMYT
2	NEJMAH-14	ICARDA	22	SB109	CIMMYT
3	SHIHAB-12	ICARDA	23	SB169	CIMMYT
4	AL-ZEHRAA-2	ICARDA	24	SsrT02	CIMMYT
5	BAASHA-21	ICARDA	25	SsrT09	CIMMYT
6	AMIR-2	ICARDA	26	SsrT14	CIMMYT
7	ATTILA	CIMMYT	27	SsrT16	CIMMYT
8	SOKOLL	CIMMYT	28	SsrT17	CIMMYT
9	GLADIUS	AUSTRALIE	29	SsrW35	CIMMYT
10	AUS30354	CIMMYT	30	SsrW47	CIMMYT
11	AUS30355	CIMMYT	31	ARREHANE	MAROC
12	AUS30518	CIMMYT	32	ACHTAR	MAROC
13	AUS30523	CIMMYT	33	MARCHOUCH	MAROC
14	QG-170-4.1	CIMMYT	34	KANZ	MAROC
15	QG-58-5.1	CIMMYT	35	AMAL	MAROC
16	HARTOG	AUSTRALIE	36	MASSIRA	MAROC
17	DRYSDALE	AUSTRALIE	37	AGUILAL	MAROC
18	SB003	CIMMYT	38	BT05A104	MAROC
19	SB165	CIMMYT	39	BT05A106	MAROC
20	SB069	CIMMYT	40	RAJAE	MAROC

2.3 Greenhouse experiment

Experimental design: The glasshouse experiment was conducted at Taoujdate farm station during 2012–2013 season. The 40 genotypes were evaluated using a split plot design with four replications under each water regime (stressed and non-stressed conditions). The stressed treatment involved withholding irrigation to 50% field capacity (FC) from stem elongation to flowering stage in order to simulate mid-drought stress. Agronomic practices were carried out following standard guidelines for wheat production. Fungicide treatments were applied twice during the crop season to avoid stresses overlapping.

Data collection: Root weight (RW) and volume (RV) were calculated at each stage from tillering to maturity. Roots and shoots were oven dried in 70 °C for 48 h to determine root dry weight and calculate relative water content (RWC). At maturity, the soil with roots in each pot was separated into two sections 0–20 cm and above 20 cm to evaluate root weight (RW20, RW + 20) and root volume (RV20, RV + 20) in topsoil and subsoil respectively. Roots number (RN), root length (RL) and roots: shoot (R/S) ratio were evaluated at harvest. Then, root length density (RLD), root tissue mass density (TMD) and specific root length (SRL) were calculated as follows:

$$\text{RLD (cm/cm}^{-3}) = \text{RL/soil volume}$$

$$TMD \text{ (mg/cm}^{-3}) = \text{root dry mass/RV}$$

$$SRL \text{ (m/g)} = \text{RL/root dry mass}$$

Regarding shoot parameters, productive spikes number (FSN), grains number (GN), grain number per fertile spike (GNFS), biomass (BY), harvest index (HI) and grain yield (GY) were recorded at maturity stage. Plant height (PH), peduncle length (PL), flag leaf area (FLA), leaf temperature (LT), canopy temperature (CT), stomatal conductance (SC), chlorophyll fluorescence (CF), chlorophyll content (Chla, Chlb, Carotenoides) and leaf relative water content (LRWC) were assessed during the cropping cycle.

2.4 Field experiments

Experimental design: The field experiments were carried out under contrasting weather conditions in two INRA Moroccan experimental stations, Taoujdate (Favorable, >450 mm) and Sidi El Aidi (semi-arid, <300 mm), for two years 2014 and 2015, making four testing environments hereafter referred to as T15 (Taoujdate, 2015), T14 (Taoujdate, 2014), S15 (Sidi El Aidi, 2015), and S14 (Sidi El Aidi, 2014). Following an alpha lattice design with two replications, the genotypes were planted in 6 rows plots of 3 m length, with 0.25 m spacing between rows. Agronomic management was performed according to recommended local practices at each location. To avoid the overlapping of stresses, chemical treatments against foliar diseases and weeds were performed as needed during the crop cycle.

Data collection: Agronomic data were recorded at maturity stage. Grain yield (GY) was driven from 4.5 m^2 of harvested plot and converted to the standard unit at metric ton per hectare (t/ha). The grain yield (GY) and aboveground biomass (BY) at each plot were used to calculate the harvest index (HI). The number of productive spikes (NFS) and grains number (GN) were counted to calculate the fertile spikes number per plant (NFSP) and grains number per fertile spike (GNFS). Finally, the thousand kernel weight (TKW) was assessed considering a random seed sample from harvested yield. For morphological and physiological traits, plant height (PH), peduncle length (PL), spike length (SL), awns length (AWL) and flag leaf area (FLA) were computed using a standard ruler. Plant vigor (PV) were estimated based on a visual scoring ranging from 1 (worst) to 5 (extreme vigor). Ground cover (GC) represents soil cover percentage using digital camera photos and Sigma Scan software. The canopy temperature depression (CTD) was evaluated using the infrared thermometer IR 1000 at 1 linear meter for each plot. Chlorophyll content (CC) and fluorescence (CF) were evaluated using respectively the chlorophyll meter SPAD 502 and the fluorometer.

2.5 Statistical analysis

Recorded data were subjected to analysis of variance (ANOVA) using GenStat for Windows 18th edition (VSN International, Hemel Hempstead, UK). Using SPSS software (IBM SPSS, v24), correlation, regression analysis and path analysis were performed to evaluate the relationships between different parameters and their interactions.

3 Results and discussion

3.1 Phenotypic evaluation of roots and shoot traits under controlled conditions at different growth stages

Roots phenotyping at germination stage under laboratory experiment: Based on ANOVA, large differences between treatments and significant genotype by environment interactions were expressed for all traits (P <0.05); except for germination capacity. Generally, Mean germination time (MGT), germination rate (GR) and germination value (GV) were consistently correlated to each other at all treatments (r >0.70), except between GV and GR at the first PEG treatment (-3 MPa) and between MGT and GV at the second PEG treatment (-7 MPa). The vigor index (VI) was strongly correlated with root length (RL) at all treatments; while its correlation with coleoptile length (CL) became highly significant at the first PEG treatment (r = 0.67) and was maintained to a less content at the second treatment (r = 0.49). On the other hand, root number (RN) had a positive moderate significant association with VI (r = 0.49) at the first PEG treatment. GR had negative correlation with root weight content (RWC) at stress conditions only. This correlation was highly significant at moderate stress (r = -0.47), and non-significant at severe stress (r = -0.26).

Shoots phenotyping under greenhouse experiment: ANOVA analysis demonstrated significant decrease from non-stressed condition (100% FC) to the stressed one (50% FC) for all traits (P <0.05). Under well-irrigated conditions, grain yield (GY) was positively correlated with biomass (BY), grains number (GN), productive spikes number (FSN) and harvest index (HI) (0.52 <r <0.65; P <0.001). Based on stepwise multiple regression analysis, GN and thousand kernel weight (TKW) explained more than 94% of yield variation. Although BY (0.05), HI (0.03) and FSN (0.04) had the lowest direct effects, they earned the highest indirect ones (0.53, 0.50 and 0.56 respectively) through GN. Under stressed conditions, GY presented also positive correlation with GN, GNFS, FSN and HI (0.45< r <0.92; P <0.001). HI and BY explained about 98% of yield variation. Moreover, HI detained the most important direct effect (0.99), while the effect of BY (0.43) was hidden by its negative indirect effect via HI (-0.34). The effects of GN (0.86), FSN (0.48) and GNFS (0.74) were mainly indirect through reinforcing the HI effect (Table 2).

Regarding physiological characters, significant correlations were observed between leaf temperature (LT) and GY (r = -0.37; P = 0.02) under non-stressed conditions. This trait explained 12% of yield variation and had an important negative direct effect. At stressed conditions, the regression analysis revealed that stomatal conductance (SC) (13%) associated to a lesser extent to chla (8%) and RWC (7%) could explain 28% of yield variation. These traits had respectively the highest direct effects and moderate negative correlations with yield. However, the positive effect of chla has been cancelled by the indirect effects through mainly carotenoids content and vice versa. The other effects remained very weak and non-significant. None of the morphological traits expressed a significant correlation with yield or contribute to explain yield variation under well-irrigated conditions. Under drought environment, positive correlation associated yield to plant height (PH) and peduncle length (PL) (r = 0.41 and 0.66 respectively) (Table 3).

Table 2 Direct and indirect effects of each trait and their correlation with grain yield in the two test environments

	Traits		Direct effect	Total indirect effects	Correlation with GY		Traits		Direct effect	Total indirect effects	Correlation with GY
Non-stressed environment (100% FC)	yield components	BY	0.05	0.52	0.53***	Stressed environment (50% of FC)	yield components	BY	0.43	-0,32	0.09
		HI	0.03	0.50	0.52***			HI	0.99	-0,04	0.90***
		TKW	0.86	-0.56	0.26*			TKW	0.02	-0,21	-0.14
		GN	1.11	-0.41	0.65***			GN	0.04	0,86	0.85***
		FSN	0.04	0.56	0.53***			FSN	0.09	0,48	0.50***
		GWFS	0.03	0.42	0.48***			GWFS	0.08	0,59	0.68***
		GNFS	-0.02	0.13	0.13			GNFS	-0.01	0,74	0.71***
	physiological traits	CT	-0.09	-0.04	-0.05			CT	0.09	0,02	0.08
		SC	-0.13	0.10	0.01		physiological traits	SC	-0.36	-0.03	-0.39**
		LT	-0.37	-0.01	-0.37**			LT	-0.04	0.08	0.10
		Chl*a*	-0.04	0.12	0.05			Chl*a*	0.34	-0.30	-0.29*
		Chl*b*	-0.03	-0.01	-0.05			Chl*b*	-0.04	0.03	0.11
		Caretenoides	0.06	0.15	0.20			Caretenoides	-0.24	0.24	0.14
		ChlT	-0.04	0.06	-0.004			ChlT	-0.07	0.09	0.21
		LRWC	0.2	0.14	0.27*			LRWC	0.30	0.03	-0.32*
		CF	-0.01	-0.02	-0.02			CF	0.00	-0.01	0.01

BY – biomass; HI – harvest index; TKW – thousand kernel weight; GN – grain number; SN – spike number; FSN – productive spikes; GWFS – grain weight per fertile spike; GNFS – grain number per fertile spike; CT – canopy temperature; SC – stomatal conductance; LT – leaf temperature; Chl*a* – chlorophyll *a*; Chl*b* – chlorophyll *b*, carot carotenoids; ChlT – total chlorophyll; LRWC – leaf relative water content; CF – chlorophyll fluorescence
*, **, *** significant correlation at 0.5, 0.01 and 0.001 probabilities respectively

Table 3 Summary of forward regression analysis to predict grain yield under stress and non stress conditions

Environments	Traits		Model R^2 adjusted	Regression coefficient B	Standard error	Standardized coefficients β	*t*-value	Sign	Durbin -Watson
Non-stress environment (100% FC)	yield components	GN	0.40	0.04	0.00	1.11	23.76	<0.001	2.39
		TKW	0.94	0.30	0.02	0.86	18.39	<0.001	
	physiologic traits	LT	0.12	-0.46	0.18	-0.37	-2.48	0.02	2.19
Stressed environment (50% of FC)	yield components	HI	0.80	24.7	0.85	0.99	28.93	<0.001	2.00
		BY	0.97	0.28	0.02	0.43	16.82	<0.001	
		GWFS	0.98	0.53	0.21	0.08	2.48	0.02	
	physiologic traits	SC	0.13	-0.09	0.03	-0.36	-2.65	0.01	1.97
		Chl*a*	0.21	0.87	0.35	0.34	2.51	0.02	
		LRWC	0.28	0.04	0.02	0.30	2.19	0.04	
	morphologic traits	SD	0.28	1.41	0.35	0.55	4.01	<0.001	1.35

GN – grain number; TKW – thousand kernel weight; LT – leaf temperature; HI – harvest index; BY – biomass; GWFS – grain weight per fertile spike; SC – stomatal conductance; Chl*a* – chlorophyll *a*; RWC – relative water content; SD – spike diameter

Roots phenotyping under greenhouse experiment: Concerning root parameters, root: shoot ratio (R/S), root volume at topsoil (RV20), total root weight (RW), root weigh at top soil (RW20) and subsoil (RW + 20), root tissue mass density (*TMD*) and specific root length (*SRL*) showed significant differences between dry and optimum conditions ($P < 0.05$). At 100% field capacity (FC), R/S, RW and RV were highly positively associated between them. Root length density (*RLD*) had a significant correlation with RN ($r = 0.44$; $P = 0.004$) and *RL* ($r = 0.76$; $P < 0.001$). This latter component also had a moderate correlation with RV + 20, RW + 20 and RW20 ranging from 0.42 to 0.56 ($P < 0.05$). Under stressed conditions at 50% FC, R/S showed significant correlations with RN; RV; RW; RV+20; RW20; RW + 20 and *TMD* ($0.32 < r < 0.95$; $P < 0.001$). RN is also positively associated with RV ($r = 0.43$; $P = 0.006$) and RW ($r = 0.53$; $P < 0.001$); while *TMD* is correlated with RW20 ($r = 0.60$; $P < 0.001$) and RW + 20 ($r = 0.42$; $P = 0.006$).

Under well-irrigated conditions, no significant correlations were detected between grain yield or yield components and root parameters; except between R/S, HI ($r = 0.35$; $P = 0.03$) and BY ($r = -0.44$; $P = 0.004$) and between RN and GWFS ($r = -0.33$; $P = 0.04$). Under stressed conditions, RN was negatively correlated with GY, GN, FSN ($-0.51 < r < -0.32$) and HI ($r = 0.56$; $P < 0.001$). RW, especially at the topsoil, presented moderate negative correlation with these parameters ($-0.45 < r < -0.33$; $0.03 < P < 0.004$); while RV had negative significant correlation with HI only ($r = -0.33$; $P = 0.04$). RV20 also displayed negative moderate correlation with GN and GNFS ($r = -0.32$ and -0.36 respectively). Finally, SLD demonstrated positive moderate correlation with HI ($r = 0.32$; $P = 0.04$) (Table 4). Based on multiple regression analysis, none of the root parameters was retained under well-irrigated conditions as potential predictor of grain yield. In contrast, RN enables the explanation of 31% of yield variability under stress conditions with an important negative direct effect (β = -0.67). Moreover, RW + 20 was also able to explain 8% of variability with positive direct effect (β = 0.30). Based on the path analysis, RW and R/S expressed also the highest negative indirect effect (-0.45 and -0.43 respectively).

Roots phenotype evolution over stages: From tillering to stem elongation, roots demonstrated an important development in terms of weight (73%) and volume (77%). At flowering stage, RW and RV were more important under drought stress conditions compared to non-stressed ones (+22% and +31% respectively). The same scenario was also observed at maturity stage (+42% and +16% respectively for RW and RV). From flowering to maturity, a more pronounced increase was recorded for RW especially under stress (+49%) compared to non-stressed environment (+10%); whereas RV denoted a significant decrease at both non-stressed (-26%), as well as stressed conditions (-39%).

The R/S remained stable between the tillering and stem elongation stage, while it noted an important decrease at flowering (-54% and -63% respectively at 50 and 100% FC). At maturity stage, a significant drop was observed at non-stressed conditions (-38%) in contrast with stressed conditions (+15%).

Table 4 Correlations between roots traits and yield components under stressed conditions

Traits	R/S	RV	RW	RN	*RL*	GY	BY	GN	SN	FSN	HI	GWFS	GNFS
R/S	1												
RV	0.32*	1											
RW	0.95***	0.37*	1										
RN	0.56***	0.43**	0.53***	1									
RL	-0.03	0.19	-0.03	0.11	1								
GY	-0.19	0.29	- 0,15	0.58***	-0.02	1							
BY	-0.18	0.21	0.11	-0.02	0.05	0.03	1						
GN	-0.28	-0.08	0.19	-0.39*	0.04	0.58***	0.24	1					
SN	0.25	0.22	0.27	0.02	0.19	0.22	0.04	-0.11	1				
FSN	-0.28	-0.14	-0.19	0.51***	0.09	0.63***	0.27	0.41**	0.45**	1			
HI	-0.17	-0.33	-0.22	-0.56***	-0.05	0.92***	0.30	0.42**	0.27	0.54***	1		
GWFS	-0.12	-0.27	-0.13	-0.39*	-0.07	0.78***	-0.13	0.43**	-0.06	0.09	0.75***	1	
GNFS	-0.26	0.08	-0.22	-0.29	-0.12	0.45**	0.11	0.89***	-0.31*	0.03	0.33*	0.59***	1

R/S – root/shoot ratio; RV – root volume; RW – root weight; GW – grain weight; BY – biomass; GN – grain number; SN – spike number; FSN – fertile spike number; HI – harvest index; RN – root number; RL – root length; GWFS – grain weight per fertile spike; GNFS – grain number per fertile spike
*, **, *** significant correlation at 0.5, 0.01 and 0.001 probabilities respectively

3.2 Phenotypic evaluation of shoot traits under field conditions

Climatic conditions: The 2014 season was characterized by severe periods of drought throughout the crop cycle. The favorable site (Taoujdate) was also subject to water stress and had a cumulative rainfall (November-June) of only 250 mm against 216.5 mm at Sidi El Aidi. Stress was moderate during the vegetative stage but was rather important around flowering. In contrast, the 2015 season showed a favorable rainfall pattern, generally well distributed in time and space. Taoujdate received 388 mm, while Sidi El Aidi experienced a water deficit during the vegetative stage followed by a terminal drought with a total rainfall of 258 mm.

Grain yield: Bennani et al. (2017) had already displayed grain yield variations and pattern. Taoujdate experimental site (3.94 t/ha ±0.74) was more productive compared to the semi-arid site (2.51 t/ha ±0.82); while the 2015 cropping season (3.7 t/ha ±1.02) was more favorable than the 2014 (2.64 t/ha ±0.84). The grain yield depletion was much higher under semi-arid conditions and reached 34%. The combined ANOVA indicated highly significant variability between environments and among genotypes (P <0.001) and significant environment × genotype interaction.

Secondary traits: Analysis of variance showed highly significant differences for all traits between the four environments except for CF. The genotype × environment interaction was non-significant only for HI, TKW, PH, SL, CC and CF. The yield heritability was very low (30%), compared with yield components HI, TKW, GNFS and GN (52%< h <²80%). The physiological traits PV, CC and CF exhibited also high levels of heritability ranging from 45 to 65% (Table 5).

Regarding yield components, a highly significant correlation was observed between yield, GN and BY under both rainfed and semi-arid conditions with values ranging from 0.70 to 0.94. The significant correlation between GY and HI was also permanent over all environments with more important values under favorable conditions (r = 0.74; P <0.001) in comparison with drought stressed environments (0.52< r <0.64). The effect of TKW on yield was relevant only at the dry year 2014 under both sites. Similarly, GNFS and NFSP relationships were more important at the driest one (S14) (r = 0,61 and 0.74; P <0.01 respectively). For physiological and morphological traits, PH and SL developed significant moderate positive correlation with GY under Sidi El Aidi site for both growing seasons 2015 and 2014 respectively. Similarly, GC and PV notations were also positively correlated with yield under the semi-arid site, while CTD had moderate negative association with it (Table 6).

In order to identify traits that contribute most to the variation of grain yield, the multiple regression was

Table 5 Mean square from Analysis of variance of studied traits and their heritability

Traits		Mean	Environnement (E)	Genotype (G)	G x E	Heritability (%)
Yield		3.22	135.94***	1.54***	0.63**	29.8
Yield components	HI	35.33	1950.70***	112.13***	50.99	54.5
	BY	914.9	2336440.00***	79893.00***	56578.00**	29.2
	TKW	35.24	5414.30***	83.02***	16.66	79,9
	GNFS	30.11	1140.30***	176.10***	58.79***	66.6
	GN	9256	243600000.00***	16180000.00***	7708000.00*	52.4
	NFSP	2.56	65.95***	0.93***	0.67***	28.4
Morphological traits	PH	96.38	42608.00***	266.40*	193.20	27.5
	PL	16.11	3470.50***	19.64	19.82*	0.00
	SL	10.46	112.93***	1.65	1.68	0.00
	AWL	5.98	9.50***	1.83	1.83*	0.00
Physiological traits	GC	68.86	21798.00***	164.80*	164.10**	0.42
	PV	3.38	75.30***	3.10***	1.20**	59.9
	CC	43.44	3739.70***	35.70***	12.50	64.9
	CTD	0.43	40.00**	10.90***	9.10**	17.1
	CF	0.74	0.08	0.02	0.01	44.5

HI – harvest index; BY – biomass, TKW – thousand kernel weight; GNFS – grain number per fertile spike; GN – grain number; NFSP – number of fertile spikes per plant; PH – plant height; PL – peduncle length; SL – spike length; AWL – awns length; GC – ground cover; PV – plant vigor; CC – chlorophyll content; CTD – canopy temperature depression; CF – chlorophyll fluorescence
*, **, *** significant correlation at 0.5, 0.01 and 0.001 probabilities respectively

Table 6 Direct and indirect effects of yield components and their correlation with grain yield

	Traits		Direct effect	Indirect effects	Correlation with yield		Traits		Direct effect	Indirect effects	Correlation with yield
S14 = E4	yield components	HI	-0.02	0.52	0.52**	S15 = E3	yield components	HI	-0.08	0.66	0.62**
		BY	0.04	0.82	0.83***			BY	-0.05	0.75	0.81***
		TKW	0.34	-0.45	0.02			TKW	0.40	0.31	0.77***
		GNFS	0.00	0.25	0.25			GNFS	0.02	0.56	0.61**
		GN	1.04	-0.08	0.94***			GN	0.78	0.05	0.94***
		NFSP	0.00	0.65	0.62**			NFSP	-0.03	0.67	0.74**
	morphological traits	PH	-0.03	0.19	0.26		morphological traits	PH	0.07	0.00	0.05
		FLA	-0.19	0.23	0.07			FLA	0.24-	-0.01	-0.24
		PL	-0.05	-0.02	0.40**			PL	0.18	0.10	0.19
		SL	-0.08	0.16	0.39*			SL	0.33	-0.05	0.33*
		AWL	0.01	-0.03	-0.14			AWL	0.20	-0.03	0.10
	physiological traits	GC	0.21	0.64	0.52**		physiological traits	GC	0.34	-0.19	0.55***
		PV	0.30	0.55	0.50***			PV	0.17	0.19	0.35*
		CC	0.09	0.11	0.07			CC	0.21	-0.13	0.02
		CTD	-0.46	-0.31	-0.45**			CTD	-0.03	-0.13	-0.31*
		CF	-0.11	-0.07	-0.12			CF	0.11	0.03	0.12
Taoujdate 2014	yield components	HI	0.36	0.40	0.74***	Taoujdate 2015	yield components	HI	0.04	0.67	0.64**
		BY	0.37	0.35	0.73***			BY	-0.02	0.62	0.68***
		TKW	0.05	-0.27	0.004			TKW	0.63	-0.33	0.63**
		GNFS	0.00	0.67	0.64**			GNFS	0.03	0.37	0.16
		GN	0.54	0.32	0.85***			GN	1.00	-0.24	0.79***
		NFSP	0.00	0.28	0.27			NFSP	-0.06	0.31	0.32*

HI – harvest index; BY – biomass; TKW – thousand kernel weight; GNFS – grain number per fertile spike; GN – grain number; NFSP – number of fertile spikes per plant; PH – plant height; FLA – flag leaf area; PL – peduncle length; SL – spike length; AWL – awns length; GC – ground cover; PV – plant vigor; CC – chlorophyll content; CTD – canopy temperature depression; CF – chlorophyll fluorescence
*, **, *** significant correlation at 0.5, 0.01 and 0.001 probabilities respectively

carried out independently for each environment. GN, and to a lesser extent TKW, were the best indicators for all environments accounting for more than 97% of the total variation (Table 7). The contribution of GN increases with increasing stress, while the effect of TKW was reduced by the negative indirect effect through GN. The exception was noticed during the driest year S14 where all direct and indirect effects of TKW were positive. The remaining characters contributed indirectly to yield variation, mainly BY and HI. Besides, NFSP also had a significant indirect effect (0.65 and 0.67 respectively at S15 and S14), while NGFS held a significant effect at S14 (0.56). All these effects originated from the indirect effect with GN (Table 6). Regarding morphological traits, only PH could explain 9% of yield variation at S15 with a negative effect (β = -0.34).

The regression models based on yield and physiological characters were not significant at the rainfed favorable site during both cropping cycles. For the semi-arid site, CTD explained about 21% of the yield variation during 2015. This parameter has the highest indirect effect (-0.46) followed by PV (0.30). The most important indirect effects are recorded by the GC and PV readings (varying from 0.55 to 0.81) and which reinforce the weight of CTD by an equally important indirect effect (-0.30). During 2014 season, about 20% of total yield variation was explained by PV (13%) and GC (11%) which held the highest values of direct effects (β = 0.40 and 0.34 respectively). The other direct and indirect effects remained very weak (Table 7).

A more specific understanding of plant responses to drought under the target environment is a must before implementing a selection strategy under the breeding program. The present study aims to identify the most powerful combination of traits implied in the drought tolerance breeding strategy.

Table 7 Summary of forward regression analysis to predict grain yield under stress and non stress conditions

Environments/ traits groups		Traits	Adjusted R^2	Regression coefficient B	Standard error	Standardized coefficients β	*t*-value	Signifiance	Durbin -Watson
T14	yield components	GN	0.71	0.00	0.00	0.54	4.79	0.00	2.22
		TKW	0.99	0.05	0.01	0.28	4.51	0.00	
		BY	0.99	0.00	0.00	0.37	5.86	1.18	
		HI	0.99	0.05	0.01	0.36	5.20	8.77	
T15	yield components	GN	0.61	0.00	0.00	1.00	31.73	0.00	2.54
		TKW	0.97	0.11	0.01	0.63	19.98	0.00	
	morphological traits	FLA	0.19	-0.08	0.02	-0.49	-3.73	0.00	2.51
		AWL	0.32	-0.24	0.09	-0.37	-2.82	0.01	
S14	yield components	GN	0.88	0.00	0.00	1.04	54.21	0.00	2.43
		TKW	0.99	0.09	0.00	0.34	17.52	0.00	
	morphological traits	SL	0.33	0.39	0.18	0.33	2.12	0.40	1.47
	physiological traits	CTD	0.19	-0.22	0.07	-0.46	-3.19	0.00	1.83
S15	yield components	GN	0.87	0.00	0.00	0.78	25.53	0.00	2.06
		TKW	0.98	0.07	0.01	0.40	15.25	0.00	
		HI	0.98	-0.01	0.00	-0.08	-2.71	0.01	
	morphological traits	PL	0.14	0.07	0.03	0.33	2.27	0.03	1.90
		SL	0.21	0.2	0.10	0.31	2.08	0.04	
	physiological traits	PV	0.11	0.34	0.12	0.40	2.78	0.00	1.99
		GC	0.20	0.03	0.01	0.34	2.33	0.03	

GN – grain number; TKW – thousand kernel weight; BY – biomass; HI – harvest index; FLA – flag leaf area; AWL – awns length; SL – spike length; CTD – canopy temperature depression; PL – peduncle length; PV – plant vigor; GC – ground cover

3.3 Roots traits

During the last decades, there is an increasing interest in studying roots for crop breeding purposes. Roots acquire nutrients and water resources from soil for plant growth and regulation. Hence, breeding cultivars with adapted root systems is a promising strategy to increase the resilience of wheat genotypes in drought prone environments and enlarge their plasticity to abiotic stresses (Kim et al., 2020).

Seed germination is potentially one of the most sensitive stages to drought (Queiroz et al., 2019). Several methods and mathematical expressions were developed to assess the germination process. The germination value and germination rate provide information on the speed and the spread of germination respectively. The mean germination time measures the average length of time required for maximum germination (Ranal and De Santana, 2006). The present study depicted that water stress at this early stage affects the speed and peak time of germination with the rise of osmotic potential levels, rather than germination capacity. In fact, drought negatively affects germination process through inhibition of water uptake, and induces delayed emergence. Metabolic disorders impede starch synthesis reactions and decrease ATP production and respiration for energy production process, thus impairing water uptake through the seed coat and seed imbibition for the output of the radicle (Panda et al., 2021).

The vigor index was strongly correlated with root and coleoptile length showing the importance of root parameters for further plant establishment as stated by Panda et al. (2021). The findings of the present study also revealed that the above traits increased at moderate stress, whereas root number expressed its impact on germination through the vigor index at the same level. Farooq et al. (2009) and Saha et al. (2017) supported these findings and stated that the expression of certain genes controlling root development is simulated by hormonal signals under drought conditions to deal with water deficit. However, there was a decrease in roots traits, including root number, at severe osmotic level. The plants adopted a conservative defense strategy where all shoot and root traits shrunk considerably because of reduced cellular division and elongation (Queiroz et al., 2019).

In greenhouse experiment, the vegetative stage indicated the highest expansion of roots in terms of weight and volume. In the absence of stress, the plant emphasizes on root growth in the first place through capturing nitrogen and water to ensure shoot development (Chen et al., 2020). The water shortage was applied at the flowering stage, which is the most vulnerable growth stage of plant cycle towards drought stress. According to modelling studies, wheat yield would increase by 55 kg/ha on average for each mm of water extracted from the soil after anthesis (Christopher et al., 2013). Root architecture and morphology were greatly modified under drought stress. Roots weight and volume were more developed under dry conditions, especially at deep soil, without significant impact on root length and root length density. Root mass expressed the highest negative indirect effect on grain yield (emphasised by the significant moderate correlation) and explained a significant part of yield variation with positive direct effect. Therefore, the differences in root system size were mainly due to root branching and proliferation rather than differences in rooting depth enabling, shifting from investment in roots axial development to the lateral one and enabling better access to water under stress (Fang et al., 2017; Kim et al., 2020; Panda et al., 2021). The root mass development was further reflected by the increase in root: shoot ratio under drought conditions. Furthermore, the root tissue mass density was increased under stress highlighting further the importance of root weight over the root volume, while the specific root length decreased supporting the fact that the plant developed thick roots that persist longer and produce more and larger roots branch to increase water uptake capacity (Franco et al., 2011).

The other root parameters seem to be less sensitive to the drought effect. However, the genotype x environment interaction was significant for roots number. The latter was positively correlated with the root: shoot ratio, roots volume and weight. Roots number is considered as a drought tolerant criterion contributing to better exploitation of soil moisture and good acquisition of nutrients in dry and poor soils (Idrissi et al., 2015). However, roots number had a negative association with yield under stress. Greater root mass and volume in topsoil increases root inter-competition and delays the effectiveness of roots in capturing resources under stress (Fang et al., 2017). In our case, the roots of tolerant genotypes are supposed to be thick and extensive in capturing resources under water stress, and thus could ensure a better yield.

From flowering to maturity, a more pronounced increase was recorded for root weight in parallel with a significant decrease in root volume. Generally, carbohydrate reserves are mobilised to the grain and root growth is generally altered in cultivated wheats around flowering. At maturity stage, roots increase apoplastic barriers and take up less water with age (Comas et al., 2013).

3.4 Shoot traits

Regarding pots experiment, the drought reduces plant growth and development leading to hampered flower production and grain filling and thus smaller and fewer grains (Panda et al., 2021). Harvest index and biomass were the best predictors of grain yield as emphasized through correlation outputs. The other yield components, namely grain number and spike fertility, had important indirect effects on yield through reinforcing the harvest index effect.

A more detailed study was undertaken in the field conditions under favorable and semi-arid conditions, concentrating on the main promising criteria in terms of usefulness for drought tolerance selection and ease of handling. Grain yield expressed low heritability (30%) under drought as stated by Farooq et al. (2009), Slafer et al. (2014) and Bennani et al. (2017). Grain number, biomass and harvest index were highly significantly correlated with yield under all environments, as pointed out in many studies (Slafer et al., 2014; Al-Ajilouni et al., 2016). Grain number and kernel weight were the best predictors of yield. However, the yield variation was more associated with changes in number rather than weight, especially at severe drought conditions, as reported by Foulkes et al. (2011), Slafer et al. (2014) and Al-Ajilouni et al. (2016). The effect of thousand kernel weight was relevant only at the dry year 2014 at both locations with positive direct and indirect effects, but without significant impact on yield due to the limitation of the initial grain wells (Slafer et al., 2014). The harvest index and biomass act directly under favorable conditions and indirectly in stressed environments through grain number. A good aboveground biomass enables to establish active photosynthesis and to meet plants' needs, while the harvest index represents the reproductive efficiency and has been the key factor for yield improvement since the green revolution (Richards, 2000). The remaining yield components contribute indirectly through grain number, especially at semi-arid conditions. In our study, the effects of grain number per fertile spike and especially number of fertile spikes per plant varied depending on the environment and were more relevant at the driest one. The competition between ear and stem would result in the reduction of assimilates for developing florets under stress, implicating the abortion of the florets and thus a cap of grain numbers (Foulkes et al., 2011).

Regarding physiological traits, early plant vigor showed positive correlation with grain yield under dry conditions, supported by the highest direct effect especially at the driest scenario. Early development contributes to rapid coverage of the soil surface and thereby limits the evapotranspiration and retains soil moisture, while increasing carbon capture, light interception and photosynthesis process (Rahman et al., 2016). Additionally, lower canopy temperature depression values, and therefore cooler canopies, were associated to greater grain yield under semi-arid environment. It explained a significant part of its variation, and held an important indirect value through plant vigor weights at moderate stress. A cool canopy indicates transpiring leaf area and can be an indirect measure of vascular capacity of root system. However, the use of this parameter should be taken with caution. In fact, canopy temperature is no longer associated to grain yield under severe stress, as demonstrated through non-significant correlation, direct and indirect effects. Pask et al. (2012) pointed out that the absence of water in deep soil would limit the genetic differences in terms of root capacities of water extraction, and therefore, this trait could lead to divergent conclusions.

The morphological traits were also associated to drought tolerance process. Spike and peduncle lengths were positively correlated with grain yield, especially under severe stress, probably due to the drastic diminution of tillers and spikes number leading to less competition between organs. This finding is in line with Amiri et al. (2013) outputs. The spike maintains sustained photosynthetic activity with awns and contributes to the improvement of the dry matter content (20-30%), and thereby grain yield (Amiri et al., 2013). The peduncle length represents up to half of the total stem length and represents a significant storage for nutrients and soluble carbohydrates for grain filling (Pask et al., 2012).

4 Conclusions

It is assumed that no single trait can consitute an absolute selection criteria for drought tolerance, and thus a combination of relevant shoot and root traits at different stages is more effective for a successful breeding program. The present study aims to identify potential combinations of traits that can increase plant growth under a wide variety of drought environmental conditions from germination to maturity stage. Based on our findings, germination speed and spread (expressed through germination rate and mean germination time) play a major role in determining roots development. The tolerant genotypes minimize their root system in terms of number, volume and mass. However, the size of the plant's root system should be considered in relation to the aboveground plant parts. Grain number is the most important agronomic component for yield improvement. Under stress conditions, its effect is reinforced by reduced tillers number, high spike fertility, combined with a high grain weight and to some extent high grain number per fertile spike. The biomass and ground cover should be maintained at a reasonable rate to keep the balance between the available resources and plants' needs. Moreover, spike and peduncule lengths may also be advantageous under severe stress conditions. A combined selection of these traits will be effective for developing high-yielding and drought tolerant wheat varieties.

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