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INTERACTIVE EFFECTS OF HEAT STRESS AND WATER DEFICIT ON THE PERFORMANCE OF CHICKPEA (*Cicer arietinum* L.) GENOTYPES IN SUDAN

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ABSTRACT

Climate variations pose significant challenges for chickpea production. Increases tolerance to heat stress and water deficit is an important option to increase chickpea productivity in Sudan. This study aimed to evaluate the performance, stability and correlation between different traits of nine chickpea genotypes under different conditions. The genotypes were tested at two locations; Hudeiba for three seasons (2007/08, 2009/10 and 2010/11) and Shambat during season 2008/09. To induce stresses, four treatments were used: non-stress, terminal heat stresses, water stress and combination of heat and water stresses. A split-plot design with three replications was used where the stress treatments were assigned to main plots and genotypes to subplots. The results showed that heat, water and combined stresses significantly affected all studied traits. Heat stress induced more reduction than water stress for most of the traits, however combined stress imposed the highest effect. Significant differences between genotypes for all studied traits were also found. The interactions between the genotypes and treatments were significant for most of studied traits. Some genotypes were found tolerant and stable under water deficit (Wad Hamid and Shendi), heat stress (Hwata), or combined stress (Wad Hamid) conditions. Seed yield positively and significantly correlated with number of pods/plant, number of seeds/plant, 100-seed weight and plant height and these traits could be used as selection criteria for breeding high yielding genotypes. It could be concluded that cultivars differentially responded to heat, water

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and combined stresses, indicating that further improvement in the tolerance of chickpea to these stresses could be achieved.

Keywords: Heat stress; water deficit; stress tolerance; yield stability; chickpea; Sudan

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important legume crops in the world and Sudan. It is playing a leading role in food safety in the world by covering the deficit in proteins of daily food ration of Indian and African Sub Sahara populations [1]. Chickpea is a good source of energy, protein, minerals, vitamins, fiber, and also contains potentially health-beneficial phytochemicals [2]. The traditional area of chickpea cultivation in Sudan was along the River Nile banks north of Khartoum, where winter is relatively longer and cooler. Recently, there has been a remarkable shift in the growing environment of chickpea to the warmer, short-season environments of central clay plain of Sudan. Moreover, the general farmer practice of sowing chickpea late during December subject the crop to high temperatures at the flowering and grain filling stages. Chickpea is produced under both irrigation and residual soil moisture after flood recession.

The yield of chickpea in Sudan is low due to a number of biotic and abiotic factors contribute to this low yield. The major abiotic factors identified are water deficit and heat stress whereas biotic factors include diseases (especially *fusarium* wilt, root rot and chlorotic dwarf virus), insects and weed infestation. Plant responses to high temperature vary with plant species and phenological stages [3]. High temperature affects in both plant growth and development and limits chickpea yield [4][5]. Also drought is one of the major constraints limiting crop productivity [6], with terminal drought, seed yields of chickpea can be reduced by 58–95% compared to irrigated plants and reductions in pod production and abortion are key factors impacting final seed yield [7]and causing 40-50% reduction in chickpea yield globally [8]. When combined, drought and high temperature have both additive and interactive effects on plant growth [9]. There exists a strong relationship between the plant water status and temperature, thus making it very difficult to separate the contributions of heat and drought stress under field conditions [10]. Identification of genetic variability governing the chickpea response to water deficit and heat stress is a key step for improving the crop performance under these stresses. The objectives of this study is to identify cultivars with specific or combined tolerance to water and heat stresses and therefore, it aimed to: (1) assess the individual and combined effects of heat and water stresses on chickpea yield and other related traits; (2) evaluate the interactive performance of nine chickpea genotypes and determine their single and combined tolerances to heat and water stress; (3) determine stability of nine chickpea genotypes under single and combined water and heat stress; (4) estimate the correlation between yield and yield components.

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2. MATERIALS AND METHODS

2.1 Experimental conditions and plant material

The experiment was conducted at two locations; Hudeiba Research Station Farm (located at latitude 17°34`N, longitude 33°56`E, and 350 m above sea level) for three seasons (2007/08, 2009/10 and 2010/11, hereafter designated as HUD08, HUD10 and HUD11, respectively) and Shambat demonstration Farm of the Faculty of Agriculture, University of Khartoum (lat. 15°40`N, long. 32°32`E, 380 m above sea level) in 2008/09 season (SHAM09). Means of temperature and relative humidity during the experimental period are shown in Figure 1. The genetic material used in this study comprised of nine chickpea genotypes; eight released cultivars and ICARDA drought tolerance genotype FLIP87-59C (Table 1). The four stress treatments were; non-stress (N, optimum sowing date and irrigated every 10 days), terminal heat stress (H, late sowing after a month and irrigated every 10 days), water stress (D, optimum sowing date and irrigated every 20 days) and combined heat and water stresses (HD, late sowing after a month and irrigated every 20 days). Moisture content as percentage under two water treatments **(**non-stress and water stress) for the two seasons 2007/08 and 2009/10 are shown in Figures 2 and 3.

2.2 Agronomic management

Optimum sowing date was in mid-November, whereas late sowing (heat stress) was in mid-December in all environments. Optimum irrigation was practiced every 10 days, however, water stress was applied by irrigating every 20 days, and induced from third irrigation. Each genotype was sown in two rows 4 m long and 0.6 m apart and 0.1 m between plants within the row. Urea was applied at a rate of 20 kg N/ha with the second irrigation. The plots were hand weeded twice at early stages of crop cycle.

2.3 Data collection

Data were collected on five plants selected randomly per each genotype. These included; seed yield/plant (g), number of pods/plant and number of seeds/plant. Seed yield (t/ha), 100-seed weight (g), days to 50% flowering, days to 90% maturity and plant height (cm) were also recorded.

2.4 Experimental design and statistical analysis

The experiment was arranged in a split plot design with three replications. Stress treatments were assigned as main plots and cultivars as subplots. Combined analysis of variance was carried out according to [11]. The relative yield (yield under stress/yield under non-stress condition) was used as index to measure stress tolerance. The yield stability analysis for the nine genotypes was

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carried out according to [12] for each specific stress resulting in 8 environments, as a combination of 2 treatments (non-stress combined with either water or heat or severe stress) X 4 seasons (HUD08, HUD10, HUD11 and SHAM09). Also stability was conducted for overall environments (16) as combination of 4 treatments (N D, H, & HD) X 4 seasons. General analysis was done using a computer program of GenStat12th edition package (2009).

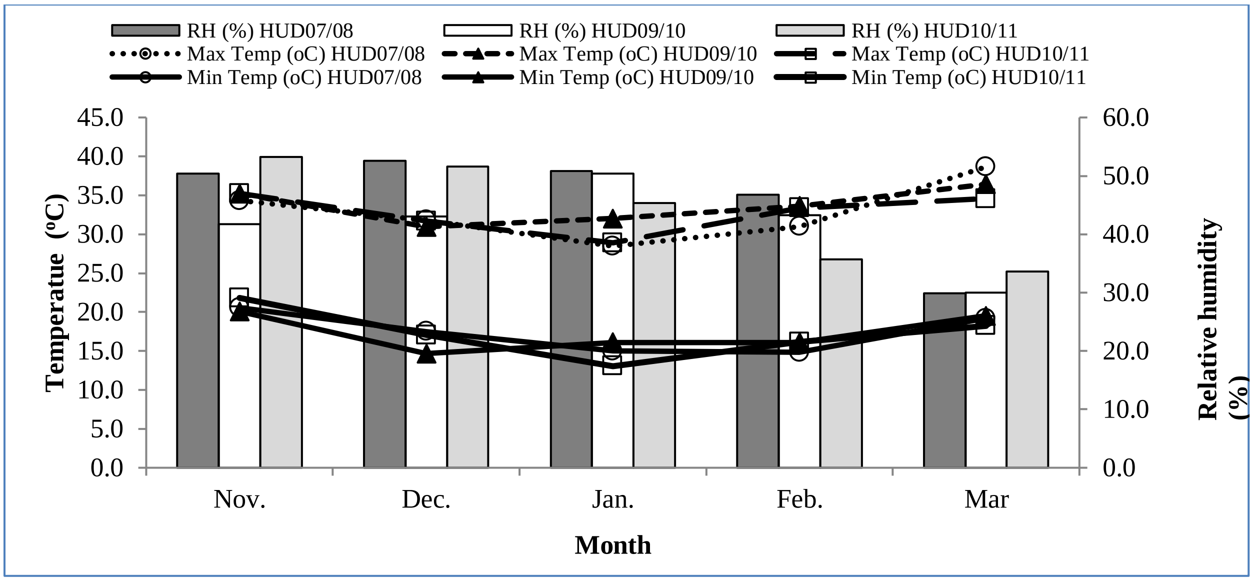

Figure 1: Monthly maximum, minimum temperature (°C) and relative humidity (%) during growing season at three environments at Hudeiba during 2007/08, 2009/10 and 2010/11

Table 1: Cultivar pedigree, year of release and their growth habit

No.	Cultivar Name	Accession No.	Year of release	Growth Habit
1	Shendi	ILC 1335	1987	Semi-spreading
2	Jabel Marra	ILC 915	1993	Semi-erect
3	Wad Hamid	ICCV 2	1996	Spreading
4	Atmor	ICCV 89509	1996	Semi-erect
5	Hwata	ICCV 92318	1998	Semi-erect
6	Burgeig	ICCV 91302	1998	Semi-erect
7	Salwa	FLIP 89-82c	1996	Spreading
8	Matama	FLIP 91-77c	1998	Semi-Spreading
9	FLIP87-59C	ICARDA (check)	breeding line	Spreading

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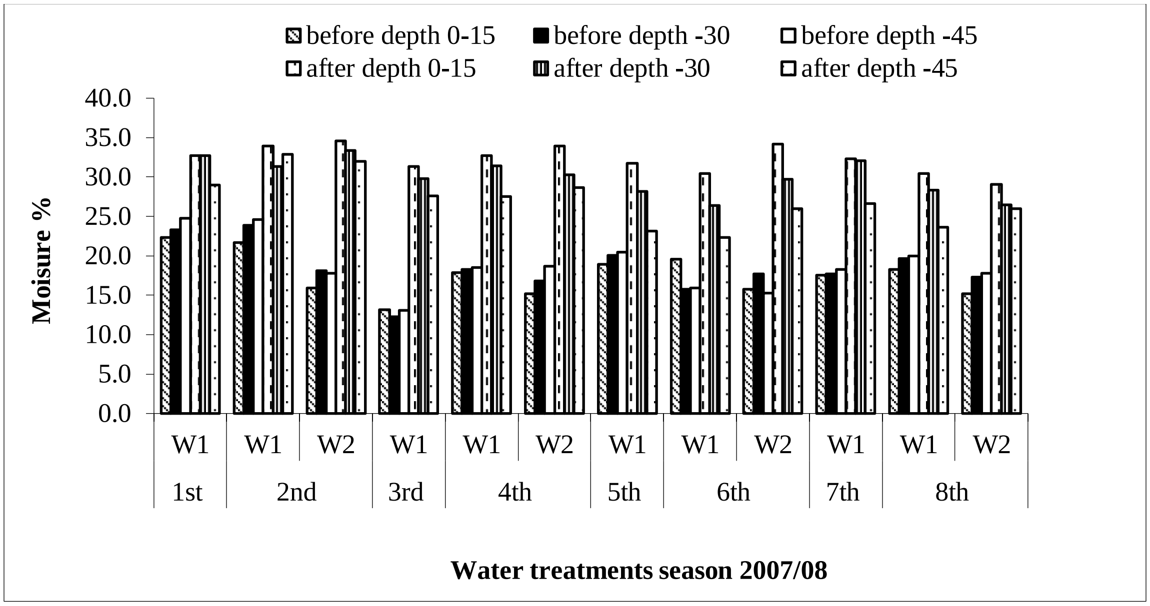

Figure 2: Moisture content as percentage under two water treatments W1 (non stress irrigated every 10 days and W2 (stress treatment, irrigated every 20 days) during season 2007/08 at Hudeiba

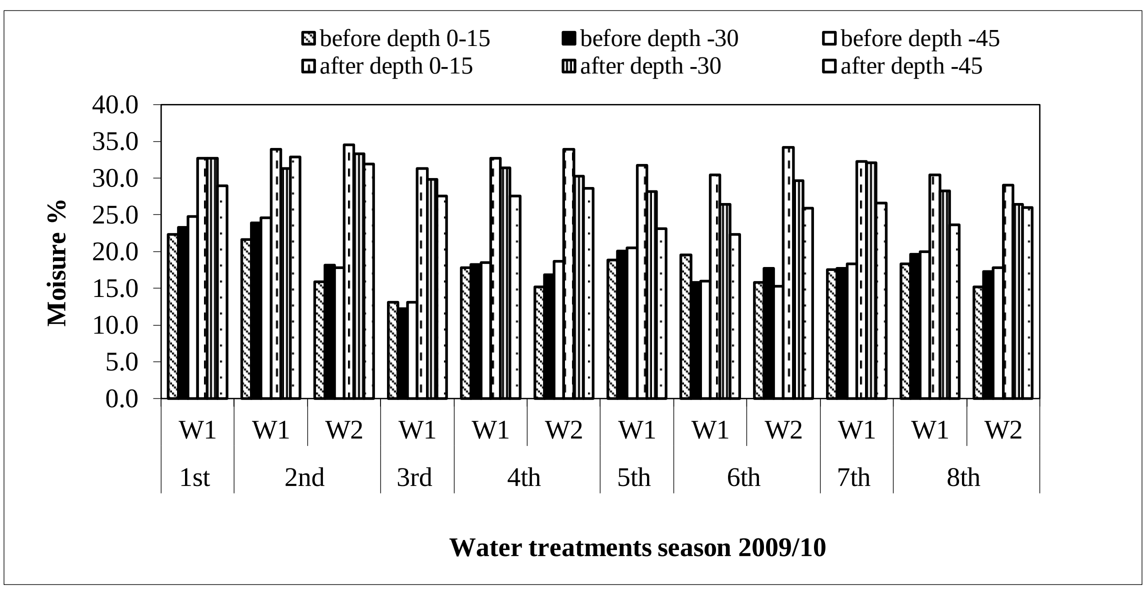

Figure 3: Moisture contents as percentage under two water treatments W1 (non stress irrigated every 10 days and W2 (stress treatment, irrigated every 20 days) during season 2009/10 at Hudeiba

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3. RESULTS

3.1 Weather

Higher maximum temperatures (>35 ºC) at the flowering stage were observed for the late sowing (heat stress) treatments (Fig. 1), compared to the non-stress (optimum sowing) treatments (<30 ºC) at Hudieba. In addition, Figures 2 and 3 show high reductions in moisture content as percentage in water stress treatments for the two seasons (2007/08 and 2009/10) compared with non-stress treatments, at Hudeiba.

3.2 Environment, Genotype, stress treatment, and their interactions

Significant differences were detected among environments, genotypes, treatments and their interaction for all studied traits, except a non-significant difference due to genotype x treatment interactions for 100-seed weight and days to 90% maturity (Table 2). Means effect of stress treatments for the studied traits is given in Table 3. Terminal heat stress induced more reduction than water stress for all studied traits, except for days to 50% flowering, and plant height. Days to 50% flowering was accelerated under water stress but delayed under heat stress. Plant height was much reduced under water stress as compared to heat stress. 100-seed weight was decreased under heat stress and combined heat and drought stresses, but was not affected by drought under optimum sowing date (Table 3).

3.3 Stress tolerance parameters

Table 4 shows the means of stress tolerance parameters of nine chickpea genotypes determined under four stress treatments. Under non-stress condition, the highest seed yield was given by Atmor cultivar and FLIP87-59C genotype. While, under water stress the highest seed yield was also exhibited by Atmor cultivar followed by Wad Hamid and Shendi. Under heat stress, the highest seed yield was revealed by Hwata cultivar. Whereas, under severe stress Burgeig and Matama cultivars showed the highest seed yield (Table 4). The cultivars were different for their tolerance to stress. The highest tolerance to water stress was obtained by Wad Hamid followed by Atomr and Shendi cultivars, while the highest heat tolerance was obtained by Hwata cultivar followed by Burgeig and Matama cultivars. The two cultivars Wad Hamid and Matama were the most tolerant under severe stress followed by Burgeig (Table 4).

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Table 2: Mean squares from combined analysis due to environments (E), treatments (T), interaction (ExT), genotype (G), interactions (GxE, GxT, GxExT) for different traits in nine chickpea cultivars evaluated over four treatments across four environments (HUD08, HUD10, HUD11 and SHAM09)

Trait	Variance components						
	E	T	ExT	G	GxE	GxT	GxExT
	d.f.						
	3	3	9	8	24	24	72
Seed yield/ha (t)	1838682*	31341274**	1635384**	390072**	431124**	218761**	211003**
Seed yield/plant (g)	1639**	1397**	475**	53.3**	41.4**	24.8**	23.4**
Number of pods/plant	22778**	25400**	7179**	1803**	602**	667**	465**
Number of seeds/plant	29701**	26124**	10371**	4171**	864**	744**	585**
100-seed weight (g)	708**	457**	24.4**	1036**	45.4**	13.2	15.3**
Days to 50% flowering	1489**	86.7*	193**	609**	164**	37.3**	38.7**
Days to 90% maturity	1740**	5825**	504**	227**	53.8**	15.3	16.3**
Plant height (cm)	5344**	5892**	252**	65.4**	50.1**	44.3**	29.3**

Table 3: Means of different traits for nine chickpea cultivars evaluated under four treatments; non stress (N), water stress (D), heat stress (H) and severe stress (HD) across four environments (HUD08, HUD10, HUD11 and SHAMB09)

Trait	Stress treatments				Mean	LSD5%	CV%
	N	D	H	HD			
Seed yield/ha (t)	1918	1213	991	642	1191	159.6	26.9
Seed yield/plant (g)	15	12.5	11	6.5	11.2	1.39	27.8
Number of pods/plant	66	52	48	29	49	6.0	28.2
Number of seeds/plant	67	54	53	29	51	6.93	31.1
100-seed weight (g)	24.4	24.3	20.4	21.3	22.6	0.61	13.1
Days to 50% flowering	47	45	48	47	47	1.54	7.5
Days to 90% maturity	109	100	95	93	100	1.61	3.3
Plant height (cm)	53.3	38.5	43.7	36.9	43.1	2.56	8.4

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Table 4: Means yield performance and stress tolerance parameters of nine chickpea cultivars evaluated at four treatments; non stress (N), water deficit (D), heat stress (H) and severe stress (HD), across four environments (HUD08, HUD10, HUD11 and SHAM09)

Cultivars	N	D	H	HD	YD/YN	YH/YN	YHD/YN
Shendi	1.77	1.27	0.95	0.56	0.79	0.59	0.38
Jabel Marra	1.98	1.20	0.70	0.55	0.61	0.37	0.30
Wad Hamid	1.73	1.29	0.95	0.68	<u>0.88</u>	0.62	<u>0.46</u>
Atmor	<u>2.17</u>	<u>1.58</u>	1.10	0.59	0.78	0.59	0.31
Hwata	2.01	1.28	<u>1.14</u>	0.72	0.80	<u>0.66</u>	0.38
Burgeig	1.84	1.23	1.13	<u>0.73</u>	0.70	<u>0.64</u>	0.43
Salawa	1.88	0.98	0.89	0.60	0.52	0.49	0.32
Matama	1.73	1.05	1.04	<u>0.73</u>	0.68	<u>0.64</u>	<u>0.46</u>
FLIP87-59C	<u>2.17</u>	1.05	1.03	0.62	0.49	0.50	0.30
Mean	1.92	1.21	0.99	0.64	0.69	0.57	0.37
EMS	$246.7^{N.S}$	83.4^{**}	44.4^{**}	$34.8^{N.S.}$	$0.141^{N.S.}$	0.043^{**}	$0.032^{N.S.}$
LSD (5%)	0.41	0.24	0.17	0.15	0.31	0.17	0.15

3.4 Yield Stability

Analysis of variance for yield Stability of the nine chickpea genotypes evaluated across four environments; heat stress, water stress, severe stress and overall of stress treatments were revealed in Table 5. Highly significant differences were found among all environments (E). Differences among genotypes were significant for heat stress and overall environments. Genotype by environment interaction was significant in all stresses. The two stability parameters; coefficient of regression (*bi*) and deviation from regression (S^2d) were estimated for seed yield/ha of all genotypes (Table 6). Stability analysis under water stress environment differentiated the cultivars Jabel Marra, Hwata, Shendi and Wad Hamid for regression coefficient (*bi*) close to 1.0 and non-significant deviation from regression. The genotype FLIP 87-59C showed the lowest deviation from regression (S^2d) and the highest regression coefficient (*bi*). Under heat stress environment, Hwata cultivar showed regression coefficient close to unity, non–significant deviation from regression with high seed yield followed by FLIP 87-59C genotype and Wad Hamid cultivar while, Atmor cultivar gave the highest regression coefficient (*bi)* with significant deviation from regression (S^2d). Under severe stress environment, Hwata, FLIP 87-59C and Wad Hamid genotypes showed regression coefficient close to unity with non–significant deviation from regression with high seed yield. Under both heat and severe stresses the cultivar Matama was adapted to adverse environment with stable yield (S^2d=0). On basis of overall environments, and based on regression coefficient (*bi*) close to 1.0 and non-significant

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deviation from regression the cultivar Hwata was the most stable for three stresses followed by FLIP 87-59C genotype and Wad Hamid cultivar. Across overall environments, the genotypes Hwata, Jabel Marra and FLIP87-59C were high stable, whereas Atmor cultivar was more adapted for favorable environments. The cultivars Shendi, Wad Hamid, Burgeig, Salwa and Matama were unstable and showed significant deviation from regression and adapted to adverse environments (Table 6).

Thus, the cultivars Wad Hamid and Shendi were considered to be highly stable and tolerant to water stress condition whereas, Hwata cultivar was stable and tolerant to heat stress conditions. The cultivar Wad Hamid was considered to be high stable and tolerant under heat and water stresses combination. Matama cultivar was the most tolerant under heat and combined of heat and water stresses and most adapted to adverse environments. The genotypes Hwata and FLIP87-59C were high stable overall environments, whereas Atmor cultivar was more adapted for favorable environments

3.5 Correlation between different traits

Seed yield (t/ha), seed yield/plant (g), number of pods/plant, number of seeds/plant, 100-seed weight, plant height and days to 50% maturity were positively and significantly correlated with each other. Negative and significant correlation was found for days to 50% flowering with 100-seed weight and plant height. Furthermore, days to 50% flowering was not significantly correlated with seed yield/ha and seed yield/plant in positive and negative direction respectively. Negative and non significant correlation was also found for 100-seed weight with number of pods/plant, number of seeds/plant and days to 90% maturity (Table 7).

Table 5: Mean squares from stability combined analysis of variance for seed yield (t/ha) of nine chickpea cultivars evaluated under specific 8 environments (2 treatments (non-stress combined with either water or heat or severe stress) X 4 seasons (HUD08, HUD10, HUD11 and SHAM09)); and across overall 16 environments (4 treatments X 4 seasons).

Source of variation	Specific 8 environments				Overall 16 environments	
	DF	Heat stress	Water stress	Heat and Water stresses	DF	
Genotypes (G)	8	0.339*	0.4837	0.16	8	0.390**
Environment (E)	7	8.688**	5.30**	14.02**	15	7.617**
GE Interaction	56	0.334**	0.359**	0.228**	120	0.257**
Environment (Linear)	1	60.81**	37.0843	98.138**	1	114.259**
GE Interaction (Linear)	8	0.384**	0.327*	0.446**	8	0.525**
Pooled Deviation	48	0.326**	0.364**	0.192	112	0.237**

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Pooled error	128	0.1455	0.165	0.1407	256	0.102**

Table 6: Estimates of stability parameters, regression coefficient (*bi*) and mean square deviation (*S^2d*) for seed yield (t/ha) of nine chickpea cultivars evaluated under specific 8 environments (2 treatments (non-stress combined with either water or heat or severe stress) X 4 seasons (HUD08, HUD10, HUD11 and SHAM09); and across overall 16 environments (4 treatments X 4 seasons).

	Water stress			Heat stress			Severe stress			Overall		
Genotype	Mean	bi	S^2d	Mean	bi	S^2d	Mean	bi	S^2d	Mean	bi	S^2d
Shendi	1.521	0.92	0.065	1.358	0.86	0.15**	1.166	1.02	0.049	1.137	0.92	0.08**
Jabel Marra	1.587	1.16	0.041	1.337	1.22	0.121*	1.266	1.09	0.041	1.105	1.12	0.07**
Wad Hamid	1.507	0.87	0.073	1.342	0.99	0.047	1.202	0.87	0.038	1.161	0.93	0.039
Atmor	1.876	1.33	0.24**	1.632	1.33	0.18**	1.377	1.37	0.16**	1.358	1.32	0.11**
Hwata	1.641	1.09	0.070	1.574	1.02	0.019	1.365	1.03	0.008	1.287	1.07	0.041
Burgeig	1.534	0.73	0.075	1.485	0.78	0.14**	1.287	0.81	0.061	1.233	0.88	0.07**
Salwa	1.426	0.85	0.23**	1.385	0.93	0.12*	1.236	0.92	0.11**	1.085	0.92	0.110**
Matama	1.389	0.59	0.14**	1.383	0.61	0.044	1.229	0.71	0.042	1.136	0.64	0.07**
FLIP87-59C	1.607	1.45	0.027	1.597	1.27	0.049	1.393	1.19	0.008	1.217	1.22	0.042

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Table 7: Phenotypic coefficients of correlation between yield and yield components for nine chickpea cultivars evaluated across four environments and under four treatments

	Seed yield (t/ha)	Seed yield /plant(g)	Number of pods/plant	Number of seeds/plant	100-seed weight (g)	Days to 50% flowering	Days to 90% maturity
Seed yield /plant (g)	0.506**						
Number of pods/plant	0.511**	0.880**					
Number of seeds/plant	0.480**	0.876**	0.937**				
100-seed Weight (g)	0.175*	0.246**	-0.014	-0.076			
Days to 50% Flowering	0.003	-0.007	0.114	0.110	-0.403**		
Days to 90% Maturity	0.641**	0.194*	0.283**	0.243**	-0.043	0.222**	
Plant height (cm)	0.463**	0.411**	0.418**	0.390**	0.234**	-0.164*	0.197*

4. DISCUSSION

Terminal heat stress, water and combined stresses induced a remarkable reduction in yield and yield components of chickpea genotypes. Terminal heat stress induced more reduction than water stress this because chickpea is usually documented as drought tolerant crop [13]. Nevertheless, [14] reported that, drought stress had a greater effect than heat stress on yield and the biochemical seed-filling mechanisms of chickpea. Chickpea is especially sensitive to heat stress at the reproductive stages [15] [16] [17]. [4] reported that, the heat stress during pod development decreased seed yield more than 53 %. Yield reduction was greater for heat stress during pod development compared with the same stress during early flowering [4]. Averaged across chickpea genotypes, seed yields were significantly decreased by 43% in response to drought stress [18]. The combined effects of heat and water stresses were more severe than the additive effects of heat and water deficit alone. Previous research on wheat has shown that the combination of stresses being more severe than either stress alone [19] [20] [21].

Water stress resulted in early flowering and high reduction in plant height, whereas heat stress delayed flowering. Earlier days for 50% flowering due to moisture stress was also obtained by [13]. Both heat and water stresses accelerated maturity. Early maturity is an important trait to

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drought avoidance to the onset of severe moisture stress. Earliness is the most important trait offering resistance to terminal drought and heat stress [6]; however, under terminal drought conditions, selection for earliness and early maturity has been highly successful [22]. In case of breeding drought and heat-tolerance chickpea, days to the first flowering and maturity should be evaluated ahead of many other phenological traits, harvest index, biological yield and pods per plant to escape terminal drought and heat stresses for increased yield [23].

The genotypes in this study exhibited variable yield performances and tolerance under water, heat and severe stresses conditions. This indicates that, tolerance to stress in chickpea varies with the incidence of heat, drought and also its severity. [24] reported that, temperature tolerance is an important component of drought resistance and a necessary attribute for varieties destined. It is interesting that the cultivar Atmor gave the highest seed yield in both water stress and non stressed conditions. It is rare that one single genotype shows good performance in two different conditions. Wad Hamid cultivar showed the highest drought tolerance whereas, Hwata cultivar revealed the highest heat tolerance followed by Burgeig and Matama cultivars. The two cultivars Wad Hamid and Matama were the most tolerant under severe stress. The predominance of GxE effects on the seed yield/ha under drought, heat and their combination conditions, showed that, genotypes stability in environments was different. According to the definition of [12], a stable preferred variety would have approximately bi =1, (S^2d= 0) and a high mean performance. Many cultivars showed stable yield under water stress however, a few number of cultivars gave stable yield under heat or combined of heat and water stress. The cultivars Jabel Marra, Hwata, Shendi and Wad Hamid were stable under drought. Only Hwata cultivar was stable under heat stress conditions. The genotypes Hwata, FLIP 87-59C and Wad Hamid were stable under severe stress. Matama cultivar was adapted to adverse environments with stable yield under both heat and severe stresses. The genotype FLIP 87-59C which has been released as a drought-resistant germplasm source [25] was highly adapted to favorable condition and gave stable yield. Based on overall environments the cultivar Hwata was stable, whereas the cultivar Atmor was adapted to favorable environments.

Analysis of agronomic and yield components and their correlation towards seed yield could provide a good opportunity for effective indirect selection of high yielding genotypes. Positive and significant association for number of pods/plant, number of seeds/plant, 100-seed weight and plant height with seed yield t/ha revealed importance of these traits in determining yield. These results were in agreement with those of [26] [27] [28]. Non significant correlation for days to 50% flowering with seed yield/ha and seed yield/plant was also found by [26]. A negative correlation for days to 50% flowering with seed yield/ha was reported by [29] [28]. Days to maturity was positively correlated with seed yield and its components in contrast to what has been reported by [26]. The negative association of number of pods/plant with 100-seed weight may be due to the compensatory phenomenon in chickpea between yield components during plant development. Similar results were also reported by [26].

5. CONCLUSION

It could be concluded that, chickpea was more sensitive to terminal heat stress as compared to water stress. The high yielding tolerant and stable cultivars were Wad Hamid and Shendi under water deficit stress, Hwata under terminal heat stress and Wad Hamid under severe stress (combination of heat and drought). However, these genotypes could be used to improve tolerance to different abiotic stresses in chickpea and making possibilities of extending production of chickpea in the non-traditional areas of Sudan (The cultivars Hwata was high stable across overall environments, whereas Atmor cultivar was more adapted for favorable environments). Moreover, the traits, such as number of pods/plant, number of seeds/plant, and 100-seed weight could be used for indirect selection of high yielding chickpea genotypes, because of their strong and positive association with seed yield.

6. ACKNOWLEDGEMENTS

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