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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF CHICKPEA (*Cicer arietinum* L.) GENOTYPES TO DIFFERENT MOISTURE STRESSES

*A.N. Md. ANAMUL KARIM, Uttam KUMER SARKER, Ahmed KHAIRUL HASAN, Najrul ISLAM, Md. ROMIJ UDDIN**

Bangladesh Agricultural University, Department of Agronomy, Mymensingh, Bangladesh
Corresponding author: romijagron@bau.edu.bd

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ABSTRACT

Moisture stress influence seed germination, growth including physiological, biochemical attributes and yield of chickpea (*Cicer arietinum* L.). Genotypes may vary in their capacity to tolerate moisture stress. Therefore, the study was undertaken to evaluate physiological and biochemical responses of selected chickpea genotypes in the drought prone ecosystems. Relative water content and carotenoids content significantly decreased when stress imposed until pod formation stage. Moisture stress imposed during pre-flowering stage significantly decreased chlorophyll a and chlorophyll b content. Proline accumulation was higher in BD-6048 compared to other genotypes under all moisture stress conditions. Phosphorus, potassium and protein content were lower under moisture stress until pod formation stage. Under moisture stress conditions the genotypes BD-6048 had the highest yield compared to other genotypes. Moisture stress until pre-flowering and pod formation stage reduced seed yield more severe than that on flowering stage.

Keywords: Chlorophyll content, field capacity, relative water content and seed yield

INTRODUCTION

Agriculture and water resource sectors are commonly affected by moisture stress. It may cause significant economic losses in the agriculture part of developed countries through diminutions in crop yield or total failure of crops (Toker and Mutlu, 2011; Sweet et al., 2017). It can also cause human migration and food crisis in developing countries under certain circumstances (Gray and Mueller, 2012; Grolle, 2015). In irrigated agricultural systems moisture stress has great impact on crop production (Vidal-Macua et al., 2018) and also troubles for urban water supply, manufacturing needs, decreases of hydropower production, etc. (Balling and Gober, 2007; Jerez et al., 2013). About 35% of land of the world is in arid and semi-arid condition. Farmers have taken on low yield set of varieties for the chickpea (*Cicer arietinum* L.) crop in rain-fed areas. The uses of optimal inputs are restricted bythe adjustment of such type of genotypes cultivation (Jackson et al., 2007). The concept of moisture stress tolerance of chickpea genotypes becoming a novel assignment for researchers due to water shortage, climate change as well as alteration of irrigated land into household for over population (Fahadet al., 2017; Eckstein et al., 2019; Jamro et al., 2020).

Recently, chickpea is the third most important pulse crop of Bangladesh in area and production. Bangladesh grows chickpea on about 4812 ha producing 5347 tons of grains with an average yield of about 1111 kg ha^{-1} in 2019 (FAOSTAT, 2021), which constitute about 0.04% of the total chickpea production in the world. Chickpea is mainly grown in the dry zone of Bangladesh. It is grown under residual soil moisture in both lowland and upland conditions. In lowland areas, it is grown as a relay or sequential crop after rice, while in upland areas it is grown mostly on fertile soil with a good water holding capacity after sesame, maize, green gram or fallow. The main constraints in yield reduction of chickpea crop are long moisture stress and sudden rain existed in the dry region where the water shortage is the main difficulty. The breeding and selection of genotypes under drought are considered an effective method to minimize the ramification of moisture stress exposure (Toker and Mutlu, 2011; Eckstein et al., 2019; Jamro et al., 2020).

Plants react to moisture stress and become adapted through various physiological and biochemical changes including changes of water use efficiency, proline content, and photosynthetic activity (Farooq et al., 2009). Moisture stress tolerance is linked with high relative water content (RWC) and low excised-leaf water loss. There is modulation of the activities of antioxidant enzymes which leads to enhanced cellular protection during the crop experiences stress conditions (Kaur et al., 2012). Plant cells respond defensively to oxidative stress by maintaining antioxidant defense compounds and osmolytes. Proline is one of the familiar osmolytes which boost in plants under

moisture stress and assist the plants to continue cell turgidity (Moayedi et al., 2011). The damage caused during stress finally stress yield. Studies on reaction of antioxidative and non-antioxidative defense systems have been reported earlier in chickpea, but changes in carotenoids, chlorophyll, phosphorus, potassium, protein and proline content due to drought stress is still lacking. Therefore, the present study aims to expose various physiological and biochemical adaptations of selected chickpea genotypes with yield attributes at different moisture stress.

MATERIALS AND METHODS

Site description

The experiments were conducted during rabi season of 2017 and 2018 in the pot yard of the Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. The experimental site was located at $23^0 59'$ latitude and $90^0 24'$longitude and at the elevation of 34.5 m above the sea level. The soil was clay loam/clay in texture. General fertility status of the soil was low having low status of organic matter, including low status of phosphorus (P), potassium (K), medium status of zinc (Zn) and boron (B). The pH of the soil is 5.6. The soil contained 1.12% organic matter, 0.054% total nitrogen, 7.6 meq phosphorus, 0.14 meq 100 g^{-1} potash, 11.4 µg g^{-1}sulphur, 0.74 µg g^{-1} zinc and 0.23 µg g^{-1} boron.

Experimental design and treatments

The experiment was laid out in factorial experimental design. All treatments including four genotypes and 11 moisture stress applications were performed as Complete Randomized Design (CRD) with three replications. Experiment was carried out at net house.

Genotypes and moisture treatments

Four genotypes (G_1- BD-6048, G_2 - BD-6045, G_3- BD-6090, G_4- BD-6092) of chickpea along with 11 moisture stresses including T_1- Control (without irrigation), T_2- 30% of Field Capacity (FC) until pre-flowering stage,T_3- 50% of FC until pre-flowering stage,T_4- 70% of FC until pre-flowering stage, T_5- 90% of FC until pre-flowering stage, T_6- 30% of FC until flowering stage,T_7- 50% of FC until flowering stage, T_8- 70% of FC until flowering stage, T_9- 90% of FC until flowering stage, T_{10}-30% of FC until pod formation stage,T_{11}- 50% of FC until pod formation stage were included in the study. Seeds were collected from Plant Genetic Resources Center (PGRC), BARI.

Pot preparation and seed sowing

At first 132 pots were set at the net house of BARI. The size of each pot was 20 cm depth and 9 cm radius. Then the prepared soil was filled in the plastic pots. Each plastic pot was contained 6.0 kg soil. After filling pots, seeds were sown in the plastic pot properly. Ten seeds were sown in each plastic pot by hand at 3 to 4 cm depth with plant spacing of about 8 cm. Before sowing the seeds, all the pots were pre-irrigated to make them in optimum soil moisture condition, necessary for germination. After the germination, three healthy seedlings per pot were kept for each pot.

Procedures of water management

An appropriate amount of water was applied to all the pots every day until the beginning of the treatments. The day before the starting of the treatments, 500 ml of water was applied to each pot so that the soil moisture content (percentage) of all the genotypes remained equal. The first water stress treatment was started on 15, December 2017 and 2018 until pre-flowering stage, 2nd water stress treatment was started on 15, February 2018 and 2019 until flowering stage and 3rd water stress treatment was started on 1st, March 2018 and 2019 until pod formation stage. The amount of water needed according to the treatments applied in each pot with the help of the measuring cylinder. Moisture was maintained on the basis of prevailing moisture of sun-dried soil in pots and pot weight every one-day interval taking consideration of weight of plants in pot.

Physiological parameters

Relative leaf water content was estimated according to the method of Weatherley (1950). 100 mg fresh leaves were kept in distilled water for 4 hours to obtained turgid weight. The turgid weight was recorded after blotting the excess water on the surface of the sample. Dry weight was obtained after drying the samples in oven at 70˚ C till constant weight occurred. The relative water content (RWC) was calculated by the formula:

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Biochemical parameters

Samples for chlorophyll and carotenoids determination were taken from chickpea leaves using a 0.8 cm diameter cork borer, weighted quickly in pre-weighted clean glass vials and 5 cm^3 of 80% acetone was added to these samples. The leaf material was bleached and decanted off. The optical density (OD) was read at λ = 663 nm, 645 and 470 nm using 80% acetone as a blank by a spectrophotometer. Content of chlorophyll a, chlorophyll b and carotenoids were calculated according to Lichtenthaler and Wellburn (1983) using the following formula:

Chlorophyll a = 12.21 OD 663 nm – 2.81 OD 645 nm

Chlorophyll b = 20.13 OD 645 nm – 5.03 OD 663 nm

Carotenoids = (1000 OD 470 nm – 3.27 chlorophyll a - 104 Chlorophyll b) / 229

Proline content was measured according to the method of Bates et al. (1973). An aliquot amount of fresh green leaf of chickpea was homogenized in 10ml of 3% sulphosalicylic acid and the homogenate was centrifuged at 5000 rpm for 15 min. Two milliliters of the supernatant were reacted with 2 ml of acid ninhydrin (1.25 g ninhydrin dissolve in 30 ml of glacial acetic acid and 20 ml of 6 M phsphoric acid) and 2 ml of glacial acetic acid for 1 hr at 100 ^{0}C and the reaction was then terminated in an ice bath.

The colored reaction mixture was extracted with 4 ml of toluene and the absorbance was recorded at 520 nm. Proline content was calculated from a standard curve. The protein contents of seeds were estimated following the procedure of Microkjeldhal (AOAC, 1965). One g seed sample was kept in a digest ion flask, with a little quantity of catalyst mixture (K_2SO_4 + $CuSO_4$), 10 ml of 96% concentrate sulphuric acid was added and kept for complete digestion. Digested sample was distilled. The distilled amount of ammonia was titrated with 0.1 N H_2SO_4.Nitrogen and protein content were calculated as per following formula:

Nitrogen (%) =Normality of H_2SO_4× V of H_2SO_4× 1.4 x100

Weight of sample

Protein (%) = Percent of nitrogen × 6.25

The content of phosphorous in the leaf of chickpea was determined by the procedure of Jackson (1975). One milliliter of aliquot of plant diacid extract in 50 ml volumetric flask, mixed with 10 ml of vanadate molybdate reagent diluted to 50 ml with distilled water and mixed well. The color was read after 30 min at 470 nm. The phosphorus concentrations were calculated using the standard curve expressed as percent. The content of potassium in the leaves of chickpea was determined by the method of Chapman and Pratt (1961). The plant extract (diacid digested) was directly read on flame photometer or after appropriate dilution as that final concentration range between 0 to 50 mg potassium per liter. A blank without sample was also run simultaneously. Result was calculated using a standard reading from potassium solution and expressed as percent potassium.

Measurement of yield and yield components

At maturity, the whole plant was cut at the ground level with a sickle. The harvested crop from each pot was bundled separately and tagged appropriately. Finally, data on yield contributing parameters such as plant height, number of pods per plant and seed yield were recorded separately.

Data analyses

Data were assessed by analysis of variance and by Duncan's multiple range test (Gomez and Gomez, 1984) with a probability $P \leq 0.05$. The values followed by the same letters are not significantly different and different letters within treatments indicate significant differences at the 0.05 probability level.

RESULTS AND DISCUSSION

Climatic condition during crop cycle

The climatic condition of the experimental plot is subtropical in nature characterized by heavy rainfall from June to September (78-92%) and scanty in winter (1-11%) and mean rainfall is around 2200 mm per year. Temperature starts rising from February and continues till September and then gradually falls from the month of October of the year. The monthly rainfall, maximum and minimum temperature sand humidity during the study period were detailed in Fig. 1 and Fig. 2.

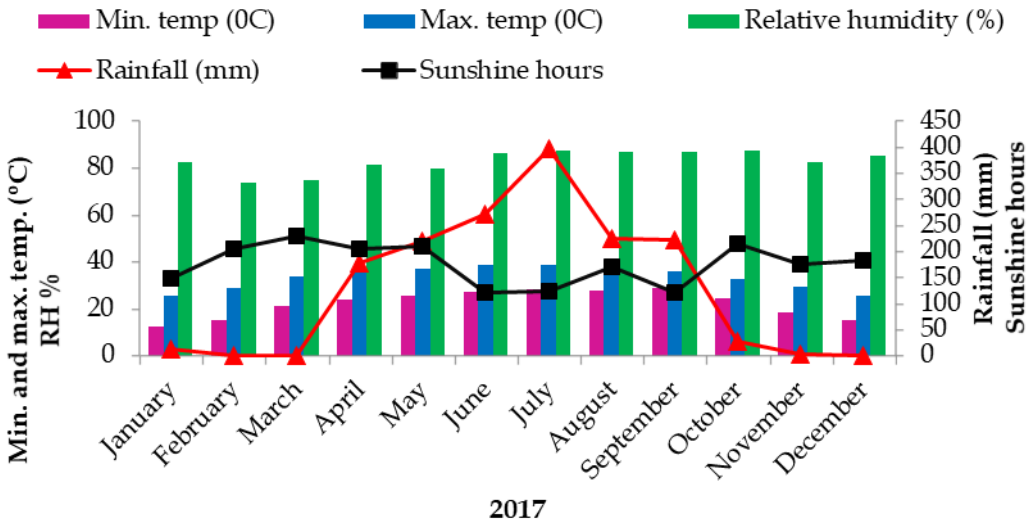

Fig. 1 Monthly average temperature, rainfall, relative humidity and sunshine hours of the experimental site in Gazipur during 2017

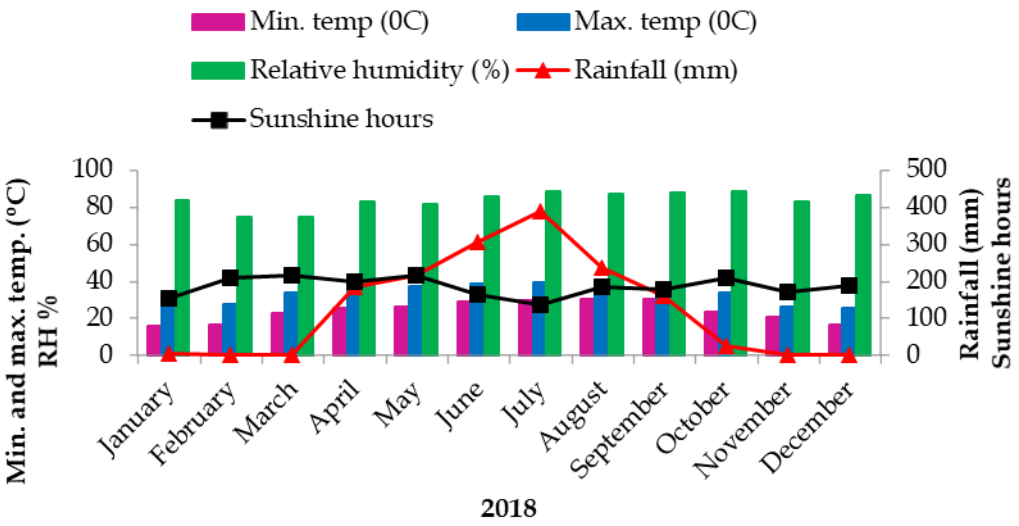

Fig. 2 Monthly average temperature, rainfall, relative humidity and sunshine hours of the experimental site in Gazipur during 2018

Interactions /Statistical significant analyses

Genotype by drought stresses and year interactions were found to be significant for RWC, carotenoids, chlorophyll a and b, protein and proline contents. ($P \leq 0.05$). Genotypic effect was significant for plant height, number of pod per plant and seed yield ($P \leq 0.05$).

Relative water content

Optimum relative water content is crucial for effective physiological functioning and growth processes of crop and is known as potential physiological marker in many crops. In the present study, RWC significantly decreased in all genotypes under moisture stress condition (Fig. 3). Among all the genotypes, BD-6048 showed maximum RWC (72.36 %) at 70% of FC until flowering stage and decreased when drought stress continued up to pod formation stage. This decline may be due to higher water loss through stomatal regulation during photosynthesis and ineffective water utilization assimilation under moisture stress (Lobato et al., 2008).

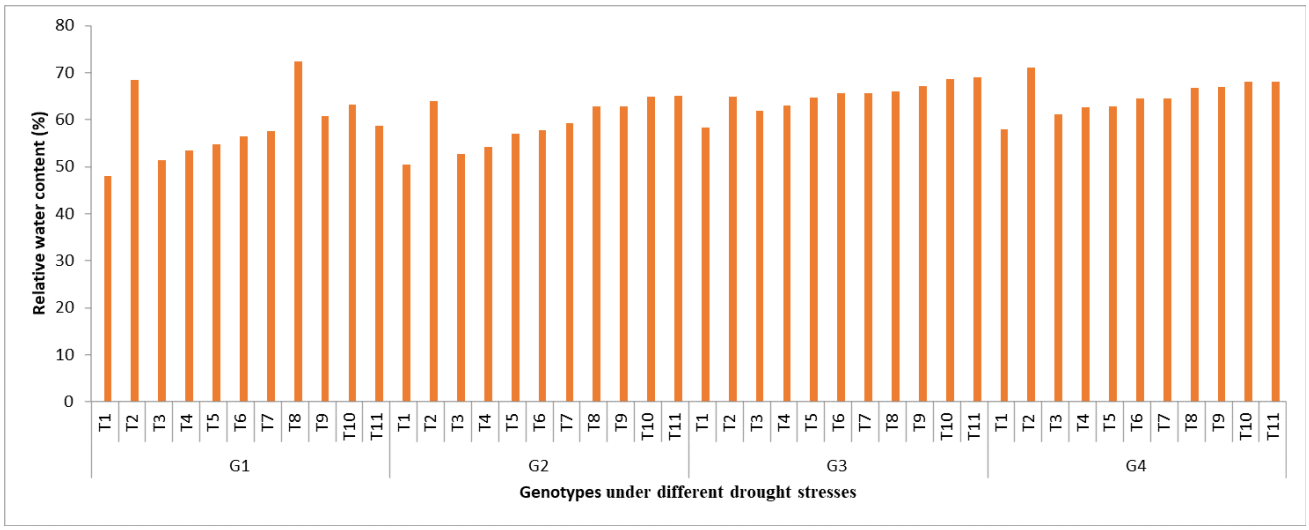

Fig 3 Relative water content (%) of genotypes under different drought stresses.

Carotenoids content

Leaf carotenoids content of chickpea under moisture stress differed in a genotype-dependent manner (Table 1). Carotenoids content of genotypes BD-6048 was highest compared to other genotypes. Moisture stress tolerant genotypes ('Yazd' and 'Shiraz') exhibited higher accumulation of carotenoids than moisture stress sensitive genotypes (Askari and Ehsanzadeh, 2015). While progressive increase in moisture stress level resulted in significant increases carotenoids content up to flowering stage and when chickpea was grown under moisture stress up to pod formation stage then it tended to decrease.

Table 1. Effect of interaction between genotypes and moisture stress on carotenoids content, chlorophyll a, chlorophyll b and potassium (%) of chickpea

Treatments	Carotenoids content (mgg^{-1})				Chlorophyll a (mgg^{-1})				Chlorophyll b (mgg^{-1})				Potassium (%)			
	G_1	**G_2**	**G_3**	**G_4**	**G_1**	**G_2**	**G_3**	**G_4**	**G_1**	**G_2**	**G_3**	**G_4**	**G_1**	**G_2**	**G_3**	**G_4**
T_1	1.28 xy	1.34vwx	1.94 ijk	1.10 z	0.62 za	0.62 za	0.76 rs	0.43 c	0.57 x	0.62z	0.75y-d	0.43C	3.16 zab	3.00 bc	2.62 d	6.02 j
T_2	2.28 e	2.03 gh	2.00 hij	1.86 lm	0.66 xy	0.66 xy	0.52 b	0.74 stu	0.67 x	0.69w	0.51z-a	0.79s	3.48 wx	3.14 zab	2.86 c	6.10 ij
T_3	1.29 xy	1.42 tuv	2.02 ghi	1.15 z	0.71 uv	0.61 za	0.59 a	0.83 p	0.73 v	0.73v	0.57y-b	0.83q	3.58 vw	3.27 yz	3.09 ab	6.19 hi
T_4	1.33 wx	1.46 stu	2.08 g	1.23 y	0.76 rs	0.62 z	0.63 yz	0.87 o	0.79 s	0.77t	0.63z	0.86p	3.69 uv	3.42 xy	3.04 b	6.29 h
T_5	1.40uvw	1.54 rs	2.18 f	1.27 xy	0.80 pq	0.70 vw	0.67 wx	0.91 mn	0.83 q	0.81r	0.66y	0.90o	3.89 st	3.59 vw	3.13 zab	6.53g
T_6	1.59 qr	1.59 qr	2.27 e	1.34vwx	0.91 lm	0.74 st	0.70 v	0.93klm	0.93 m	0.86p	0.69w	0.92mn	3.91 rst	3.69 uv	3.20 za	6.79f
T_7	1.64 pq	1.65 pq	2.32 de	1.44 tu	0.94 jkl	0.88 no	0.72 tuv	0.97 hij	1.09 g	0.92n	0.72v	0.96k	3.95 rst	3.71 uv	3.39 xy	7.00e
T_8	2.63 a	1.72 op	2.37 cd	1.49 st	1.35 a	0.96 ijk	0.78 qr	0.98fghi	1.29a	0.95l	0.76tu	0.98j	8.06a	3.80 tu	3.61 vw	7.26d
T_9	1.88klm	1.82 mn	2.45 bc	1.58 qr	0.99 fgh	1.03 e	0.82 p	1.01 efg	1.22c	0.99j	0.83q	1.02i	4.28 mn	4.05 pqr	3.97 qrs	7.60c
T_{10}	1.94 ijk	1.93 jkl	2.52 b	1.76 no	1.29 b	1.08 d	0.88 no	1.08 d	1.30b	1.10g	0.87p	1.07h	5.14 kl	4.25 no	4.16 nop	7.82b
T_{11}	1.72 op	2.01ghij	2.65 a	1.82 mn	0.98 ghi	1.15 c	1.01 ef	1.12 c	1.15d	1.13e	0.99j	1.11f	4.10 opq	5.04 l	4.42 m	5.23k
CV (%)	1.78	1.78	1.78	1.78	2.27	2.27	2.27	2.27	0.92	0.92	0.92	0.92	2.10	2.10	2.10	2.10
LSD ($_{0.05}$)	0.08	0.08	0.08	0.08	0.031	0.031	0.031	0.031	0.12	0.12	0.12	0.12	1.53	1.53	1.53	1.53

In a column, means followed by same letters are not significantly different at 5 % probability level by Duncan's Multiple Range Test (DMRT),G_1 = BD-6048, G_2 = BD-6045, G_3 = BD-6090, G_4 = BD-6092, T_1 = Control (without irrigation), T_2 = 30% of FC until pre flowering stage, T_3 = 50% of FC until pre flowering stage, T_4 = 70% of FC until pre flowering stage, T_5 = 90% of FC until pre flowering stage, T_6 = 30% of FC until flowering stage, T_7 = 50% of FC until flowering stage, T_8 = 70% of FC until flowering stage, T_9 = 90% of FC until flowering stage, T_{10} = 30% of FC until pod formation stage, T_{11} = 50% of FC until pod formation stage.

Chlorophyll a and chlorophyll b content

The interactions between genotypes by moisture stress treatments were significant for chlorophyll contents($P \leq 0.05$).Moisture stress imposed up to the pre-flowering stage, significantly decreased chlorophyll *a* and chlorophyll *b* content whereas moisture stress imposed up to flowering and pod formation stage also influenced these contents. The restricted water supply up to pre-flowering is liable for reducing chlorophyll content and during flowering stage and pod formation stage it had a mild effect on these contents. The moisture stress also indicated that chlorophyll *b* is not more sensitive to moisture stress than chlorophyll *a* (Table 1).The genotype BD-6048 showed a higher chlorophyll *a* and *b* content than the other genotypes up to flowering stage (Table 1). The results are accord with Nyachiro et al. (2001), depicted a significant decrease of chlorophyll *a* and *b* caused by water deficit in six *Triticum aestivum* L. cultivars. Decreased or unchanged chlorophyll level during moisture stress has been stated in other species, depending on the interval and severity of moisture stress (Kpyoarissis et al., 1995). A decrease of chlorophyll with moisture stress means a lowered capacity for light harvesting.

K and P content

The impact of moisture stress on P and K of the plant leaf was shown in Table 1 and 2.K content was higher when moisture stress imposed up to 70% of FC until flowering stage and tended to decrease at moisture stress up to pod formation stage. P contents showed considerable variation among the stress treatments and genotypes. Decreases in P content were larger for moisture stress up to pod formation stage compared to drought imposed at the pre-flowering and flowering stage. This might be expected with decreasing mobility of soil nutrients and plant water uptake as stress progressed. Novak and Voinovich (2000) stated that nutrient loss mechanism uptake of nutrients during the latter parts of the vegetation period was related to the differences in the estimated dry-weight biomass. In addition, Radersma et al. (2005) found that lower soil water contents caused less P uptake (through hampered diffusion) and decreased maize biomass growth.

Proline content

Differences in proline content or interactions between genotypes by moisture stress treatment were significant($P \leq 0.05$). The proline content increased where moisture stress imposed up to pre flowering stage than up to both flowering and pod formation stages in all genotypes of chickpea (Table 2). The proline content due to moisture stress was more in genotype BD-6048. The proline content depends on plant age, leaf age, leaf position or leaf part (Chiang and Dandekar, 1995). Under vegetative stage, moisture stress improved proline content, these increasing roles due to osmotic compatible and adjust osmotic potential which resulted in moisture stress avoidance in chickpea. Proline was found to be accumulated in a large level under moisture stress (Kalefetoglu, 2006; Tan et al.,2006; Ceyhan et al., 2012). Proline accumulation play adaptive roles in plant stress tolerance (Verbruggen and Hermans, 2008). For selection of stress tolerance, accumulation of proline has been considered as a parameter (Jaleel et al., 2007).

Protein content

Percent protein content was reduced with receding moisture stress (Table 2). At maintaining moisture stress up to flowering stage, the increase in leaf protein content was observed in BD-6048. With the further reduction in water levels, the protein content decreased in BD-6048 in comparison with control. Johal et al. (2020) elucidated that stress sensitive genotypes recorded maximal reduction (20.86 %) in comparison with tolerant accession at 75% receding moisture level.

Plant height and pod number per plant

Moisture stress had a significant effect on plant height and the number of pods per plant. Plants were generally tallest and had the highest number of pods when they were grown without moisture stress. Interactions between genotypes by moisture stress treatment were significant for plant height and pod number. The effect of the moisture stress on plant height up to pre flowering stage was severe and it had less effect when stressed up to flowering and pod formation stage (Table 3). Averaged across treatments BD-6048 showed the highest plant height. Pod number was also affected by moisture stress. The severity was more when stressed continued up to pod formation stage. BD-6048 had the highest pod numbers irrespective of treatments (Table 2). The yield of grain legumes grown under moisture stress conditions is largely depending on the number of pods plant per plant (Lopez et al., 1996; Pilbeam et al., 1992).

Seed yield per plant

The yield reaction to drought stress of chickpea is given in Table 3. The yield of all for genotypes of chickpea was affected by moisture stress. Interactions between cultivars by moisture stress treatment were significant. Plants stressed until pre-flowering stage and pod formation stage, gave a significantly lower yield than plants stressed during flowering stage. The highest yield was obtained from BD-6048 when stress imposed 70% of FC until flowering stage. Seed yield under moisture stress at 50% of FC until pod formation stage showed 19.94 % less than that under stress treatment at 50% of FC until flowering stage.

Table 2. Effect of interaction between genotypes and moisture stress on phosphorus (%), proline content and protein content (%) of chickpea

Treatments	Phosphorus (%)				Proline content (mg100g^{-1})				Protein content (%)			
	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4
T_1	1.02 a	0.72 d	0.68 e	0.75 c	219.39a	214.52b	205.33d	193.38i	20.88 yz	22.42 n-r	21.09 yz	18.27 yzo
T_2	0.68 e	0.66 fgh	0.61 jkl	0.51 tuv	208.18c	202.37f	196.32h	186.39l	22.31o-s	22.98 i-m	21.62 vwx	20.81 z
T_3	0.74 cd	0.66 e-h	0.67 efg	0.67 ef	203.41e	196.33h	193.65i	183.44no	22.78 k-n	23.09 h-l	21.70 u-x	21.32 xy
T_4	0.85 b	0.61 jk	0.65 gh	0.68 e	199.30g	191.48j	191.46j	181.50q	22.84j-n	23.26 g-j	21.81 t-w	21.57 wx
T_5	0.74 cd	0.59 lmn	0.64 hi	0.65 h	195.35h	184.50m	188.71k	178.44s	23.82 def	23.40 f-i	22.05 r-v	21.89 s-w
T_6	0.68 e	0.58mno	0.62 ij	0.62 jk	191.26j	182.51opq	186.90l	176.68t	23.93 cde	23.54 e-h	22.14 q-u	22.20 q-t
T_7	0.54 rs	0.53 st	0.60k-n	0.61 jk	186.65l	177.46st	183.56mn	173.70u	24.13 cd	23.65 efg	22.26 p-s	22.26 p-s
T_8	0.31 a	0.51 uv	0.60j-m	0.58 nop	182.69nop	171.31v	181.69pq	171.46v	24.72 a	23.73 def	22.29 p-s	22.70 l-p
T_9	0.38 y	0.49 v	0.58 nop	0.56 opq	178.44s	168.67x	179.72r	168.57x	24.36 abc	23.77 def	22.59m-q	22.95 i-m
T_{10}	0.35 z	0.46 w	0.56 pqr	0.55 qrs	174.23u	163.28z	176.57t	166.38y	24.62 ab	23.67 efg	22.75 k-o	23.16 h-k
T_{11}	0.49 v	0.41 x	0.53 s	0.53 stu	170.24w	159.30a	174.28u	163.38z	24.18 bcd	23.10 h-l	22.82 j-n	23.26 g-j
CV (%)	2.35	2.35	2.35	2.35	0.34	0.34	0.34	0.34	1.22	0.34	1.22	0.34
LSD ($_{0.05}$)	0.023	0.023	0.023	0.023	1.03	1.03	1.03	1.03	0.451	1.03	0.451	1.03

In a column, means followed by same letters are not significantly different at 5 % probability level by Duncan's Multiple Range Test (DMRT), G_1 = BD-6048, G_2 = BD-6045, G_3 = BD-6090, G_4 = BD-6092,T_1 = Control (without irrigation), T_2 = 30% of FC until pre flowering stage, T_3 = 50% of FC until pre flowering stage, T_4 = 70% of FC until pre flowering stage, T_5 = 90% of FC until pre flowering stage, T_6 = 30% of FC until flowering stage, T_7 = 50% of FC until flowering stage, T_8 = 70% of FC until flowering stage, T_9 = 90% of FC until flowering stage, T_{10} = 30% of FC until pod formation stage, T_{11} = 50% of FC until pod formation stage.

Table 3. Effect of interaction between genotypes and moisture stress on yield and yield contributing characters of chickpea

Treatments	Plant height (cm)				Number of pod plant^{-1}				Seed yield plant^{-1} (g)			
	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4
T_1	43.07m-q	42.61pq	41.02r	39.21s	65.57rs	64.13stu	63.71tu	59.34w	15.06j-m	14.12mn	11.56qr	11.25r
T_2	44.10 h-n	43.15 m-q	42.95 n-q	40.52r	69.89j-n	66.46pqr	63.35uv	61.80v	16.50fgh	14.39lmn	12.43pq	11.80qr
T_3	44.13 h-n	43.71 k-p	43.05 m-q	42.48q	70.95 h-l	68.46no	65.13rst	63.14uv	16.01hij	15.72h-k	12.93op	12.17pqr
T_4	45.85 c-f	43.60 l-q	43.46 l-q	42.70opq	71.91ghi	69.64j-n	67.47opq	66.43pqr	16.45f-i	15.99hij	14.15mn	13.58no
T_5	45.95cde	44.08 h-n	43.75 k-p	43.47 l-q	74.71cde	70.31-m	65.15rst	65.88qrs	15.92h-k	14.87klm	13.67no	13.67no
T_6	46.10cd	45.01d-j	44.24 g-m	43.93 i-o	75.65bcd	73.43efg	70.36i-m	67.70op	18.62bc	17.42def	15.42i-l	15.49hijk
T_7	47.00bc	46.08cd	45.21d-h	45.27d-h	76.91ab	74.30de	71.30h-k	69.52lmn	19.46ab	17.76cde	15.50h-k	15.85hijk
T_8	48.70a	48.03ab	46.62c	45.92cde	78.37a	76.37bc	73.83ef	71.86ghi	20.37a	19.85a	18.46bcd	17.90cde
T_9	45.99cde	45.91cde	45.02d-j	43.94 i-n	76.91ab	74.42de	70.81h-m	70.84h-m	18.02cde	18.73bc	17.45def	17.28efg
T_{10}	45.35d-g	44.86 e-k	45.09d-i	44.66 f-l	71.36hij	72.46fgh	69.59k-n	69.70j-n	16.29ghi	17.99cde	15.71h-k	15.74h-k
T_{11}	44.40g-l	44.45 g-l	43.75 k-p	43.84 j-p	73.67ef	71.14 h-l	69.18mno	67.46opq	15.58h-k	16.49fgh	16.21ghi	14.24mn
CV (%)	3.93	3.93	3.93	3.93	1.55	1.55	1.55	1.55	4.17	4.17	4.17	4.17
LSD ($_{0.05}$)	1.23	1.23	1.23	1.23	1.75	1.75	1.75	1.75	1.06	1.06	1.06	1.06

In a column, means followed by same letters are not significantly different at 5 % probability level by Duncan's Multiple Range Test (DMRT), G_1 = BD-6048, G_2 = BD-6045, G_3 = BD-6090, G_4 = BD-6092, T_1 = Control (without irrigation), T_2 = 30% of FC until pre flowering stage, T_3 = 50% of FC until pre flowering stage, T_4 = 70% of FC until pre flowering stage, T_5 = 90% of FC until pre flowering stage, T_6 = 30% of FC until flowering stage, T_7 = 50% of FC until flowering stage, T_8 = 70% of FC until flowering stage, T_9 = 90% of FC until flowering stage, T_{10} = 30% of FC until pod formation stage, T_{11} = 50% of FC until pod formation stage.

CONCLUSIONS

In conclusion, chickpea genotypes showed significant variation based on studied parameters for moisture stresses, which highlight the usability of these genotypes for future research programs. With the current outcome, it can be accomplished that moisture stress hinders the growth and metabolic action of chickpea genotypes. On the basis of analysis of chickpea genotypes, we concluded that there was considerable difference in physiological, biochemical attributes and seed yield. BD-6048 had higher RWC, chlorophyll, carotenoids and proline content in comparison to other genotypes up to moisture stress at pre-flowering stage. These parameters also demonstrated substantial variability under different moisture stress conditions. This research may assist to understand some adaptive mechanisms developed by chickpea genotypes and may useful to categorize valuable traits for chickpea breeding programs.

LITERATURE CITED

AOAC. 1965. Official Methods of Analysis of Association of Official Analytical Chemists.15. ed, Washington D.C.

Askari, E. and P. Ehsanzadeh. 2015. Drought stress mitigation by foliar application of salicylic acidand their interactive effects on physiological characteristics of fennel (*Foeniculum vulgare* Mill.) genotypes. Acta. Physiol. Plant.37:1-14.

Balling, R.C. and P. Gober. 2007. Climate variabilityand residential water use in the city of Phoenix,Arizona. J. Appl. Meteorol. Clim. 46(7): 1130-1137.

Bates, L.S., R.P. Waldren and L.D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant Soil. 39: 205-207.

Ceyhan, E., A. Kahraman, M. Onder, M.K. Ates, S. Karadas, R. Topak and M.A. Avci. 2012. Physiological and biochemical responses to drought stress of chickpea genotypes. World Aca. Sci. Eng. Technol. 66: 383-388.

Chapman, H.D. and P.R. Pratt. 1961. Methods of Analysis for Soils, Plants and Water. Univ. of Calif., Berley. pp. 169-170.

Chiang, H.H. and A.M. Dandekar. 1995. Regulation of proline accumulation in *Arabidopsis thaliana* (L.) Heynh during development and in response to desiccation. Plant. Cell Environ. 18: 1280–1290.

Eckstein, D., V. Kunzel, L. Schafer and M. Winges. 2019. Global climate risk index 2020. Bonn Ger. 2019: 9.

Fahad, S., A.A. Bajwa, U. Nazir, S.A. Anjum, A. Farooq, A. Zohaib, S. Sadia, W. Nasim, S. Adkins, S. Saud, M.Z. Ihsan, H. Alharby, C. Wu, D. Wang and J. Huang. 2017. Crop production under drought and heat stress: Plant response sand management options. Front. Plant Sci. 8:1147.

FAOSTAT, 2021. Statistical database of the United Nations Food and Agriculture Organization, http://faostat fao.org/download/O/OA/E. (Accessed April23, 2021)

Farooq, M.A. Wahid, N. Kobayashi, D, Fujita and S.M.A. Basra. 2009.Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29: 185-212.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. A Wiley International Science Published by John Wiled and Sons New work, Brisbane Singapore pp. 139-240.

Gray, C. and V. Mueller. 2012. Drought and Population Mobility in Rural Ethiopia. World Dev. 40(1): 134-145.

Grolle, J. 2015. Historical case studies of famine sand migrations in the West African Sahel and their possible relevance now and in the future. Popul. Environ. 37(2): 181-206.

Jackson, L.E., U. Pascual and T. Hodkin. 2007. Utilizing and conserving agro biodiversity in agricultural landscapes. Agro Ecol. Syst. Environ. 121: 196-210.

Jackson, M.L. 1975.Soil Chemical Analysis Prentice Hall of India Pvt. Ltd. New Delhi.

Jaleel, C.A., R. Gopi, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharanand R. Panneerselvam. 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South Afr. J. Bot. 73: 190–195.

Jamro, S., F.N. Channa, G.H. Dars, K. Ansari and N.Y. Krakauer. 2020. Exploring the Evolution of Drought Characteristics in Balochistan, Pakistan. Appl. Sci. 10(3): 913.

Jerez, S. and R.M. Trigo. 2013. Time-scale andextent at which large-scale circulation modesdetermine the wind and solar potential in the Iberian Peninsula. Environ. Res. Lett. 8(4):044035.

Johal, N., J.Kaur, S.K. Grewal, S. Singh and A. Kushwah.2020. Physiological and Biochemical Responses of Chickpea Accessions at Reproductive Stage Under Receding Moisture Conditions. Agric. Res. 9(4):554–567.

Kalefetoglu, T. 2006. Characterization of chickpea (*Cicer arietinum*L.) cultivars and lines tolerance to drought stress. Master Thesis, Hacettepe Universiy, Ankara, Turkey.

Kaur, S., M. Arora, A.K. Gupta and N. Kaur. 2012. Exploration of biochemical and morphological diversity in chickpea seeds to categorize cold stress-tolerant and susceptible genotypes. ActaPhysiol. Plant.34: 569-580.

Kpyoarissis, A., Y. Petropoulou and Y. Manetas. 1995. Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: avoidance of photo inhibitory damage through decreased chlorophyll contents. J. Exp. Bot.46: 1825–1831.

Lichtenthaler, A. and R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem. Soc. Trans.11 (5): 591–592.

Lobato, A.K.S., R.C.L. Costa, C.F. Oliveira Neto, B.G. Santos Filho, F.J.R. Cruz, J.M.N. Freitas and F.C. Cordeiro. 2008. Morphological changes in soybean under progressive water stress. Int. J. Bot.4: 231-235.

Lopez, F.B., C. Johansen, Y.S. Chauhan. 1996. Effect of timing of drought stress on phenology, yield and yield components of as hort-duration pigeon pea. J Agron.Crop Sci. 177: 311-320.

Moayedi, A.A., A. Nasrulhaq-Boyce and H. Tavakoli. 2011. Application of physiological and biochemical indices for screening and assessment of drought tolerance in durum wheat genotypes. Aust. J. Crop Sci.5: 1014-1018.

Novak, V. and J. Vidovic. 2000. Transpiration and nutrient uptake dynamics in maize (*Zea mays* L.). Ecol. Model. 166:99–107.

Nyachiro, J.M., K.G. Briggs, J. Hoddinottand, A.M. Johnson-Flanagan. 2001. Chlorophyll content, chlorophyll fluorescence and water deficit in spring wheat. Cereal Res. Commun. 29: 135–142.

Pilbeam, C.J., J.K. Akatse, P.D. Hebblethwaite and C.D. Wright. 1992. Yield production in two contrasting forms of spring-sown faba beans in relation to water supply. Field Crops Res. 29:73-287.

Radersma, S., B. Lusianaand M. van Noordwijk. 2005. Simulation of soil drying induced phosphorus deficiency and phosphorus mobilization as determinants of maize growth near tree lines on a Ferral soil. Field Crops Res. 91:171–184.

Tan, Y., Z. Liang, H. Shao and F. Du. 2006.Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of *Radix astragali* at seeding stage. Colloid Surf. B. 49: 59-64.

Toker, C and N. Mutlu. 2011. Breeding for abiotic stresses.In: Biology and Breeding of Food Legumes, Pratap, A.; Kumar, J.Eds, CaB Int., Wallingford, UK, pp: 241-261.

Verbruggen, N. and C. Hermans. 2008.Proline accumulation in plants: a review. Amino Acids.35: 753- 759.

Vidal-Macua, J.J., M. Ninyerola, A. Zabala, C. Domingo-Marimon, O. Gonzalez-Guerrero and X. Pons. 2018. Environmental and socioeconomic factors of abandonment of rainfed and irrigated crops in northeast Spain. Appl. Geogr. 90: 155-174.

Weatherley, P.E. 1950. Studies in the water relations of the cotton plant. I. The ûeld measurement of water deûcits in leaves. NewPhytol.49: 81-97.