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Effect of moisture regimes on physiological and biochemical parameters of chickpea (*Cicer arietinum* L.) genotypes

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Abstract
Parameter viz., number of primary and secondary branches per plant, plant height, number of nodules, biomass per plant, CGR, RGR, days to maturity, total chlorophyll, nitrate reductase activity and protein content were studied at 55, 75 and 95 DAS whereas, biological yield, yield and yield components were studied at harvest the results revealed that genotype KWR-108, RSG-888 and KSJD-884 were drought tolerant and PG-5, K-850 and JG-74 were drought susceptible genotypes. The drought tolerance capacity of tolerant genotypes was associated with retention of high RWC, less membrane damage, high total chlorophyll content and higher osmotic potential under stress conditions. Drought tolerant varieties have less reduction in maintained NAR, RGR and CGR for longer duration. Regarding sink potential, the tolerant genotypes recorded less reduction in yield attributes like pods per plant, seeds per plant, 100-seed weight, straw yield, harvest index and grain yield under conditions of water stress over the susceptible genotypes.

Keywords: Chickpea, rainfed, irrigated, genotype

Introduction
Chickpea is hardy crop and acclimatized a wide range of soils and environmental conditions. So it is imperative to ascertain the optimal water requirements of chickpea crop for maximum and sustainable grain yield. The ever increasing population of the country coupled with inadequate progress in the pulse production is posing serious challenges on the food front. The progressive decline in per capita availability of pulses (69 g in 1961 to 30 g in 2002) in India is a matter of great concern. At present the developing countries account for 87% of the total pulses area and contribute 71% to the global production with an average yield of 645 kg/ha. Chickpea (*Cicer arietinum* L.) is the third most important food legume grown in over 45 countries in all continents of the world. The crop occupies 8.74 million hectares with production of 8.25 million tonnes and productivity is 943 kg/ha in India (Anonymous, 2011) [1]. Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra, Gujarat, Andhra Pradesh, and Karnataka are the major chickpea producing states sharing over 95% area. The year 2009-10 marked significant increase in area under chickpea (8.74 million ha) which is highest in last 10 years. Similarly, the chickpea production (7.35 million tonnes) also surpassed last 50 years record with productivity of 8.40 q ha^{-1}. The area under chickpea has increased from 6.45 million ha to 8.74 million ha in 2009-10. Water deficit is also known to alter a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation (Hu and Schmidhalter 1998) [8].

Materials and methods
The present investigation was carried out at main experiment station, Department of Crop Physiology, Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad (U.P.). under natural condition during Rabi season of 2012-13. A set of 6 cultivars of Chickpea was the test material of the present study. These cultivars were obtained from the germplasm collection center of Pulse section, Department of G.P.B., N.D.U.A. &T., Kumarganj, Faizabad. The experiment was laid out in Randomized Block Design with three replications, growing in rainfed and irrigated condition. Five tagged plants were randomly uprooted from each treated plot at growth stage on i.e. 55, 75, 95 DAS. Plant height, number of branches, leaf area, dry matter partitioning, and root length as well as volume and number of nodules were recorded.

Relative water content (RWC) per cent

The relative water content (RWC) was determined by the method described by Weatherly (1965). Select second leaf from lip for RWC measurement three leaves from each replication was sampled before on set drought Simlarly sample were colleted from drought eaposed field from each treatments seperatly.

$$\text{RWC (\%)} = \frac{\text{Fresh weight (g)-Dray weight (g)}}{\text{Saturated weight (g)-Dray weight (g)}}$$

Crop growth rate

Crop growth rate represented total dry matter productivity of the community per unit land area over a certain time spam. The crop growth rate have been calculated by using following equation given by Watson (1947) and reported as kg m^{-2} $week^{-1}$ or kg m^{-2} $month^{-1}$.

$$CGR = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{1}{L}$$

Where,

W_1 and W_2 are plant dry weight at times t_1 and L is land area

Relative growth rate

An index of the amount of growing material per unit dries weight of plant per unit times. Fisher (1921) [5] showed that if W_1 and W_2 are the total dry weight at times t_2 and t_1 respectively, the mean value of RGR for time interval t_2-t_1 can be calculated as bellow:

$$RGR = \frac{\text{Log e } W_2 - \text{Log e } W_1}{(t_2 - t_1)}$$

It is also called efficiency index (Y) and can be expressed as mg g^{-1} day^{-1}

Drought Susceptibility Index (DSI)

This parameter was worked out by the formula of Chakherehaman *et al.*, 2009 [4].

$$DSI = [1 - (Ysi/Ypi)/ SI]$$

Where,

Ysi	=	yield of cultivar in stress condition
Ypi	=	yield of cultivar in normal condition
SI	=	1- (Ys/Yp)
Ys	=	Total yield mean in stress condition
Yp	=	Total yield mean in normal condition

Samples for chlorophyll determination were taken from chickpea leaves using a 0.8 cm diameter cork borer, weighted quickly in pre-weighted clean glass vials and 5 cm3 of 80% acetone was added to these samples. The leaf material was bleached and decanted off. The optical density was read at λ = 663, 645 and 470 nm using 80% acetone as a blank by a spectrophotometer (Spectronic Genesys-5 Milton Roy). Content of chlorophyll *a*, chlorophyll *b* and carotenoids (µg g–1) was calculated according to using the method of Arnon (1949) [2] and expressed as mg per g fresh weight.

$$\text{Chlorophyll a} = 12.7 \times \text{O.D. (663)} - 2.69 \times \text{O.D. (645)} \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9 \times \text{O.D. (645)} - 4.68 \times \text{O.D. (663)} \times \frac{V}{1000 \times W}$$

$$\text{Total Chlorophyll} = 20.2 \times \text{O.D. (645)} + 8.02 \times \text{O.D. (663)} \times \frac{V}{1000 \times W}$$

Nitrate reductase activity was recorded according to the method of Jaworski (1971), and expressed as µ g nitrite produce g^{-1} fresh weight. Which is important role in physiological functions. The soluble and insoluble protein content was estimated by using method of Lowry *et al.* 1951. Harvest index (%) was calculated by using the relationship of Grains yield/ Total biomass* 100. Data were subjected to Analysis of variance to determine the significance of differences between treatments by using the software MSTATC.

Results and Discussion

Parameters viz., number of branches (primary and secondary) per plant, plant height, dry matter partitioning, number of nodules, CGR, RGR, relative water content, total chlorophyll, nitrate reductase enzyme were studied at 55, 75, 95 DAS and at maturity stages. Whereas, dry matter production, yield and yield components were studied at harvest. The results obtained are being summarized here as under (Table 1, 2 & 3).

The results of the experiment revealed that genotype KWR 108, RSG 888 and CSJD 884 were drought tolerant and K 850, PG 5 and JG 74 were drought susceptible genotypes. The drought tolerance capacity of tolerant genotypes was associated with retention of high RWC, less membrane damage, high total chlorophyll content and higher osmotic potential under stress conditions. The higher osmotic potential was associated with more accumulation of osmolite solutes like nitrate reductase contents in tolerant genotypes under stress condition. Regarding sink potential, the tolerant genotypes recorded less reduction in yield attributes like pods per plant, seeds per plant, 100-seed weight, harvest index and grain yield under conditions of water stress over the susceptible genotypes. High relative water content (RWC) at all stages coupled with less reduction in total chlorophyll as well as nitrate reductase activity.

Linearity of chickpea was greatly affected by both irrigated and rainfed conditions. In irrigated environment, JG 74, PG 5 and K 850 showed greater height than rest of the varieties while genotypes like JG 74, PG 5 and K 850 showed better height performance in rainfed. This shows that response of tolerance and susceptible genotypes of chickpea showed different response in both the environments up to more than 20% more height than susceptible one. Tolerant genotypes had less reduction (19-26%) than susceptible genotypes (37-41%). Genotype like K-850 maintained better plant height and number of branches in irrigated conditions but not responsive to rainfed environment. This shows that JG 74 had maintained better growth due to initial faster shoot and root growth in rainfed condition than other genotypes.

Number of branches was variably affected by irrigated and rainfed environments. In general, number of branches increased with the increase of age of crop. Under irrigated condition JG 74, PG 5 and K 850 showed maximum branches than other genotypes while, JG 74, K 850 and PG 5 possessed maximum branching in rainfed condition. This shows that response of tolerant and susceptible genotypes gave different response in both the environment. The increment of branch number in tolerant genotypes seems to increase more photosythates which maximize the yield potential under rainfed environment where assimilate is limiting. Similar findings were also reported in chickpea by Reddy and Ahlawat (1998), Yousefi *et al.* (1997) [22], Singh *et al.* (2005) [17], Sharma *et al.* (2006) [15], Saha *et al.* (2006) [13], Macar *et al.* (2009) [11] and Ulemale *et al.* (2013) [20]. The tolerant genotypes like JG 74, PG 5 and K 850 had maintained, maximum root length which seems directly comes in contact with lower strata of soil moisture in deeper soil profile and RGR and CGR which are indirect measurement of photosynthetic efficiency of plants also decreased (9-42% and 10-41%, respectively) under rainfed environment than irrigated environment. Higher CGR and RGR at earlier stage of crop growth also observed in chickpea by Sudhakar *et al.* (2006), Sharma, *et al*. (2007) [14], Kapoor *et al*. (2007), Pannu *et al*. (2011) [12], Ghanepour *et al.* (2014) [7], Shivakumar *et al.* (2014),. Under rainfed condition, variety K-850 recorded low value of RGR (42%) and CGR (41%) than other varieties, while, variety PG 5 maintained better and less decrease than control in CGR (10%) and RGR (9%) than other varieties. This might be attributed to inherent genotypic variations. The CGR and RGR were comparatively lower at early stages (55-75 DAS) than the later stages of crop growth

Relative water content (RWC) is one of the most important parameter to measure water status of the tissue (Barrs and Weatherley, 1962). In general, RWC increased up to 95 DAS, after that reduction was noticed. The effects of moisture regimes on RWC per cent was recorded significantly higher in irrigated condition (55-74%) over rainfed (30-45%) at all the crop growth stage, except 55 DAS. Under rainfed environment, the maximum reduction in RWC was recorded with genotype K-850 (59.35%) and minimum in PG 5 (14%). The reduction in RWC was more in susceptible genotypes (51-60%) as compared to tolerant genotypes (14-25%) in chickpea.

The maintenance of chlorophyll 'a, and 'b' and total chlorophyll contents are crucial parameters to maintain photosynthetic rate under rainfed environment. The nitrate reductase activity increased upto 95 DAS and decreased thereafter under both irrigated and rainfed conditions. Genotypes, CSJD 884 had the less reduction in NR activity under rainfed condition. Maximum nitrate reductase activity (57.46%) was recorded in PG 5. While, under irrigated condition K 850 maintained higher NR activity.

The protein content in seed was higher under irrigated condition in compression to rainfed, but there was no significant reduction was noticed. The maximum reduction was noticed in genotypes K 850 and minimum was genotypes CSJD 884. It is concluded that among chickpea genotypes JG 74 gave higher yield under rainfed condition followed by PG 5 and RSG 888. Under irrigated condition, genotype K 850 performed best followed by CSJD 884 and KWR 108. The present investigation clearly indicate that genotype KWR 108, RSG 888 and CSJD 884 are relatively drought tolerant compared to other varieties as they showed relatively less reduction in grain yield due to moisture stress.

Table 1: Effect of moisture ecosystems on Crop Growth Rate (g m^{-2} day^{-1}) and Relative Growth Rate (mg g^{-1} day^{-1}) of chickpea genotypes.

Variety	Crop Growth Rate						Relative Growth Rate					
	55-75 DAS			75-95 DAS			55-75 DAS			75-95 DAs		
	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean
KWR 108	0.180	0.198	0.189	0.31	0.34	0.33	0.068	0.075	0.072	0.045	0.050	0.048
PG 5	0.190	0.238	0.214	0.33	0.41	0.37	0.072	0.090	0.081	0.048	0.060	0.054
RSG 888	0.183	0.215	0.199	0.32	0.37	0.35	0.069	0.081	0.075	0.046	0.054	0.050
K 850	0.181	0.222	0.201	0.31	0.39	0.35	0.068	0.084	0.076	0.046	0.056	0.051
CSJD 884	0.151	0.166	0.158	0.26	0.29	0.27	0.057	0.063	0.060	0.038	0.042	0.040
JG 74	0.157	0.201	0.179	0.27	0.35	0.31	0.060	0.076	0.068	0.040	0.051	0.045
Mean	0174	0.206	0.190	0.30	0.36	0.33	0.066	0.078	0.072	0.044	0.052	0.048
SEm±	0.003	0.005	0.008	0.005	0.009	0.013	0.001	0.002	0.003	0.001	0.002	0.002
CD at 5%	V-0.009	I-0.016	V×I-NS	V-0.015	I-0.026	V×I-NS	V-0.004	I-0.006	V×I-NS	V-0.003	I-0.005	V×I-NS

Table 2: Effect of moisture ecosystems on Relative water content (%) and Total chlorophyll content (mg g^{-1} fr. wt.) of chickpea genotypes

Variety	Relative water content									Total chlorophyll content								
	55 DAS			75 DAS			95 DAS			55 DAS			75 DAS			95 DAS		
	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean
KWR 108	76.5	74.2	75.35	47.4	50.3	48.86	42.3	45	43.69	1.86	1.98	1.92	2.16	2.29	2.22	1.97	2.09	2.03
PG 5	78.8	76.25	75.52	88.9	91.2	90.05	79.3	81.2	80.24	3.49	3.58	3.54	4.04	4.15	4.09	3.68	3.78	3.73
RSG 888	73.35	71.35	72.35	55.5	60.1	57.78	49.4	53.6	51.49	2.18	2.36	2.27	2.52	2.73	2.63	2.29	2.48	2.39
K 850	75.7	73.4	74.55	77.8	81.2	79.48	69.4	72.5	70.94	3.05	3.19	3.12	3.53	3.69	3.61	3.22	3.37	3.30
CSJD 884	74	71.8	72.90	61.8	67	64.37	55.2	59.8	57.45	2.43	2.63	2.53	2.81	3.04	2.93	2.56	2.78	2.67
JG 74	72.2	70	71.10	94.2	95.8	94.98	84	85.5	84.77	3.68	3.76	3.92	4.28	4.35	4.32	3.90	3.97	3.94
Mean	75.09	72.83	73.06	70.92	74.26	72.59	80.45	84.25	64.76	2.78	2.92	2.85	3.22	3.38	3.30	2.94	3.08	3.01
SEm±	0.003	1.149	1.625	0.924	1.601	2.264	0.884	1.532	2.710	0.044	0.076	1.108	0.050	0086	0.122	0.041	0.070	0.100
CD at 5%	V-1.945	I-3.369	V×I-NS	V-2.711	I-4.695	V5INS	V-2.594	I-4.493	V×I-NS	V-0.129	I-0.224	V×I-NS	V-0.146	I-0.252	V×I-NS	V-0.119	I-0.207	V×I-NS

Table 3: Effect of moisture ecosystems on Nitrate Reductase Activity (µ mole $hr^{-1}g^{-1}$ fr. wt.), Protein content, seed yield and harvest index in seed of chickpea.

Variety	Nitrate Reductase Activity (µ mole $hr^{-1}g^{-1}$ fr. wt.)									Protein content in seed			seed yield and harvest index						DSI
	55 DAS			75 DAS			95DAS						Seed yield (gm) $plant^{-1}$			Harvest Index (%)			
	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean	RF	IR	Mean	
KWR 108	33.55	37.00	35.27	47.91	52.84	50.37	31.94	35.23	33.58	21.20	21.30	21.25	21.50	24.30	22.90	29.23	29.80	29.52	7.69
PG 5	35.52	44.45	39.99	50.73	63.49	57.11	33.82	42.43	38.07	21.40	21.50	21.45	23.40	29.75	26.58	29.17	29.79	29.48	6.83
RSG 888	34.15	40.11	37.13	48.77	57.28	53.03	32.51	38.19	35.35	21.50	21.70	21.16	22.15	26.55	24.35	28.52	29.57	29.05	7.25
K 850	33.77	41.42	37.60	48.23	59.15	53.69	32.16	39.43	35.80	21.40	21.60	21.50	21.80	27.56	24.68	27.61	29.25	28.43	7.71
CSJD 884	28.18	30.93	29.56	40.24	44.18	42.21	26.83	29.45	88.14	21.60	21.80	21.70	18.00	19.75	18.88	28.33	28.72	28.53	7.92
JG 74	29.38	37.53	37.46	41.97	53.60	47.49	27.58	35.74	31.86	21.45	21.70	21.58	18.90	24.77	21.84	28.73	29.60	29.17	6.63
Mean	29.43	38.57	35.05	46.31	55.09	50.70	30.87	36.73	33.80	21.43	21.60	21.51	20.96	25.45	23.20	28.60	29.46	29.03	-
SEm±	0.611	1.058	1.497	0.834	1.445	2.044	0.551	0.954	1.350	0.338	0.585	0.828	0.384	0665	0.941	0.505	0.874	1.236	-
CD at 5%	V-1.792	I-3.104	V×I-NS	V-2.447	I-4.238	V×I-NS	V-1.616	I-2.799	V×I-NS	V-0.991	I-1.717	V×I-NS	V-1.127	I-1.952	V×I-NS	V-1.480	I-2.563	V×I-NS	-

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