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Effect of cold stress on morphological traits of chickpea (*Cicer arietinum* L.) genotypes at flowering stage

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Abstract
Cold stress in chickpea mostly affects the Northern parts of India as temperatures drop below 15 °C at flowering stage which can limit chickpea growth and vigor. In the present investigation the effects of cold stress on morpho-physiological traits in chickpea genotypes were studied at reproductive stage. Pot experiment was conducted simultaneously in the control and treated conditions respectively at Division of Plant Physiology, Chatha, and field trial were conducted in October, 2020-2022 at ACRA Dhiansar SKUAST, Jammu. The factors to be studied 32 chickpea genotypes and cold stress at flowering stage. Analysis for plant height, no of branches per plant, no. leaves per plant, dry weight of stem, leaves, and SPAD value of control and cold-stressed plants were recorded. The results showed that cold stress induced at flowering stage declined the Plant height, number branches, number of leaves per plant and dry weight in stress condition ICCV 88506 genotype followed by Pusa 362 and ICCV 96030 and other chickpea genotypes were at par whereas RSG 896 and PBG 5 were performed minimum during cold stress condition in pot as well as in field. This study imply that cold stress greatly influences morphological and physiological functions that affect plant growth and yield production in chickpea genotypes.

Keywords: cold stress, plant height, chickpea, dry weight, stem

Introduction
Chickpea (*Cicer arietinum* L.) generally also known as gram, Chana, Bengal gram and Garbanzo beans is the second most important food legume after soybean, widely cultivated in tropical, sub-tropical and temperate regions of the world, as a source of protein (Akibode, 2011) [14]. India is the largest chickpea-producing country, with a 75 percentage share of global production (Gaur *et al.*, 2019) [1]. In India, total area under chickpea is around 105.73 lakh hectares with the production of 111.58 lakh tonnes and productivity of 1056 kg/ha during 2017-18. Particularly in J&K, total area under pulses cultivation is around 26.78 thousand hectares, whereas, production around 144 thousand quintals. Cold stress, which includes both chilling (less than 10° Celcius) and freezing injury (less than 0° Celcius), is one of the most significant abiotic stresses of agricultural plants, causing micro-organelle disruption, phase transition in cell membrane lipids, reactive oxygen species (ROS) production, as well as the inhibition of crop growth and development, which consequently reduces crop yield and production. Chickpea lacks cold tolerance and is sensitive to chilling temperatures (>8 °C), especially at its reproductive phase (Srinivasan *et al.*, 1998; Bakht *et al.*, 2006 Croser *et al.*, 2003; Kaur *et al.*, 2008; Thakur *et al.*, 2010; Kumar *et al.*, 2013) [2, 3, 10, 11, 12, 13]. The reproductive structures can withstand temperature of 8 °C minimum to 22 °C maximum during the coldest period. The most advantageous temperature ranges for normal flowering, fertilization and seed set is 10 to 14 °C average minimum temperature and 25 to 31 °C average maximum temperature. Temperature within the chilling range can limit the growth and vigor of chickpea at all phenological stages but is considered most damaging to yield at reproductive stage. During the reproductive phase, low temperature is detrimental to normal flowering and pod development, which causes prolonged reproductive phase, floral abortion, poor pollen germination, impaired ovule development, failure in pod set and reduction in seed filling that drastically affects the crop productivity. It would therefore, be important to identify the morpho-physiological traits for low temperature tolerance at the reproductive stage because the reproductive phase in chickpea is especially more sensitive to cold stress. The proposed research is to to evaluate the relative response of the chickpea genotypes to cold stress during the reproductive stages and reasons related to differential cold sensitivity of these genotypes.

Materials and Methods

32 chickpea genotypes were studied with cold stress at flowering stage. Pot experiment was conducted simultaneously in the control and treated conditions respectively at Division of Plant Physiology, Chatha, SKUAST, Jammu along with Field experiment. Pot experiment was exposed with low temperature below the threshold temperature (less than 8 ºC) at reproductive stage. Sampling done above threshold temperature (8 ºC) was taken as control in pot experiment and destructive sampling was done after the plants experiencing temperature lower than threshold point i.e., less than 8 ºC during flowering stage. Parameters such as plant height, number of primary branches per plant, number of leaves per plant, dry weight of leaves and stem and physiological parameter *i.e.*, SPAD value were taken after exposure to cold stress. SPAD (Soil Plant Analysis Development) measurements were made with a SPAD-502 instrument. SPAD readings taken around the midpoint of each leaf, upper, middle and lower leaf on one side of the midrib and values were averaged.

Results and Discussion

Genotypic variation in plant height (cm) among genotypes was recorded in the range of 49 cm to 79 in field plants. In treated plants it varied from 18.2 cm to 30.7 cm (Fig. 1) and in control condition it varied from 25.1 to 38.0 cm. Besides in cold stress condition the plant height was declined significantly, minimum plant height was observed in PBG 5 (18.2) and RSG 896 (20.3) and maximum was observed in ICCV 88506 (30.7) followed by Pusa 362 (30.4) and ICCV 96030 (28.8). The genotypes showed significant differences for plant height in field, control and cold stress condition.

Similarly, the genotypes showed significant differences for number of branches per plant, number of leaves per plant, dry weight of stem and leaves per plant in control, field and cold stress experiments. The cold stress significantly reduced all the morphological traits under study. The mean number of branches was less from field followed by control to cold stress condition *i.e.*, 4.42, 3.55 to 3.31 respectively (Table 1). Maximum number of branches were observed in ICCV 88506 (5.11) followed by Pusa 362 (4.89) and ICCV 96030 (4.72), other genotypes were at par and minimum in PBG 5 (2.96) genotypes. Whereas, average maximum numbers of leaves (Table 2) were observed in ICCV 88506 (394.8) followed by Pusa 362 (392.7), ICCV 96030(390.9) and minimum in PBG 5 (243.0) genotypes.

Similarly Figure 2 and 3 depicts dry weight of stem and leaves respectively. Maximum dry weight of leaves was observed in the chickpea genotype ICCV 88506 (3.83 g) followed by Pusa 362 (3.12 g) and ICCV 96030 (2.65 g) and minimum in PBG 5 (0.72 g) at field condition. There was decline in dry weight of leaves at cold stress condition *i.e.*, minimum dry weight of leaves was observed in PBG 5(0.55 g) followed by RSG 896 (0.69 g) and maximum was found in ICCV 88506 (2.46 g) followed by Pusa 362 (2.05 g) and ICCV 96030 (1.95 g). Although in control similar trend was found. Likewise, maximum average dry weight of stem was observed in the chickpea genotype ICCV 88506 (1.54 g), followed by Pusa 362 (1.45 g) and minimum in PBG 5 (0.70 g) (Fig. 2). Plant indices and their assessment are frequently used to track how the comparison of several genotypes has progressed with crop age. Our findings demonstrated that, under various conditions, there were considerable differences in the plant's height (Fig. 1), the number of branches, and the dry weight of the leaves and stem (Table 1; Fig 2, 3). In our experiment, the chickpea genotypes ICCV 88506 and Pusa 362 in field conditions showed the highest dry weight of leaves and stem. Our results in agreement of Kumar *et al.* (2005) [4] a decrease in plant height and a delay in reproductive growth was observed during cold stress and the weight of the cold-stressed plants, both fresh and dry weight decreased significantly. This may be due to low temperatures, which inhibit plant growth, the plant's dry weight decreased, which is consistent with past studies on chickpea in this area (Srinivasan *et al.*, 1998, Nayyar *et al.*, 2007) [2, 5]. The quantitative quality of yield is the end product of numerous physiological and biochemical processes. The relationship between yield and features that contribute to yield and environmental impacts may be dynamic. Numerous studies have found similar findings in the germplasm of the chickpea, cowpea, and lentil (Qureshi *et al.*, 2004; Hegde and Mishra; 2009; Malik *et al.*, 2010; Gul *et al.*, 2013) [6, 7, 8, 9].

Table 1: Number of primary branches per plant in chickpea genotypes affected by cold stress at flowering stage

Number of primary branches per plant					
S. NO.	Genotypes	POT		Field	Mean
		Control	Cold stress		
1	Pusa 212	3.67 ± 0.3	3.50 ± 0.5	4.67 ± 0.3	3.94
2	JG 74	4.00 ± 0.0	3.80 ± 0.2	5.33 ± 0.3	4.38
3	Pusa 244	3.67 ± 0.3	3.33 ± 0.3	4.67 ± 0.3	3.89
4	Pusa 362	4.67 ± 0.3	4.33 ± 0.3	5.67 ± 0.3	4.89
5	BG 396	4.00 ± 0.6	3.67 ± 0.3	5.00 ± 0.6	4.22
6	PG 96006	3.33 ± 0.3	3.00 ± 0.0	4.00 ± 0.6	3.44
7	GG 2	3.33 ± 0.3	3.33 ± 0.3	4.33 ± 0.3	3.67
8	BDG 75	3.67 ± 0.3	3.50 ± 0.5	4.67 ± 0.3	3.94
9	ICCV 92944	3.33 ± 0.3	3.00 ± 0.0	4.00 ± 0.0	3.44
10	JG 12	3.67 ± 0.3	3.33 ± 0.3	4.67 ± 0.3	3.89
11	JG 315	3.67 ± 0.3	3.33 ± 0.3	4.67 ± 0.3	3.89
12	L 550	3.67 ± 0.3	3.33 ± 0.3	4.67 ± 0.3	3.89
13	BG 276	3.33 ± 0.3	3.18 ± 0.4	4.33 ± 0.3	3.62
14	Pusa 240	3.33 ± 0.3	3.17 ± 0.2	4.33 ± 0.3	3.61
15	RSG 896	3.00 ± 0.0	2.83 ± 0.2	3.33 ± 0.3	3.06
16	Saki 9516	3.67 ± 0.3	3.33 ± 0.3	4.33 ± 0.3	3.78
17	PG 186	3.33 ± 0.3	3.08 ± 0.1	4.33 ± 0.3	3.58
18	Pusa Green 112	3.00 ± 0.0	2.90 ± 0.1	3.67 ± 0.3	3.19

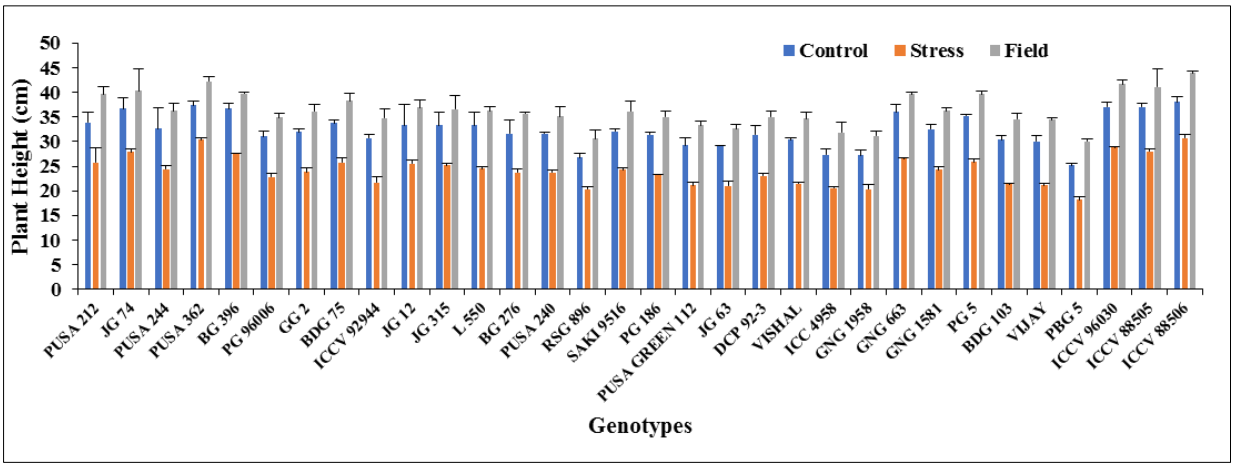
19	JG 63	3.00 ± 0.0	2.90 ± 0.1	3.67 ± 0.3	3.19
20	DCP 92-3	3.33 ± 0.3	3.00 ± 0.6	4.00 ± 0.0	3.44
21	Vishal	3.33 ± 0.3	3.00 ± 0.0	4.00 ± 0.6	3.44
22	ICC 4958	3.00 ± 0.0	2.85 ± 0.1	3.33 ± 0.3	3.06
23	GNG 1958	3.00 ± 0.0	2.83 ± 0.2	3.33 ± 0.3	3.06
24	GNG 663	4.00 ± 0.0	3.67 ± 0.3	5.00 ± 0.0	4.22
25	GNG 1581	3.67 ± 0.3	3.33 ± 0.3	4.33 ± 0.3	3.78
26	PG 5	4.00 ± 0.0	3.67 ± 0.3	5.00 ± 0.0	4.22
27	BDG 103	3.00 ± 0.0	3.00 ± 0.0	3.67 ± 0.3	3.22
28	Vijay	3.00 ± 0.0	2.92 ± 0.1	3.67 ± 0.3	3.19
29	PBG 5	2.92 ± 0.1	2.62 ± 0.3	3.33 ± 0.3	2.96
30	ICCV 96030	4.33 ± 0.3	4.15 ± 0.5	5.67 ± 0.3	4.72
31	ICCV 88505	4.00 ± 0.0	3.83 ± 0.2	5.33 ± 0.3	4.39
32	ICCV 88506	4.67 ± 0.3	4.33 ± 0.3	6.33 ± 0.3	5.11
Mean		3.55	3.31	4.42	
CD at 5%		Treatment = 0.15 Genotypes = 0.49 Treatment X Genotypes = 0.85			

Values are mean ± SE of three replicates

Table 2: Number of leaves plant^{-1} (g) in chickpea genotypes affected by cold stress at flowering stage

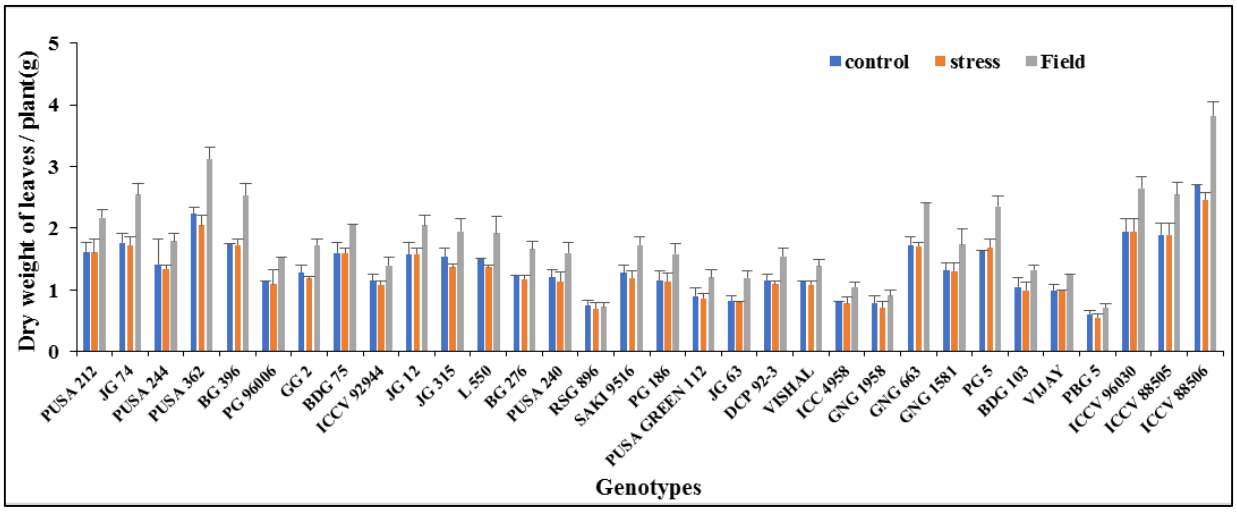
Number of leaves per plant					
S. NO.	**Genotypes**	**POT**		**Field**	**Mean**
		Control	**Cold Stress**		
1	Pusa 212	342.2 ± 31.1	240.7 ± 7.0	481.7 ± 28.3	354.9
2	JG 74	354.7 ± 18.7	252.4 ± 30.1	538.0 ± 22.3	381.7
3	Pusa 244	308.0 ± 22.0	220.0 ± 29.1	433.3 ± 43.3	320.4
4	Pusa 362	357.8 ± 35.8	254.0 ± 0.0	566.2 ± 40.4	392.7
5	BG 396	351.0 ± 19.9	242.5 ± 12.5	536.7 ± 0.0	376.7
6	PG 96006	283.3 ± 28.3	182.0 ± 0.0	385.3 ± 55.6	283.6
7	GG 2	298.7 ± 12.3	204.3 ± 3.5	420.0 ± 42.0	307.7
8	BDG 75	340.0 ± 10.0	239.9 ± 14.1	460.0 ± 10.0	346.6
9	ICCV 92944	277.3 ± 10.0	176.9 ± 5.1	384.2 ± 20.2	279.5
10	JG 12	336.0 ± 15.0	235.7 ± 10.7	455.0 ± 35.0	342.2
11	JG 315	315.0 ± 19.0	233.3 ± 30.9	455.0 ± 37.5	334.4
12	L 550	310.4 ± 28.2	233.3 ± 16.7	433.3 ± 43.3	325.7
13	BG 276	293.3 ± 18.3	201.7 ± 18.3	420.0 ± 42.0	305.0
14	Pusa 240	290.9 ± 26.4	199.1 ± 28.4	398.2 ± 28.4	296.1
15	RSG 896	256.9 ± 32.1	134.4 ± 12.2	355.6 ± 35.6	249.0
16	Saki 9516	304.0 ± 8.1	220.0 ± 22.0	426.7 ± 61.6	316.9
17	PG 186	288.9 ± 30.6	192.5 ± 15.9	391.1 ± 35.6	290.8
18	Pusa Green 112	264.4 ± 26.4	161.3 ± 14.7	384.2 ± 29.6	270.0
19	JG 63	264.4 ± 26.4	158.9 ± 12.2	378.9 ± 13.1	267.4
20	DCP 92-3	284.4 ± 28.4	183.3 ± 18.3	390.0 ± 30.0	285.9
21	Vishal	276.0 ± 20.0	176.0 ± 25.4	384.2 ± 29.6	278.7
22	ICC 4958	263.1 ± 7.1	158.3 ± 22.0	375.7 ± 31.0	265.7
23	GNG 1958	260.7 ± 15.3	154.0 ± 22.0	369.8 ± 28.4	261.5
24	GNG 663	348.4 ± 43.6	241.8 ± 51.3	522.7 ± 37.3	371.0
25	GNG 1581	304.5 ± 10.5	220.0 ± 20.0	433.3 ± 43.3	319.3
26	PG 5	342.2 ± 31.1	240.8 ± 14.2	498.3 ± 21.7	360.5
27	BDG 103	266.7 ± 26.7	174.2 ± 24.3	384.2 ± 20.2	275.0
28	Vijay	266.7 ± 26.7	166.7 ± 16.7	384.2 ± 29.6	272.5
29	PBG 5	256.7 ± 23.3	122.2 ± 12.2	350.0 ± 40.4	243.0
30	ICCV 96030	356.2 ± 48.3	253.3 ± 35.3	563.3 ± 43.3	390.9
31	ICCV 88505	355.6 ± 35.6	252.7 ± 2.7	547.5 ± 26.3	385.2
32	ICCV 88506	364.0 ± 0.0	254.2 ± 23.1	566.2 ± 43.6	394.8
Mean		305.7	205.6	439.8	
CD at 5%		Treatment = 13.4 Genotypes = 44.0 Treatment X Genotypes = 76.3			

Values are mean ± SE of three replicates



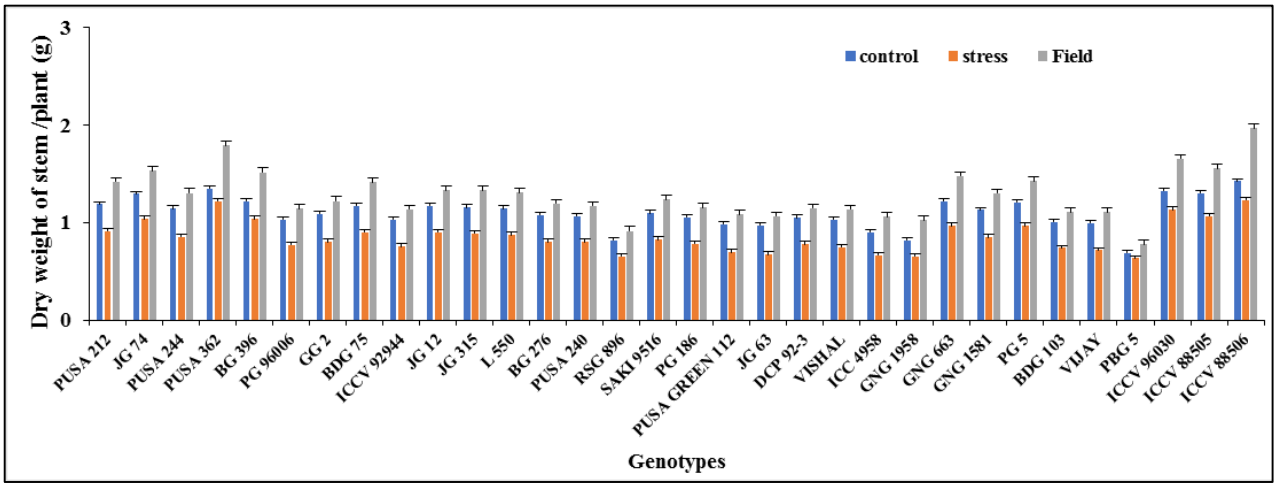
CD at 5% Treatment =0.93
Genotypes =3.04
Treatment X Genotypes =5.27

Fig 1: Changes in plant height (cm) in chickpea genotypes at maturity as affected by cold stress. Vertical bars indicate ± SE mean



CD at 5% Treatment =0.06
Genotypes =0.20
Treatment X Genotypes =0.35

Fig 2: Changes in dry weight of leaves/plant in chickpea genotypes as affected by cold stress. Vertical bars indicate ± SE mean



CD at 5% Treatment =0.06
Genotypes =0.08
Treatment X Genotypes =0.49

Fig 3: Changes in dry weight of stem/plant in chickpea genotypes as affected by cold stress. Vertical bars indicate ± SE mean

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