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J. Environ. Biol.
32, 189-194 (2011)
ISSN: 0254-8704
CODEN: JEBIDP

Comparative studies on morphological and biochemical characters of chickpea genotypes under chilling stress

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Publication Data

Paper received:
22 October 2009

Revised received:
16 March 2010

Accepted:
15 April 2010

Abstract

Comparison of chickpea (*Cicer arietinum* L.) genotypes for morphological and biochemical attributes was done. Morphological characters *viz*. Plant height, number of branches and number of leaves were recorded highest in chilling tolerant genotypes at early stages of development (30 and 60 DAS) whereas at later stages (90 and 120 DAS) these characters were recorded highest in chilling sensitive genotypes. Pollen viability percentage at 5 and 10°C temperatures were recorded highest in chilling tolerant genotypes as compared to sensitive genotypes. Biochemical characters *viz.* electrolyte leakage (%), total soluble sugars and total free amino acids were recorded highest in chilling tolerant genotypes as compared to sensitive genotypes.

Key words

Chickpea, Genotypes, Chilling stress, Abiotic stresses, Low temperature

Introduction

Chickpea (*Cicer arietinum* L.) ranks 2nd among the world's food legumes in terms of area and is grown principally as a cool-season crop. The production of the cool season grain legume chickpea is constrained by low temperatures across much of its geographical range. Chilling range temperatures for chickpea is between -1.5 and 15°C. Temperatures upto 15°C have been demonstrated to cause flower and pod abortion in parts of the Indian subcontinent and Australia (Srinivasan *et al*., 1998; Clarke, 2001). Low temperature (<15°C) affects both the development and function of reproductive structures in the chickpea flower. The function of pollen derived from chilling sensitive plants is affected by low temperature stress particularly during the growth of the pollen tubes down the style before fertilization occurs. In contrast, pollen tubes derived from chilling tolerant plants continue to grow down the style under low temperature stress (Clark and Siddique, 2004). Wery *et al.* (1994) observed the altered membrane functions during chilling stress by lipid peroxidation and leakage of electrolytes from chickpea cells. Survival at low temperature depends to a large degree on the ability of plants to maintain the integrity of membranes as they constitute the main damage site. Adaptation to all environmental stresses is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, betaines and proline (Greenway and Munns 1980; Yancey *et al.,* 1982). Cellular and metabolic changes that occur during cold acclimatization in general include increased level of sugars, soluble proteins and organic acids as well as appearance of new isoforms of proteins with changed lipid composition (Hughes and Dunn, 1996). Keeping this in view, the present study was undertaken to compare different genotypes of chickpea for their morphological and biochemical parameters under chilling stress *i.e.* below 15°C.

Materials and Methods

The experiment was conducted in the experimental area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Ten chilling tolerant (CT) genotypes *viz*., ICC-424275, IC-327572, IC-327404, IC-327412, EC-548083, WACPE-2072, WACPE-2075, ICCV-88503 & ICCV-93929 and two chilling sensitive (CS) genotypes *viz.*, PBG-1 and GPF-2 were selected for the experiment. Seeds of chickpea genotypes were sown on 31st October, 2007 (appropriate time for sowing in Punjab). The crop was raised in randomized block design with three replications for each treatment in plot size of 4 m x 1.2 m = 4.8 sq.mt. Sowing was done in rows 30 cm apart and plant to plant spacing was maintained at 15 cm. Morphological observations *viz*., plant height (cm), number of branches per plant and number of leaves

per plant were recorded at 30 (vegetative stage), 60 (flowering stage of chilling tolerant genotypes), 90 (flowering and seed filling stages of chilling sensitive and chilling tolerant genotypes respectively) and 120 days after sowing (DAS) (maturity stage of all the genotypes) stages from randomly tagged five plants from each plot. Pollen viability percentages of pollens from all the 12 genotypes were recorded when the temperatures remained below 5 and 10°C (during January). The pollen viability percentage was calculated using the following formula:

$$\text{Pollen viability (\%)} = \frac{\text{Total viable pollen in three microscopic fields}}{\text{Total pollen in three microscopic fields}} \times 100$$

Biochemical estimations *viz.*, electrolyte leakage (%) from the 4th leaf from apex, total soluble sugars (Dubois *et al.,* 1956) and total free amino acids (Lee and Takahashi, 1966) from seeds of the genotypes were recorded at 15 (seed formation stage) and 40 (seed maturity) days after anthesis (DAA) stages. The data on different crop parameters was statistically analysed, using Randomized block design (RBD) design (Gomes and Gomes, 1984).

Results and Discussion

Three key elements in characterization of an abiotic stress are intensity and duration of stress, rate of stress development and phenological timing of the stress. Plant height is an important index of plant growth and its measurement used to monitor the comparison between different genotypes. Plant height was significantly higher in chilling tolerant genotypes at 30 and 60 DAS when temperature was low (Table 1). At 30 DAS stage, the highest plant height was recorded in chilling tolerant genotype ICC-424275 (16.2 cm) closely followed by IC-548083 (15.8 cm) and WACPE-2072 (15.8 cm) whereas in chilling sensitive genotypes (PBG-I and GPF-2) the height recorded (10.0 and 10.4 cm, respectively) was approximately 30 to 60% less than that of the chilling tolerant genotypes. At 90 and 120 DAS chilling sensitive genotypes showed lead in plant height in comparison to chilling tolerant genotypes and it is attributed to increase in the ambient temperature.

Branching pattern is an important parameter which leads to increase in final yield. Number of branches per plant in general was higher in chilling sensitive genotypes as compared to chilling tolerant genotypes at 90 and 120 DAS stages when temperature was high (Table 1) though initially *i.e.* at 30 and 60 DAS stages, number of branches per plant was higher in chilling tolerant genotypes as compared to chilling sensitive genotypes. The highest number of branches per plant was counted in chilling tolerant genotype EC-548083 (14.7) and lowest in chilling sensitive genotype GPF-2 (7.9) at 30 DAS. At 60 DAS, the highest number of branches was observed in chilling tolerant WACPE-2078 (20.3) and the lowest in GPF-2 (12.1) whereas at 90 DAS stage there was drastic increase in number of branches in chilling sensitive genotypes as compared to chilling tolerant genotypes. The highest number of branches per plant (36.7) was recorded in chilling sensitive genotype PBG-1 which was significantly higher than other chilling tolerant genotypes at 90 DAS. Similar trend was observed at 120 DAS. Chilling temperature limits vegetative growth at initial stage in chilling sensitive genotypes *i.e.* PBG-1 and GPF-2 as is evident in the present study. However, with the increase in temperature chilling sensitive genotypes also gained in height. Crosser *et al.* (2003) also observed that chilling range temperature had pronounced negative effect on plant growth (plant height, number of branches and number of leaves) and dry matter production at the vegetative stage. Sudden increase in plant height in chilling sensitive genotypes at later stages could be ascribed to avoiding the chilling temperature at vegetative stage. Wery *et al.* (1994) suggested that rooting depth reduced branching and osmotic adjustments as a mechanism of adaptation to chilling stress. It has a positive correlation with yield. Berger *et al.* (2006) also suggested that extending the vegetative phase under

Table - 1: Plant height (cm), number of branches and number of leaves at various stages of development (30, 60, 90 and 120 DAS) in chilling tolerant and sensitive chickpea genotypes

	Plant height				Number of branches				Number of leaves			
Chickpea genotypes	30 DAS	60 DAS	90 DAS	120 DAS	30 DAS	60 DAS	90 DAS	120 DAS	30 DAS	60 DAS	90 DAS	120 DAS
A. Chilling tolerant												
ICC-424275	16.2	24.9	39.2	50.4	12.1	15.7	26.4	27.7	31.0	39.0	60.0	65.0
IC-327412	13.8	26.7	40.6	47.6	12.0	16.1	28.0	29.6	43.0	50.1	67.0	74.7
IC-327404	13.6	29.8	49.4	51.1	9.0	17.8	31.0	33.0	36.6	42.0	69.8	75.2
IC-327572	15.2	30.4	40.6	51.3	10.3	19.4	28.2	30.4	36.7	42.1	70.6	77.3
EC-548083	15.8	32.6	45.9	50.9	14.7	20.1	32.9	35.6	45.0	58.4	78.5	82.0
WACPE-2072	15.8	34.2	49.5	51.8	9.9	17.9	30.0	33.0	39.2	45.4	70.1	76.2
WACPE-2075	14.8	24.1	48.3	48.6	13.6	19.0	28.0	30.6	40.6	50.1	76.2	80.0
WACPE-2078	13.9	24.1	47.9	48.2	9.5	20.3	31.0	32.1	36.7	43.4	72.1	75.4
ICCV-88503	14.2	26.7	48.6	50.3	12.6	19.4	24.0	26.4	41.6	50.1	76.9	80.6
ICCV-93929	13.9	28.4	42.3	49.1	9.6	20.1	22.0	23.1	37.3	47.4	74.6	75.9
B. Chilling sensitive												
PBG-1	10.0	17.8	52.4	62.1	8.3	13.4	36.7	46.0	24.2	30.4	88.4	99.0
GPF-2	10.4	19.9	50.1	58.2	7.9	12.1	32.0	36.4	21.1	32.4	87.5	90.1

DAS= Days after Sowing

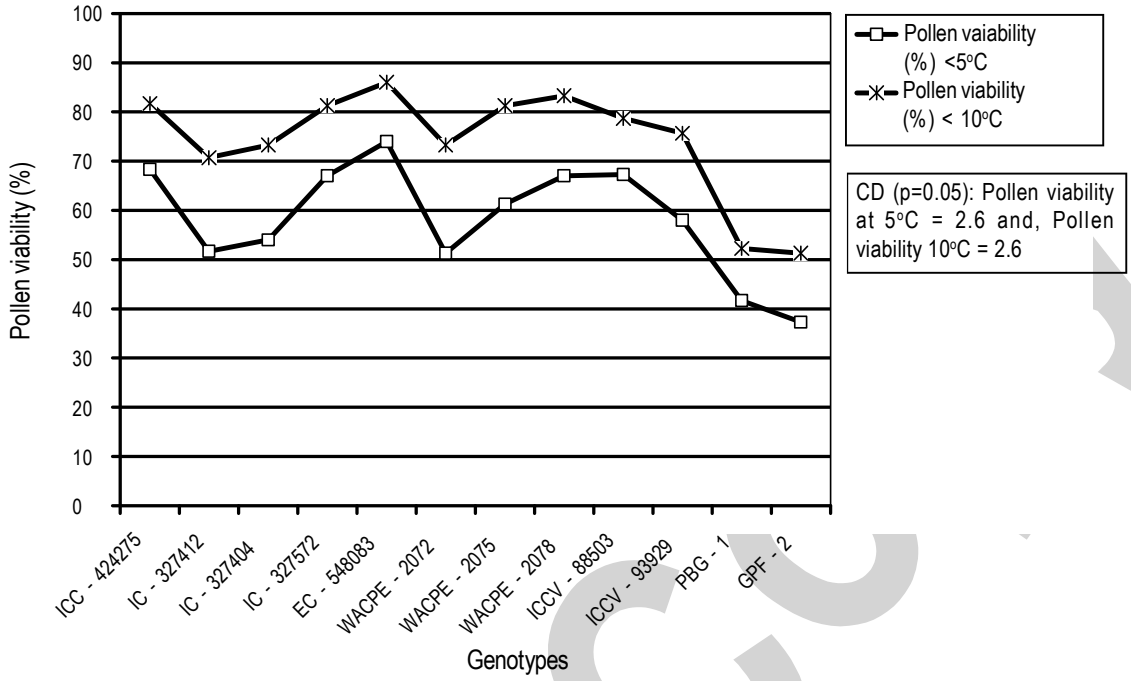

Fig. 1: Pollen viability (%) in chickpea genotypes

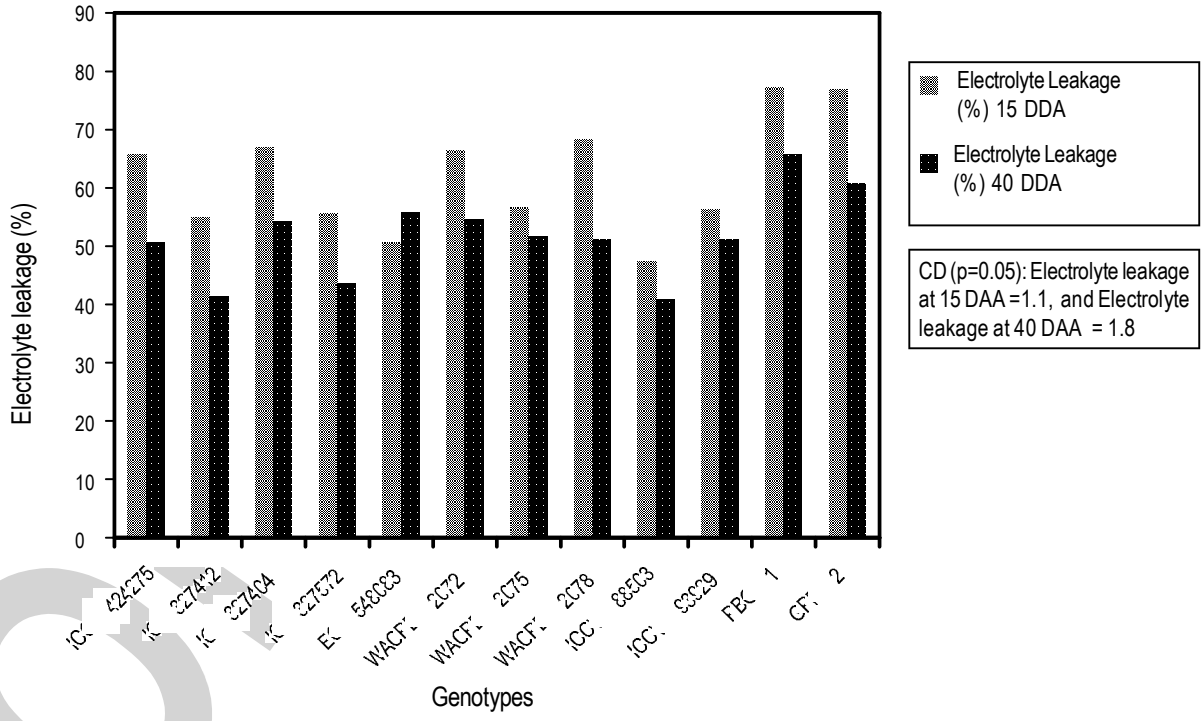

Fig. 2: Electrolyte leakage (%) from leaves of chickpea genotypes

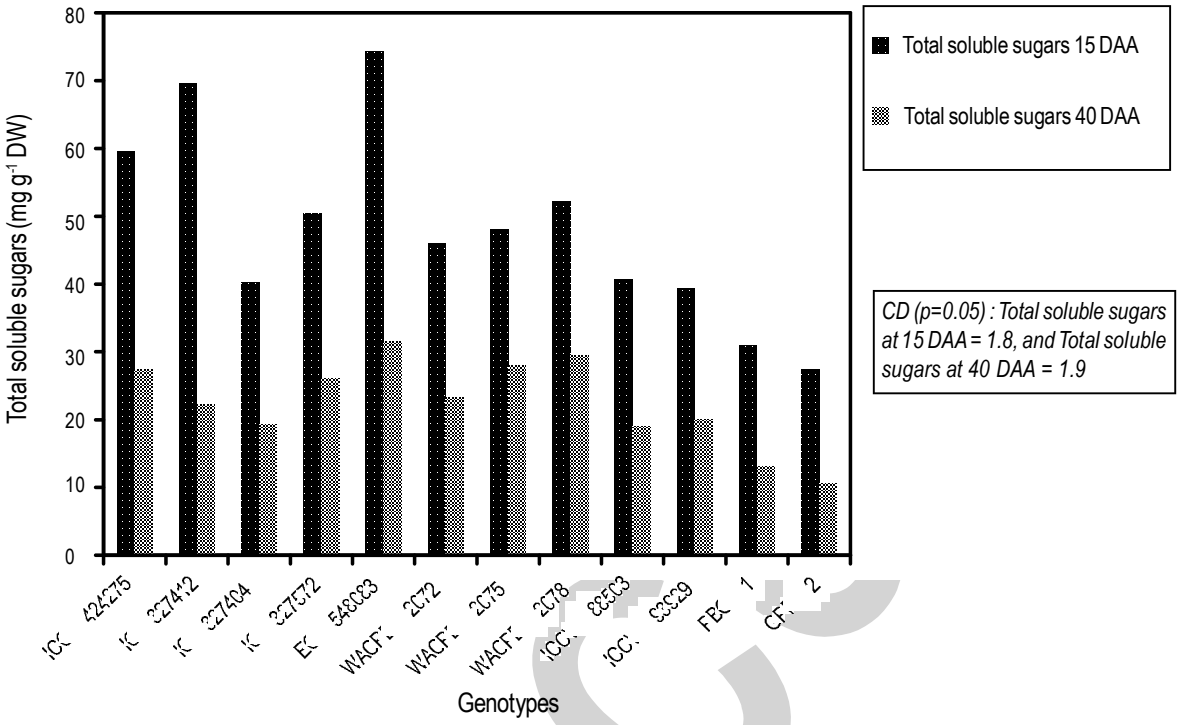

Fig. 3: Total soluble sugars (mg g^{-1} DW) from seeds of chickpea genotypes

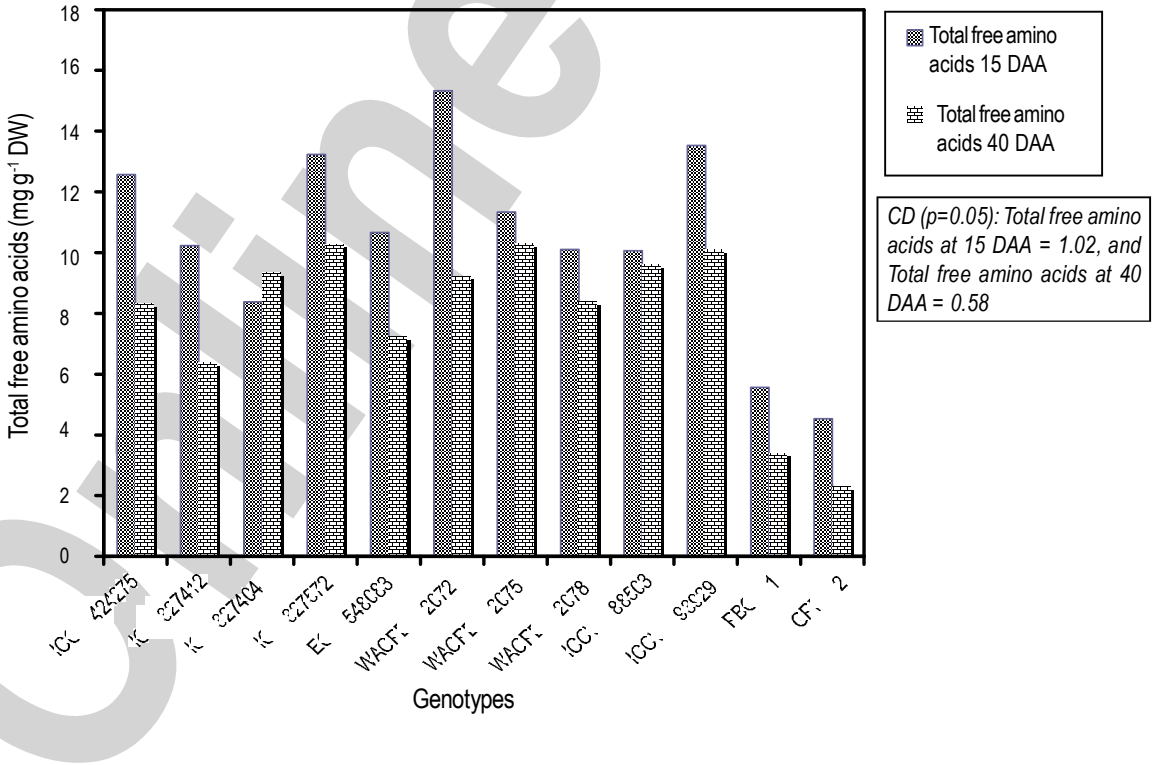

Fig. 4: Total free amino acids (mg g^{-1} DW) from seeds of chickpea genotypes

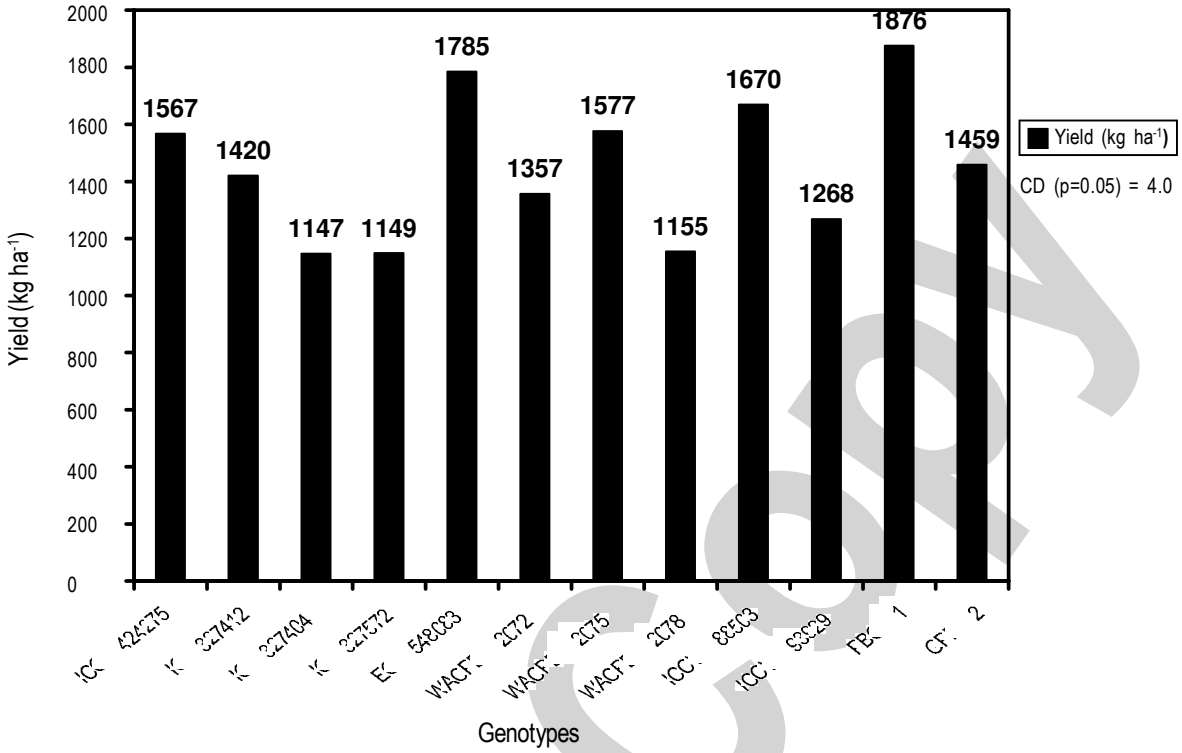

Fig. 5: Yield (kg ha^{-1}) in chickpea genotypes

long season conditions increased biomass accumulation prior to reproduction and flowering was delayed until temperature became sufficiently warm to support the pod set in chickpea favouring acclimation and increase in yield. Our study also corroborated these observations.

Leaf is the source of photosynthates and thus the number of leaves has a direct bearing on the capacity of sink and hence determines the yield of the plant. The data on number of leaves per plant taken at 30, 60, 90 and 120 DAS is presented in Table 1. Significant increase in number of leaves per plant was observed in chilling tolerant genotypes as compared to chilling sensitive genotypes at 30 and 60 DAS (Table 1). Maximum number of leaves per plant were observed in chilling tolerant EC-548083 (45.0 and 58.4) at 30 and 60 DAS respectively whereas minimum number of leaves per plant was observed in chilling sensitive genotype GPF-2 (21.1) at 30 DAS and PBG-1 (30.4) at 60 DAS. However at 90 DAS, in chilling sensitive genotypes PBG-1 and GPF-2 number of leaves per plant increased drastically and took the lead by producing 88.4 and 87.5 leaves respectively and the least in chilling tolerant genotype ICC-424275 (60.0) at 90 DAS. Other chilling tolerant genotypes also recorded increases in number of leaves at this stage (90 DAS) but it was comparatively lesser than the chilling sensitive genotypes PBG-1 and GPF-2. Similar results were observed 120 DAS. PBG-1 recorded significantly higher number of leaves as compared to other genotypes at 120 DAS. In our results chilling sensitive genotypes recorded slow vegetative growth (number of leaves) from 30 to 60 DAS (at chilling temperature below 10°C) and at 90 and 120 DAS, number of leaves increased tremendously with the increase in temperature. Yadav *et al.* (1998) also recorded reduction in number of branches, number of leaves, relative growth rate and biomass production at low temperature and found that kabuli types were more sensitive to temperature as compared to desi chickpea types.

Pollen viability studies revealed that below 5 and 10°C temperatures, GPF-2 (CS) recorded low pollen viability percentage (37.3 and 51.3%, respectively) as compared to PBG-1 (41.7 and 52.3%, respectively) (Fig. 1). Chilling tolerant genotype (EC-548083) recorded highest pollen viability percentage below 5 and 10°C temperatures. In general chilling tolerant genotypes recorded higher pollen viability percentage as compared to chilling sensitive genotype. Low temperature at flowering is a major constraint to improved yield of chickpea. Clarke and Siddique (2004) also recorded that low temperature (<15°C) affected the pollen tube growth and fertilization.

Estimated electrolyte leakage was found to be maximum *i.e.* 77.3 and 65.8% in PBG-1 (chilling sensitive genotype) at 15 DAA (Days after anthesis) and 40 DAA, respectively, which was significantly higher than other chilling sensitive (GPF-2) and all chilling tolerant genotypes (Fig. 2). Minimum electrolyte leakage was estimated in ICCV-88503 (47.3%) at 15 DAA. At 40 DAA its lowest value was recorded in ICCV-88503 (40.8%) and IC-327412 (41.4%). Thus, the results showed that electrolyte leakage was significantly higher in the leaves of chilling sensitive genotypes as

compared to chilling tolerant genotypes. Wery *et al*. (1993) proposed mechanism of resistance to temperature related stress. They considered membrane stability, an important mechanism of tolerance to chilling frost, heat and dehydration to adjust the number of seeds according to the level of stress and osmotic adjustment, which is involved in cold avoidance and tolerance. Similar results were obtained in our study *i.e.*, maximum membrane stability at low temperature provides cold tolerance in the chilling tolerant genotypes. Membrane stability means membrane integrity and is defined as the percent electrolyte leakiness from the cell which is the ratio of conductivity after 24 hr of immersion (of leaves) in water to conductivity after boiling. Hence membrane activity is directly related to electrolyte leakage.

Total soluble sugars were recorded the highest in chilling tolerant genotypes at both the stages *i.e.* 15 and 40 DAA (Fig. 3). Chilling tolerant genotype EC-548083 recorded the highest total soluble sugar content (74.3 and 31.5 mg g^{-1} dry weight at 15 and 40 DAA respectively) which was significantly higher than other genotypes at both stages. The significantly lowest soluble sugar content (27.3 and 10.5 mg g^{-1} dry weight at 15 and 40 DAA respectively) was recorded in chilling sensitive GPF-2 as compared to other chilling tolerant genotypes. Hudges and Dunn (1996) suggested that cellular and metabolic changes that occur during cold acclimation include increased level of sugars, soluble proteins, proline and organic acids as well as appearance of new isoforms of proteins and altered lipid membrane composition. At low temperature stress, total soluble sugar content increased in early maturing chickpea genotype ICCV-96029 (Nayyar, 2005). Our study corroborated these results.

Total amino acid content was recorded highest at 15 DAA stage and declined till 40 DAA stage (Fig. 4). The chilling sensitive genotypes contained significantly less total free amino acid content as compared to chilling tolerant genotypes at both the stages. The highest amount of free amino acids was recorded in chilling tolerant genotype WACPE-2072 at 15 DAA (15.33 mg g^{-1} dry weight) whereas at 40 DAA, WACPE-2075 recorded the highest amino acid content (10.30 mg g^{-1} dry weight). The lowest amount of free amino acids was recorded in chilling sensitive genotype GPF-2 at 15 DAA as well as 40 DAA stages (4.53 and 2.30 mg g^{-1} dry weight respectively). Amino acid concentration increases due to environmental stress *viz*. cold, water and salt stresses etc. Amino acid concentration provides osmotic adjustment under chilling stress (Rai, 2002). Maximum increase in free amino acids accumulation was recorded the highest during protein deposition stage (15 DAA) which declined toward maturity (40 DAA). This could be ascribed to the fact that the free amino acids pool is used up towards maturity for formation of other proteins.

Yield is the result of complex physiological processes and yield attributes have dynamic relationship with ever changing environmental factors. Yield was significantly higher in chilling sensitive genotype PBG-1 (1876 kg ha^{-1}) as compared to all other genotypes (Fig. 5). Minimum yield was recorded in chilling tolerant genotype IC-327404 (1147 kg ha^{-1}).

Thus by comparing the chilling tolerant and sensitive chickpea genotypes, we can conclude that during the initial stages (30 and 60 DAS) when the temperature is in the chilling range (0-15°C) chilling tolerant genotypes performed well in terms of morphological characters (plant height, number of branches and number of leaves) as compared to sensitive genotypes by increasing their osmoprotectants (electrolyte content, total soluble sugars and total free amino acids) under chilling stress and thus maintaining the osmotic pressure of the cells. Chilling sensitive chickpea genotypes extend their vegetative phase and overcome the negative effect of chilling stress at maturity stage and finally increase their yield. Pollen viability studies depicted that GPF-2 was more sensitive to chilling as compared to PBG-1, whereas chilling tolerant genotypes recorded sustained increase in the pollen viability percentage. Pollen viability has no correlation with the final yield; however pollen viability has correlation with chilling sensitivity.

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