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Chilling/Freezing Stress

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Low Temperature Effects during Seed Filling on Chickpea Genotypes (*Cicer arietinum* L.): Probing Mechanisms affecting Seed Reserves and Yield

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With 1 figure and 6 tables

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Abstract

Chickpea is sensitive to cold conditions (< 15 °C), particularly at its reproductive phase and consequently it experiences significant decrease in the seed yield. The information about the effects of cold stress on chickpea during the seed filling phase is lacking. Moreover, the underlying metabolic reasons associated with the low temperature injury are largely unknown in the crop. Hence, the present study was undertaken with the objectives: (i) to find out the possible mechanisms leading to low temperature damage during the seed filling and (ii) to investigate the relative response of the *microcarpa* (Desi) and the *macrocarpa* (Kabuli) chickpea types along with elucidation of the possible mechanisms governing the differential cold sensitivity at this stage. At the time of initiation of the seed filling (pod size ~1 cm), a set of plants growing under warm conditions of the glasshouse (temperature: 17/28 ± 2 °C as average night and day temperature) was subjected to cold conditions of the field (2.3/11.7 ± 2 °C as average night and day temperature), while another set was maintained under warm conditions (control). The chilling conditions resulted in the increase in electrolyte leakage, the loss of chlorophyll, the decrease in sucrose content and the reduction in water status in leaves, which occurred to a greater extent in the *macrocarpa* type than in the *microcarpa* type. The total plant weight decreased to the same level in both the chickpea types, whereas the rate and duration of the seed filling, seed size, seed weight, pods per plant and harvest index decreased greatly in the *macrocarpa* type. The stressed seeds of both the chickpea types experienced marked reduction in the accumulation of starch, proteins, fats, crude fibre, protein fractions (albumins, globulins, prolamins and glutelins) with a larger decrease in the *macrocarpa* type. The accumulation of sucrose and the activity levels of the enzymes like starch synthase, sucrose synthase and invertase decreased significantly in the seeds because of the chilling, indicating impairment in sucrose import. Minerals such as calcium, phosphorous and iron as well as several amino acids (phenylalanine, tyrosine, threonine, tryptophan, valine and histidine) were lowered significantly in the stressed seeds. These components were limited to a higher extent in the *macrocarpa* type indicating higher cold sensitivity of this type.

Key words: chickpea — chilling — *Cicer arietinum* — Desi — Kabuli — seed quality — yield

Introduction

Chickpea (*Cicer arietinum* L.) is one of the major cool season food legumes, which is grown in tropical, sub-tropical and temperate regions of the world (Singh and Ocampo 1997). During its growth, chickpea faces low temperature conditions that result in the considerable yield loss (Singh et al. 1993, Croser et al. 2003, Nayyar et al. 2005). The reproductive phase in chickpea is especially more sensitive to cold stress and consequently shows bud abscission, floral abortion, poor pod set, infertile pods and the reduced seed size that substantially limit the production potential of this crop (Srinivasan et al. 1998, Croser et al. 2003, Clarke and Siddique 2004, Nayyar et al. 2005). The underlying reasons governing cold sensitivity in chickpea are not well understood (Croser et al. 2003). The reduced pollen tube growth in the style because of chilling is cited as one of the vital reasons impairing fertilization resulting in flower abortion and poor pod set (Clarke and Siddique 2004). The elevation of abscisic acid (ABA)

coupled with decreased sucrose content in the flowers of cold-stressed plants is reportedly linked with the abortion of flowers (Nayyar et al. 2005).

Seed filling is one of the principal events in the reproductive phase of a plant, which directly determines the seed yield (Turner et al. 2001). The abiotic stresses including sub- or supra-optimal temperature conditions are known to impede the seed development (Saini and Westgate 2000). The inhibitory effects of abiotic stresses on the seed growth may arise because of the altered carbohydrate and nitrogen metabolism in source and sinks (Wilhelm et al. 1999, Nayyar et al. 2005), impaired photosynthesis (Krapp and Stitt 1995, Munier Jolain et al. 1998; Nayyar et al. 2005), senescence of source leaves (Yang et al. 2001), hormonal imbalance (Nayyar and Walia 2004) as well as vascular restrictions and reduced uptake of assimilates into seeds (Ahmadi and Baker 2001). The information on low temperature effects during the seed filling on chickpea, especially on its seed quality is lacking in chickpea (Croser et al. 2003, Nayyar et al. 2005), which is imperative to know the basis of cold sensitivity of this phase.

Chickpea exists as two types: (i) *microcarpa* also called 'Desi' type has small, angular and dark-brown coloured seeds and (ii) *macrocarpa* also called 'Kabuli' type has large, rams-head shaped and light brown seeds; both the types have slight variation in their seed composition (Malhotra et al. 1987). The earlier reports based on seed yield suggests that the *microcarpa* types possess less freezing tolerance than the *macrocarpa* types (Singh et al. 1993, 1995), which has been attributed to the traditional cultivation and the selection of the former type in relatively warmer climate like that of the Indian subcontinent and Ethiopia. On the other hand, the *macrocarpa* types have been observed to be more sensitive to drought stress than the *microcarpa* types in terms of pod abortion (Leport et al. 2006).

The present study was undertaken with these objectives: (i) to assess the impact of the chilling stress during seed development on the processes associated with the seed filling and the consequent effects on the seed composition as well as the yield and (ii) to evaluate the relative response of the *macro*- and the *microcarpa* chickpea types to cold stress during the seed filling and reasons related to differential cold sensitivity of these types. It was hypothesized that: (a) cold stress induced inhibitory effects on the seed yield might occur because of aberrations in the photosynthesis as well as the sucrose metabolism in source (leaves) and sink (seeds) organs and (b) the differential cold sensitivity of the two chickpea types might arise because of variations in these metabolic effects.

Materials and Methods

Raising plants

The experiment was conducted at Chandigarh (India), (30.74°N, 76.79°E and elevation 321 m). Chickpea genotypes, *microcarpa* (GPF2) and *macrocarpa* type (L550) were procured from Punjab Agricultural University, Ludhiana, India. The plants were raised in earthen pots (30 cm height, 25 cm diameter and 14.72 l volume) having a mixture of air dry soil, sand and farm yard manure in ratio of 2 : 1 : 1 (v/v). The soil was loam with a pH of 7.1 and available N, P and K at 54, 43 and 158 kg ha^{-1} respectively. The seeds were inoculated with *Rhizobium ciceri* at recommended rate of 1.95 g kg^{-1} seeds. Four seeds were planted in each pot in November and after emergence, the plants were thinned to two per pot. The plants were grown in warm conditions of the glass house (temperature: 17/28 ± 2 °C as average night and day temperatures; light intensity: 1350 μ mol m^{-2} s^{-1}; relative humidity: 60–65%) till initiation of seed filling (pod size ~1 cm). Thereafter, 100 pots having two plants of each genotype were moved to the field for exposure to natural chilling conditions (2.3/11.7 ± 2 °C as average night and day temperature; Fig. 1 for details; 14 December to 15 January) to impose cold stress during subsequent seed development. The potted plants shifted from the glasshouse were kept in open conditions above the soil surface having a distance of about 30 cm among the pots. The pots were rotated frequently for randomization. The plants were retained under cold conditions for subsequent growth till maturity. It is noteworthy that the temperature regime at the experimental location did not fluctuate much during the observations on chilling effects,

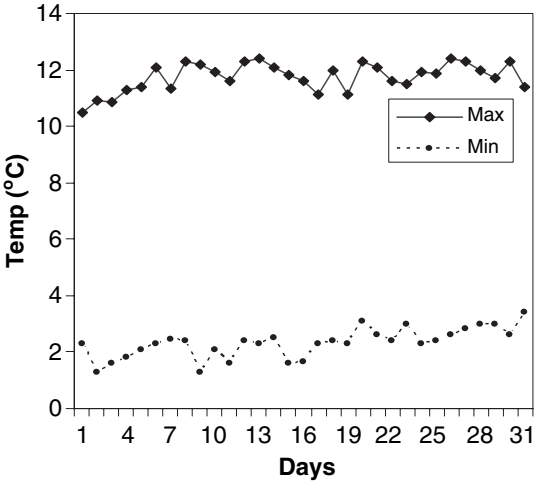

Fig. 1: Temperature profile of the field during stress period (14 December to 15 January) (location coordinates −30.74°N, 76.79°E, elevation 321 m)

which appears to make it an ideal location for such experiments.

Stress injury

It was examined 15 days after exposure to stress using the electrolyte leakage (EL; Lutts et al. 1996) and the chlorophyll content (Arnon 1949) of the uppermost newly emerged leaves (three leaves per plant from three different plants). The water status of the leaves was measured in the stressed and the control plants between 10.00 and 11.00 AM according to Barrs and Weatherly (1962).

Enzyme assays

The activity levels of the enzymes (sucrose synthase, soluble starch synthase and invertase) were assayed from fresh seeds harvested at physiological maturity from pods of upper three nodes of the control and the stressed plants growing in the field (20 days after stress). For enzymes assays, samples (500 mg; three replications) were homogenized in the presence of ice-cold 200 mM pH 7.8 HEPES/KOH buffer, containing 3 mM of EDTA $Na_2.2H_2O$, 3 mM of magnesium acetate, 10 mM of dithiothreitol and 1% (w/v) of polyvinyl-pyrrolidone. The homogenate was centrifuged (8400 ***g***) for 20 min at 4 °C and the supernatant was used directly as enzyme and protein sources. The activity of invertase (EC 3.2.1.26), soluble starch synthase (EC 2.4.1.21) and sucrose synthase (EC 2.4.1.13) were assayed according to Xu et al. (1996) and Sung et al. (1989) respectively. The assays were performed at 25 °C in a final volume of 1 ml.

Analysis of seed reserves

The pods of the branches at upper three nodes were tagged for analysis of the seed parameters. The mature seeds (including seed coats) of the control and the stressed plants were examined for analysis of various seed reserves. The soluble sugars and starch were extracted with ethanol 95% (v/v) and 30% (v/v) perchloric acid respectively. Both the components were quantified by the phenol–sulphuric acid method of Dubois et al. (1956) using glucose (Sigma, St. Louis, MO, USA; D9434) as a standard. Ash content, crude protein crude fat, crude fibre and elements were analysed by standard AOAC procedures (Helrich 1990). The sucrose, glucose and fructose were measured according to the method of Liu and van Staden (2001). The protein fractions albumins, globulins, prolamins and glutelins were sequentially extracted from seeds (along with seed coats) according to the method of Triboï et al. (2000). The protein content of each fraction was determined according to Lowry et al. (1951). The amino acid analysis followed the method of Bourgoin (1993).

Seed growth rate and seed filling duration

For investigation of the seed growth rate and seed filling duration, 25 plants growing under control and stressed conditions were examined. Five pods per plant were tagged at the beginning of pod filling (pod size ~1 cm) and followed until physiological maturity of seeds. The dry weight of the seeds was recorded at 7 days after initiation of pod filling and at physiological maturity. The seeds were oven dried at 45 °C for 5 days and the difference in their initial and final weight divided by number of days indicated the seed growth rate (mg $seed^{-1}$ day^{-1}). The time (days) required to complete the seed filling was noted in tagged pods.

Yield parameters

The number of pods (single seeded, double seeded and infertile) was recorded from 25 plants of each type and treatment. The seed weight and seed size (width) were recorded in 100 pods of each treatment. The observations were replicated thrice and the data were analysed for means and standard errors. ANOVA was conducted for each parameter and least significant difference (LSD) was calculated.

Results

The leaves of the cold-stressed plants experienced a marked increase in EL along with reduction in chlorophyll and sucrose content (Table 1). The *macrocarpa* genotype experienced significantly higher EL than the *microcarpa* type (Table 1).

Table 1: Chilling stress effects during seed filling on the stress injury (as electrolyte leakage), chlorophyll content, sucrose content and relative leaf water content in the leaves of the chickpea genotypes

	Microcarpa		Macrocarpa	
Parameter	Control	Stressed	Control	Stressed
Stress injury (electrolyte leakage; %)	13.4 ± 1.8 c	51.3 ± 2.9 b	15.3 ± 2.1 c	68.2 ± 3.6 a
Sucrose (μ mol g^{-1} DM)	156.3 ± 10.6 a	96.6 ± 8.2 b	162.3 ± 11.3 a	72.6 ± 7.3 c
Chlorophyll (mg g^{-1} fresh weight)	2.34 ± 0.13 a	1.75 ± 0.16 b	2.29 ± 0.12 a	1.33 ± 0.14 c
Relative leaf water content (%)	76.6 ± 2.2 a	67.2 ± 2.4 b	78.3 ± 2.8 a	61 ± 2.1 c

Values represent mean ± standard error. Values in a row followed by same letter are not significantly different from each other ($P < 0.05$).

Table 2: Chilling stress effects during seed filling on yield traits of the control and the stressed plants of the chickpea genotypes

Parameter	Microcarpa		Macrocarpa	
	Control	Stressed	Control	Stressed
Total weight plant^{-1} (including roots) (g)	12.6 ± 1.1 b	9.7 ± 1.2 d	15.7 ± 1.3 a	11.1 ± 1.2 c
Seed yield plant^{-1} (g)	5.2 ± 0.5 b	3.2 ± 0.8 c	6.1 ± 0.6 a	2.8 ± 0.6 c
Harvest index	0.41 ± 0.08 a	0.32 ± 0.05 b	0.38 ± 0.07 a	0.23 ± 0.06 c
Seed growth rate (mg seed^{-1} day^{-1})	8.2 ± 0.56 b	6.3 ± 0.63 c	9.0 ± 0.61 a	6.4 ± 0.54 c
Seed fill duration (days)	20.2 ± 1.3 a	14.1 ± 1.4 b	21.2 ± 1.2 a	12.9 ± 1.3 c
Average seed weight (mg)	104 ± 2.2 c	62.4 ± 3.6 d	233 ± 2.9 a	117 ± 5.6 b
Average seed size (mm)	6.6 ± 0.5 b	5.5 ± 0.6 b	8.0 ± 1.2 a	6.1 ± 1.3 b
Pods plant^{-1}	24.9 ± 2.0 a	17.7 ± 2.3 b	18.6 ± 2.4 b	10.1 ± 3.0 c
Seed number 100 pods^{-1}	121.7 ± 3.1 a	97.5 ± 2.6 c	103.4 ± 3.1 b	66.9 ± 4.1 d
Single-seeded plant^{-1}	20.2 ± 2.4 a	13.7 ± 2.3 c	15.8 ± 1.5 b	8.6 ± 1.6 d
Double-seeded plant^{-1}	4.6 ± 1.0 a	4.0 ± 0.6 a	2.8 ± 0.8 b	1.5 ± 0.3 c

Values represent mean ± standard error. Values in a row followed by same letter are not significantly different from each other ($P < 0.05$).

Compared with the control, the decrease in chlorophyll and sucrose was also greater in the stressed plants of the *macrocarpa* type (chlorophyll: 1.33 mg per g FW; sucrose: 72.6 μmol per g DM) than in the *microcarpa* type (chlorophyll: 1.75 mg per g FW; sucrose: 96.6 μmol per g DM) implying greater sensitivity of the former type (Table 1). The cold-stressed plants of the *macrocarpa* type experienced larger reduction in leaf water status than the *microcarpa* type. The total plant weight decreased because of stress in both the genotypes, the degree of reduction over the control varied only slightly between the two genotypes (Table 2).

The seed yield (Table 2) decreased because of cold stress and to a greater extent in the *macrocarpa* (control: 6.1 g per plant; stressed: 2.8 g per plant) than in the *microcarpa* type (control: 5.2 g per plant; stressed: 3.2 g per plant). Consequently, the harvest index decreased largely in the stressed plants of the *macrocarpa* (0.23) than in the *microcarpa* (0.32) type. A 23% and 28% inhibition occurred in the seed growth rate of the stressed plants over the control in the *microcarpa* and the *macrocarpa* types respectively. As a result the size and weight of the seeds decreased greatly in the *macrocarpa* type than the *microcarpa* type. The duration of the seed fill in the stressed plants dropped by 6.1 days in the *microcarpa* type and 8.3 days in the *macrocarpa* type. The seed number per 100 pods decreased from 121.7 and 103.4 in the controls of the *microcarpa* and *macrocarpa* types to 97.5 and 66.9 in the stressed plants respectively. The number of total pods per plant in the stressed plants decreased significantly over controls. The number of the single-seeded pods had a larger decrease than the double-seeded pods with higher effect in the *macrocarpa* type.

A proximate analysis of seeds of both the chickpea types indicated that the stressed seeds experienced considerable reduction in the accumulation of all the seed components. In the *microcarpa* type, the amount of starch, proteins and soluble sugars decreased to 31.6%, 14.7% and 4.6% while in the *macrocarpa* type, these components reduced to 26.3%, 10.3% and 4.1%, respectively with significant difference between the two types (Table 3). The accumulation of the fats, crude fibre and ash decreased because of stress in the seeds of both the chickpea types. Proportionately, the accumulation of these substances was inhibited to a higher extent in the *macrocarpa* than in the *microcarpa* type, but the differences were significant only for the ash content (Table 3).

An assessment of the protein fractions in seeds revealed significant inhibition in their accumulation in both the chickpea types because of cold stress with larger impact on the globulins than other fractions (Table 4). In the stressed seeds of the *microcarpa* type, the content of the albumins, globulins, glutelins and prolamins was observed to be 8.1%, 34.6%, 17.1% and 3.9%, respectively compared with 6.9%, 29.1%, 11.3% and 2.3% in the *macrocarpa* type, the variations were significant between the two chickpea types for all the fractions. The sucrose content in the seeds decreased because of stress with greater reduction in the

Table 3: Various seed reserves (g 100 g^{-1}; %) in the control and the cold-stressed seeds of the chickpea genotypes

	Microcarpa		Macrocarpa	
Parameter	Control	Stressed	Control	Stressed
Starch	43.2 ± 4.6 a	31.6 ± 3.2 b	47.6 ± 5.1 a	26.3 ± 3.4 c
Proteins	26.2 ± 2.6 a	14.7 ± 2.4 b	24.3 ± 2.8 a	10.3 ± 2.1 c
Soluble sugars	5.8 ± 0.4 a	4.6 ± 0.3 c	6.2 ± 0.5 a	4.1 ± 0.3 d
Fat	4.1 ± 0.4 a	3.1 ± 0.3 b	4.6 ± 0.5 a	2.9 ± 0.2 b
Crude fibre	5.2 ± 1.3 b	4.1 ± 1.1 c	7.6 ± 1.2 a	3.1 ± 1.2 c
Ash	2.4 ± 0.8 a	1.8 ± 0.4 b	2.6 ± 0.7 a	1.0 ± 0.5 c

Values represent mean ± standard error. Values in a row followed by same letter are not significantly different from each other ($P < 0.05$).

Table 4: Protein fractions (%) in the control and the cold-stressed seeds of genotypes

	Microcarpa		Macrocarpa	
Parameter	Control	Stressed	Control	Stressed
Albumins	11.5 ± 1.8 a	8.1 ± 1.1 b	10.6 ± 2.1 a	6.9 ± 1.0 c
Globulins	55.1 ± 3.1 a	34.6 ± 2.4 b	51.6 ± 3.6 a	29.1 ± 2.6 c
Glutelins	20.3 ± 1.3 a	17.1 ± 2.1 b	19.2 ± 1.4 a	11.3 ± 1.8 c
Prolamins	5.2 ± 1.2 a	3.9 ± 0.8 b	4.3 ± 1.0 a	2.3 ± 0.8 c

Values represent mean ± standard error. Values in a row followed by same letter are not significantly different from each other ($P < 0.05$).

Table 5: Chilling stress effects during seed filling on the soluble sugars, enzymes and minerals of the seeds harvested at physiological maturity from the control and the stressed plants of the chickpea genotypes

	Microcarpa		Macrocarpa	
Parameter	Control	Stressed	Control	Stressed
Sucrose (μ mol g^{-1} DM)	37.3 ± 2.1 a	29.1 ± 3.4 b	35.5 ± 2.3 a	17.6 ± 2.2 c
Glucose (μ mol g^{-1} DM)	7.0 ± 1.4 a	8.6 ± 1.1 a	7.9 ± 1.4 a	9.1 ± 1.2 a
Fructose (μ mol g^{-1} DM)	6.8 ± 1.5 b	8.1 ± 1.3 a	7.1 ± 1.0 b	8.3 ± 1.3 a
Sucrose synthase (nmol min^{-1} mg^{-1} protein)	60.2 ± 3.2 b	47.3 ± 2.7 c	66.2 ± 3.5 a	40.3 ± 3.5 d
Soluble starch synthase (nmol min^{-1} mg^{-1} protein)	2145 ± 12.4 b	1875 ± 21.6 c	2232 ± 50.3 a	1452 ± 48.6 d
Invertase (nmol min^{-1} mg^{-1} protein)	1956 ± 36.1 b	1523 ± 39.2 c	2041 ± 46.1 a	1231 ± 40.2 d
Calcium (mg 100 g^{-1})	221 ± 7.3 b	201 ± 8.1 c	271 ± 7.4 a	198 ± 6.3 d
Phosphorous (mg 100 g^{-1})	246 ± 6.7 a	178 ± 6.1 b	241 ± 5.8 a	141 ± 5.4 c
Iron (mg 100 g^{-1})	19.6 ± 3.4 a	12.2 ± 1.8 b	17.2 ± 1.3 a	9.6 ± 1.2 c

Values represent mean ± standard error. Values in a row followed by same letter are not significantly different from each other ($P < 0.05$).

macrocarpa (17.6 μmol per g DM) than in the *microcarpa* seeds (29.1 μmol per g DM) (Table 5). On the other hand, the glucose and the fructose levels increased marginally because of stress in both the chickpea types, the variations were insignificant between the two types. There was a marked attenuation in the activity of the soluble starch synthase (starch synthesizing enzyme), sucrose synthase and invertase (sucrose hydrolyzing enzymes) in the stressed seeds, with greater inhibition in the *macrocarpa* type. The minerals such as Ca, P and Fe had a considerable drop in the stressed seeds of both the types. Thus, the *microcarpa* seeds possessed 201, 178 and 12.2 mg per 100 g of Ca, P and Fe, respectively compared with 198, 141 and 9.6 mg per 100 g in the *macrocarpa*

Table 6: Chilling stress effects during seed filling on the amino acid composition (μmol g^{-1} DM) of the seeds of the control and the stressed plants of genotypes

	Microcarpa		Macrocarpa	
Parameter	Control	Stressed	Control	Stressed
Arginine	4.0 ± 0.3 b	4.8 ± 0.2 a	4.8 ± 0.2 a	5.1 ± 0.3 a
Glutamic acid	1.4 ± 0.2 b	1.6 ± 0.3 b	1.52 ± 0.12 b	2.3 ± 0.11 a
Glycine	1.9 ± 0.4 b	1.5 ± 0.4 b	2.3 ± 0.2 a	1.6 ± 0.16 b
Isoleucine	3.9 ± 0.5 a	3.3 ± 0.6 b	3.1 ± 0.13 b	2.5 ± 0.2 c
Leucine	6.0 ± 0.3 b	6.6 ± 0.2 a	5.6 ± 0.2 c	4.6 ± 0.3 d
Lysine	4.2 ± 0.6 a	3.8 ± 0.4 a	4.3 ± 0.3 a	2.8 ± 0.3 b
Methionine + cystine	1.8 ± 0.4 a	1.5 ± 0.2 a	1.9 ± 0.2 a	1.2 ± 0.2 b
Phenylalanine + tyrosine	6.8 ± 0.5 b	6.1 ± 0.4 c	7.3 ± 0.2 a	5.8 ± 0.4 c
Proline	4.6 ± 0.5 b	6.4 ± 0.5 a	4.1 ± 0.4 b	6.8 ± 0.5 a
Threonine	3.6 ± 0.7 a	2.8 ± 0.2 a	2.9 ± 0.2 a	2.0 ± 0.3 b
Tryptophan	1.6 ± 0.4 a	0.9 ± 0.15 b	1.4 ± 0.3 a	0.5 ± 0.1 c
Valine	3.1 ± 0.12 a	2.2 ± 0.13 c	2.7 ± 0.18 b	2.0 ± 0.16 d
Alanine	2.6 ± 0.8a	2.2 ± 0.7 a	2.0 ± 0.4 a	1.3 ± 0.3 b
Histidine	2.6 ± 0.8 a	2.4 ± 0.6 a	2.2 ± 0.4 a	1.4 ± 0.4 b
Aspartic acid	1.4 ± 0.7 a	1.1 ± 0.8 a	1.2 ± 0.3 a	0.9 ± 0.2 a
Serine	1.4 ± 0.5 a	1.3 ± 0.7 a	1.5 ± 0.3 a	1.0 ± 0.3 a

Values represent mean standard error. Values in a row followed by same letter are not significantly different from each other ($P < 0.05$).

type. Pertinently, Fe as compared with other minerals showed larger reduction in both the types because of stress (Table 5). There was a slight increase in the amino acids arginine, glutamic acid and proline in the stressed seeds, the differences between the two chickpea types were insignificant for these amino acids (Table 6). The leucine content increased in the *microcarpa* type but showed decrease in the *macrocarpa* type. The stressed seeds of the *macrocarpa* type possessed significantly lower contents of the isoleucine, lysine, methionine + cystine, threonine, tryptophan, valine, alanine and histidine than the *microcarpa* type, while the difference was insignificant for aspartic acid and serine between the two chickpea types.

Discussion

The leaves of cold-stressed plants in both the chickpea genotypes showed a significant decrease in chlorophyll and sucrose content along with elevation of stress injury (measured as EL). These aberrations were possibly responsible for decreasing the quantitative and qualitative aspects of the seeds developing under chilling stress. Low temperatures are known to cause damage to chlorophyll because of photo-oxidation and consequently the photosynthesis (Ying et al. 2000). The elevation of EL in leaf tissues of cold-stressed plants is possibly because of photo-oxidation as reported in other plant species (Lidon et al. 2001, Janowiak et al. 2002). Chilling is reported to invoke the formation of lipid peroxides in the membranes leading to their disorganization and hence leakage (Lidon et al. 2001, Janowiak et al. 2002). In previous studies, we reported that chilling-induced EL of chickpea leaves was associated with increased oxidative stress (Nayyar and Chander 2004). Sucrose reduction in stressed leaves might be ascribed to decreased photosynthesis as well as its impaired biosynthesis as noticed in other plant species experiencing cold-stress conditions (Perz et al. 2001). We also observed a significant reduction in leaf water content in cold-stressed plants indicating dehydration that might have accentuated the chilling injury. Previous studies (McWilliam et al. 1982) report that water status of the leaves decline because of reduction in hydraulic conductance of roots that decrease the stomatal conductance.

Seed yield decreased markedly in the stressed plants which occurred because of stress-induced abortion of pods resulting in lower number of seeds. Moreover, the reduced rate of seed filling

and the decrease in filling duration because of stress lowered the seeds weight. In this context, our findings are similar to previous studies reporting the inhibitory effects of cold stress on seed yield in chickpea (Singh et al. 1993, Srinivasan et al. 1999, Nayyar et al. 2005) as well as in other plant species such as pea (Lansac et al. 1996), soya bean (Spears et al. 1997) and cereals (Demotes Mainard et al. 1995; Ying et al. 2000). The abortion of pods and decrease in seed weight might occur because of restrictions in the availability of assimilates for the developing pods and seeds because of impairment in photosynthesis as indicated by lowered sucrose content in leaves and seeds of stressed plants. The sucrose levels in the leaves may become limiting because of decrease in photosynthesis *per se* because of cold stress as well as inhibition in the activity of sucrose synthesizing enzymes like sucrose phosphate synthase that has relatively higher sensitivity to low temperature (Perz et al. 2001). This enzyme was not examined in the present studies but may form a part of the future studies.

The levels of sucrose in developing seeds depend upon its import that is governed by sucrolytic enzymes like sucrose synthase and invertase in the seeds, which maintain steady state levels of sucrose in the seeds (Castonguay and Nadeau 1998). Here, the activity of these two enzymes decreased in the seeds of cold-stressed plants that might impede the sucrose utilization and hence its import in the seeds (Saini and Westgate 2000). The starch content showed a substantial reduction in stressed seeds that resulted because of inhibition in activity of its biosynthetic enzyme namely soluble starch synthase. As starch is a major component of chickpea seeds reserves, it is possible that constraints in its synthesis may affect the development of seed *per se* as well as inhibit the accumulation of proteins and other constituents.

Cold stress inhibited the accumulation of storage proteins, minerals and amino acids. The decrease in minerals and amino acids in the seeds because of chilling might be the result of limitations in their transport into the seeds (Mitchell and Madore 1992). The composition of amino acids altered in the stressed seeds, which matches the observations of Mossé et al. (1985) in wheat as well as our earlier report (Nayyar et al. 2005) and has been associated with changes in total quantity of nitrogen because of stress. In general, a decrease was noticed in the content of amino acids in stressed seeds of both the chickpea types. On the other hand, proline increased significantly because of stress. Elevated proline may serve as a cryo-protective function in cold-stressed tissues (Ruiz et al. 2002). The reduction in content of protein fractions in the stressed seeds is in agreement with the findings of Graybosch et al. (1996) and Triboï et al. (2000) who noticed similar situation in wheat seeds because of environmental aberrations. It was attributed to limitations in the accumulation of total quantity of nitrogen per grain. In legumes, environmental constraints impair the accumulation of protein fractions (Rong et al. 1996) to lower the nutritional quality of their seeds.

The present findings also revealed that the two chickpea types varied distinctively for their response to cold stress. Our observations suggested that the *macrocarpa* type was more sensitive to cold than the *microcarpa* type, which is in contrast to previous findings of Singh et al. (1993, 1995) who reported greater cold sensitivity of the *microcarpa* types than the *macrocarpa* types. It is relevant to mention here that the observations of these authors pertain to the sensitivity of early vegetative phase to freezing stress, while we investigated the effects of chilling stress on reproductive phase, which might explain the variations in our observations from the earlier studies. We found a greater stress injury (as EL), lower chlorophyll and sucrose content in the leaves of the *macrocarpa* type than in the *microcarpa* type which might have caused a larger reduction in the seed yield in the former type. Previous studies on the response of two chickpea types to water stress have reported that the *macrocarpa* genotypes in comparison with the *microcarpa* ones transported less assimilates to seeds during stress conditions (Leport et al. 1999), a similar possibility might exist under cold conditions too that needs to be investigated. A greater decrease in sucrose utilizing enzymes like sucrose synthase and invertase in seeds of the *macrocarpa* type implies its inferior ability to utilize sucrose in them. These observations match those of Tognetti et al. (1990) who also noticed lower activity levels of sucrose synthase and invertase in the cold-sensitive wheat genotypes as compared with the cold-tolerant ones. These observations suggest that the activity levels of sucrose synthase and the soluble starch synthase can be employed as indicators of cold sensitivity, while screening chickpea genotypes for cold tolerance during seeds filling. The difference between the two chickpea types with respect to other components like proteins, amino

acids and fats might be a consequence of variation in their ability to mobilize assimilates into the seeds, which requires to be examined.

Thus, the present findings indicated that the chilling induced decrease in the seed yield of chickpea was associated with the reduction in chlorophyll and the sucrose content in the leaves, which consequently inhibited the metabolic processes linked to the accumulation of seed reserves. These studies also implied that the nutritive components of the chickpea seeds, especially the proteins would fall considerably because of its cultivation in regions experiencing cold stress during seed filling. The *macrocarpa* type of chickpea as compared with the *microcarpa* type is found to be more cold sensitive during the seed development as indicated by various parameters. These observations suggest the necessity to take into consideration the differential cold sensitivity levels of two chickpea types while making Desi × Kabuli introgressions.

References

Ahmadi, A., and D. A. Baker, 2001: The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. Plant Gr. Regul. **35,** 81—91.

Arnon, D. I., 1949: Copper enzyme in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. Plant Physiol. **24,** 1—15.

Barrs, H. D., and P. E. Weatherly, 1962: A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. **15,** 413—428.

Bourgoin, R. F., 1993: Determining amino acid composition. Biotechnology **11,** 1302—1303.

Castonguay, Y., and P. Nadeau, 1998: Enzymatic control of soluble carbohydrate accumulation in cold-acclimated crowns of alfalfa. Crop Sci. **38,** 1183—1189.

Clarke, H. J., and K. H. M. Siddique, 2004: Response of chickpea genotypes to low temperature stress during reproductive development. Field Crops Res. **90,** 323—334.

Croser, J. S., H. J. Clarke, K. H. M. Siddique, and T. N. Khan, 2003: Low temperature stress: Implications for chickpea (*Cicer arietinum* L.) improvement. Critical Rev. Plant Sci. **22,** 185—219.

Demotes Mainard, S., G. Doussinault, and J. M. Meynard, 1995: Effects of low radiation and low temperature at meiosis on pollen viability and grain set in wheat. Agronomie **15,** 357—365.

Dubois, M. K., A. Giles, and J. K. Hamilton, 1956: Colorimetric method for determination of sugars and related substances. Anal. Chem. **28,** 350—356.

Graybosch, R. A., C. J. Peterson, D. R. Shelton, and P. S. Baenziger, 1996: Genotypic and environmental modification of wheat flour protein composition in relation to end-use quality. Crop Sci. **36,** 296—300.

Helrich, K., 1990: Official Methods of Analysis of the Association of Official Analytical Chemists, 15th edn. Association of Official Analytical Chemists., Washington, DC, USA.

Janowiak, F., B. Mass, and K. Dorffling, 2002: Importance of abscisic acid for chilling tolerance of maize seedlings. J. Plant Physiol. **159,** 635—643.

Krapp, A., and M. Stitt, 1995: An evaluation of direct and indirect mechanisms for sink-regulation of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady state transcript levels after cold girdling source leaves. Planta **195,** 313—323.

Lansac, A. R., C. Y. Sullivan, and B. E. Johnson, 1996: Accumulation of free proline in sorghum (*Sorghum bicolor*) pollen. Can. J. Bot. **74,** 40—45.

Leport, L., N. C. Turner, R. J. French, M. D. Barr, R. Duda, S. L. Davies, D. Tennant, and K. H. M. Siddique, 1999: Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. Eur. J. Agron. **11,** 279—291.

Leport, L., N. C. Turner, S. L. Davies, and K. H. M. Siddique, 2006: Variation in pod production and abortion among chickpea cultivars under terminal drought. Eur. J. Agron., **24,** 236—246.

Lidon, F. C., G. Ribeiro, H. Santana, H. Marques, K. Correia, and S. Gouveia, 2001: Photoinhibition in chilling stressed Leguminosae: comparison of *Vicia faba* and *Pisum sativum*. Photosynthetica **39,** 17—22.

Liu, T., and J. van Staden, 2001: Partitioning of carbohydrates in salt-sensitive and salt-tolerant soybean callus cultures under salinity stress and its subsequent relief. Plant Gr. Regl. **33,** 13—17.

Lowry, O. H., Rosebrough N. J., A. J. Farr, and R. J. Randall, 1951: Protein measurement with the folin-phenol reagents. J. Biol. Chem. **193,** 265—275.

Lutts, S, J. M. Kinet, and J. Bouharmont, 1996: NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot. **78,** 389—398.

Malhotra, R. S., R. P. S. Pundir, and A. E. Slinkard, 1987: Genetic resources of chickpea. In: M. C. Saxena, and K. B. Singh, eds. The Chickpea, pp. 67—81. International Cambrian News Ltd, Aberystwyth, UK.

McWilliam, J. R., P. J. Kramer, and R. L. Musser, 1982: Temperature induced water stress in chilling sensitive plants. Aust. J. Plant Physiol. **9,** 343—352.

Mitchell, D. E., and M. A. Madore, 1992: Patterns of assimilate production and translocation in muskmelon (*Cucumis melo* L.). II. Low temperature effects. Plant Physiol. **99,** 966—971.

Mossé, J., J. C. Huet, and J. Baudet, 1985: The amino acid composition of wheat grain as a function of nitrogen content. J. Cereal Sci. **3,** 115—130.

Munier Jolain, N. G., N. M. Munier Jolain, R. Roche, B. Ney, and C. Duthion, 1998: Seed growth rate in grain legumes I. Effect of photoassimilate availability on seed growth rate. J. Exp. Bot. **49,** 1963—1999.

Nayyar, H., and S. Chander, 2004: Protective effects of polyamines against oxidative stress induced by water and cold stress in chickpea. J. Agron. Crop Sci. **190,** 355—365.

Nayyar, H., and D. P. Walia, 2004: Genotypic variation in wheat in response to water stress and abscisic acid-induced accumulation of osmolytes in developing grains. J. Agron. Crop Sci. **190,** 39—45.

Nayyar, H., T. Bains, and S. Kumar, 2005: Low temperature induced floral abortion in chickpea: relationship to abscisic acid and cryoprotectants in reproductive organs. Env. Exp. Bot. **53,** 39—47.

Perz, P., R. Morcuende, I. M. D. Molino, L. S. D. Paunte, and R. Martnez Carrasco, 2001: Contrasting responses of photosynthesis and carbon metabolism to low temperatures in tall fescue and clovers. Physiol. Plant. **112,** 478—486.

Rong, L., J. J. Volenec, B. C. Joern, and S. M. Cunningham, 1996: Seasonal changes in nonstructural carbohydrates, protein, and macronutrients in roots of alfalfa, red clover, sweet clover and birdsfoot trefoil. Crop Sci. **36,** 617—623.

Ruiz, J. M., E. Sanchez, P. C. Garcia, L. R. Lopez Lefebre, R. M. Rivero, and L. Romero, 2002: Proline metabolism and NAD kinase activity in greenbean plants subjected to cold-shock. Phytochemistry **59,** 473—478.

Saini, H. S., and M. E. Westgate, 2000: Reproductive development in grain crops during drought. Adv. Agron. **68,** 59—95.

Singh, K. B., and B. Ocampo, 1997: Exploitation of wild *Cicer* species for yield improvement in chickpea. Theor. App. Gen. **95,** 418—423.

Singh, K. B., R. S. Malhotra, and M. C. Saxena, 1993: Relationship between cold severity and yield loss in chickpea (*Cicer arietinum* L.). J. Agron. Crop Sci. **170,** 121—127.

Singh, K. B., R. S. Malhotra, and M. C. Saxena, 1995: Additional sources of tolerance to cold in cultivated and wild *Cicer* species. Crop Sci. **35,** 1491—1497.

Spears, J. F., D. M. Tekrony, and D. B. Egli, 1997: Temperature during seed filling and soybean seed germination and vigour. Seed Sci. Tech. **25,** 233—244.

Srinivasan, A., C. Johansen, and N. P. Saxena, 1998: Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): characterization of stress and genotypic variation in pod set. Field Crops Res. **57,** 181—193.

Srinivasan, A., N. P. Saxena, and C. Johansen, 1999: Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): genetic variation in gamete development and function. Field Crops Res. **60,** 209—222.

Sung, S. S., P. P. Kormanik, D. P. Xu, and C. C. Black, 1989: Sucrose metabolic pathways in sweetgum and pecan seedlings. Tree Physiol. **5,** 39—52.

Tognetti, J. A., G. L. Salerno, M. D. Crespi, and H. G. Pontis, 1990: Sucrose and fructan metabolism of different wheat cultivars at chilling temperatures. Physiol. Plant. **78,** 554—559.

Triboï, E., A. Abad, A. Michelena, J. Lloveras, J. L. Ollier, and C. Daniel, 2000: Environmental effects on the quality of two wheat genotypes. I. Quantitative and qualitative variation of storage proteins. Eur. J. Agron. **13,** 47—64.

Turner, N. C., G. C. Wright, and K. H. M. Siddique, 2001: Adaptation of grain legumes (pulses) to water – limited environments. Adv. Agron. **71,** 193—231.

Wilhelm, E. P., R. E Mullen, P. L. Keeling, and G. W. Singletary, 1999: Heat stress during grain filling in maize: effects on kernel growth and metabolism. Crop Sci. **39,** 1733—1741.

Xu, J., W. T. Avigne, D. R. McCarty, and K. E. Koch, 1996: A similar dichotomy of sugar modulation and development expression affects both paths of sucrose metabolism: evidence from maize invertase gene family. Plant Cell **8,** 1209—1220.

Yang, J., J. Zhang, Z. Wang, Q. Zhu, and L. Liu, 2001: Water deficit – induced senescence and its relationship to the remobilization of pre-stored carbon in wheat during grain filling. Agron. J. **93,** 196—201.

Ying, J., E. A. Lee, and M . Tollenaar, 2000: Response of maize leaf photosynthesis to low temperature during the grain-filling period. Field Crops Res. **68,** 87—96.