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CHILLING/FREEZING STRESS

Cold Stress Injury during the Pod-Filling Phase in Chickpea (*Cicer arietinum* L.): Effects on Quantitative and Qualitative Components of Seeds

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Abstract

Chilling stress (<10 °C) is detrimental for chickpea, especially at the reproductive phase and leads to abortion of flowers, pods and impaired seed filling, causing severe reduction in yield. The information on the effects of low temperature during different pod-filling stages on quality and quantity of developing seeds is lacking in chickpea and hence this study. In this study, chickpea plants growing under warm conditions of the glasshouse were subjected to cold conditions of the field at the two stages, (a) early pod-filling and (b) late pod-filling, and subsequently analysed for stress injury in terms of electrolyte leakage (EL), 2,3,5-triphenyl tetrazolium chloride reduction, relative leaf water content and total chlorophyll content in the leaves of control and cold-stressed plants. Cold stress caused elevation of EL but reduced all the other parameters. Sucrose content decreased significantly in the leaves of cold-stressed plants. The differences between the effects of stress at two stages on the total plant dry weight were small and insignificant. The seed growth rate, seed fill duration, seed number, and average seed weight and size decreased greatly in the plants cold-stressed at the late pod-filling stage than those stressed at the early pod-filling stage. Greater reduction was observed in starch, proteins, soluble sugars, fat, crude fibre and storage protein fractions in the seeds of the plants cold-stressed at the late pod-filling stage. This coincided with a larger decrease in sucrose content, the activities of sucrose synthase, invertase and starch synthase observed at this stage. The germination and growth potential were, however, inhibited to a greater extent in seeds of plants stressed at the early pod-filling stage.

Introduction

India is one of the major producers of the legume crop, chickpea (*Cicer arietinum* L.), accounting for 75 % production in the world and ranks first in the Mediterranean basin and South Asia (Singh and Ocampo 1997). The seeds of chickpea have a high nutritional composition in terms of protein (220 g kg^{-1}), total carbohydrates (670 g kg^{-1}), starch (470 g kg^{-1}) and fat (50 g kg^{-1}). Chickpea experiences cold stress during the reproductive phase especially during flowering and podding, leading to abortion of flowers, poor pod set, infertile pods and abortion of pods (Srinivasan et al. 1998). Early-maturing genotypes of the chickpea are particularly more sensitive to cold stress because of coincidence of their flowering and pod-filling stages with chilling temperatures (Kumar et al. 2005).

Environmental constraints during seed development have a strong influence on the quantity and quality of seeds (Behboudian et al. 2001, Yang et al. 2001, Zhao et al. 2008). There is a wide variation in the results of studies reporting the effects of stress during seed fill on seed quality from no effect to severe reductions in seed yield, vigour and viability. Thus, water stress during

seed filling was reported to restrict the yield of chickpea (Davies et al. 1999), beans (Boutraa and Sanders 2001), wheat (Yang et al. 2001), rice (Yang et al. 2001) and barley (Samarah 2005). Sub- and supra-optimum temperatures during seed filling have profound negative effects in case of maize (Wilhelm et al. 1999) and wheat grains (Spiertz et al. 2006). In groundnut, Dwivedi et al. (1996) observed a decrease in oil content but increase in proteins in the seeds of water-stressed plants. In *Brassica napus* L. var oleifera, Champolivier and Merrien (1996) reported a reduction in the number of seeds per plant and a marked decrease in oil concentration, while glucosinolate concentration increased under water stress conditions. In chickpea, Behboudian et al. (2001) found that water stress during seed development in chickpea caused a mild increase in the nutritive values of seeds reflected in the higher accumulation of sugars, amino acids and proteins. In the seeds of chickpea plants cold-stressed during seed development, Nayyar et al. (2005) reported increase in accumulation of sugars but decrease in accumulation of storage proteins, starch and several amino acids in chickpea seeds. In water-stressed lupin, Carvalho et al. (2005) observed that the protein content was not affected but the oil content was reduced by 50 %.

Comparatively fewer studies have analysed the effects of low temperatures on seed development in pulses (Schori et al. 1993), especially in chickpea (Nayyar et al. 2005). Cold stress during the development of chickpea seeds has detrimental effects on seed yield in both Desi and Kabuli chickpea genotypes (Nayyar et al. 2007). Information about how, during different phases of pod-filling, are plants sensitive to cold stress in terms of its effects on composition and yield of the seeds does not exist in chickpea. Hence, the objective of the present study was to find out the relative sensitivity of pod-filling stages of chickpea to cold stress in terms of seed yield and quality of the seeds, which is hitherto unknown. In this study it was hypothesized that the sensitivity of pod-filling stages to cold stress might differ.

Materials and Methods

The seeds of chickpea genotype (GPF2), procured from Punjab Agricultural University, Ludhiana, India were raised in earthen pots (25 cm height, 25 cm diameter) having a mixture of air-dry soil : sand : farm yard in a ratio of 2 : 1 : 1 (v/v). The chickpea seeds were inoculated with *Rhizobium ciceri* (at 1.95 g kg^{-1} seeds). Three seeds were planted in the last week of October and after emergence the plants were thinned to two a pot. The potted plants were raised in the warm conditions of the glasshouse (28 ± 2 and 17 ± 2 °C as average day and night temperatures, respectively; 900–1100 μmol m^{-2} s^{-1} as light intensity). At the podding stage, two sets of plants were exposed to the cold conditions of the field (12 ± 2 and 3.4 ± 2 °C as average day and night temperatures, respectively; second fortnight of December), one set at early pod-filling (about three-fourth pods of ~0.6 cm size) and another set at late pod-filling stages (about three-fourth pods of ~1.2 cm size). The pods were tagged according to the stage of their development and at maturity the seeds were subjected to analysis of various parameters.

EL, relative leaf water content, chlorophyll and sucrose content

These traits were examined from the uppermost leaves collected from the control and stressed plants between 10:00 and 11:00 hours. EL was measured according to Lutts et al. (1996) as follows. The samples (1 g) were washed with deionized water to remove surface-adhered electrolytes. These were placed in closed vials containing 10 ml of deionized water and incubated at 25 °C on a rotary shaker for 24 h and subsequently electrical conductivity of the solution (L_1) was determined. Samples were then autoclaved at 120 °C for 20 min and the final electrical conductivity (L_2) was obtained after equilibration at 25 °C. The electrolyte leakage (%) was calculated as $(L_1/L_2) \times 100$. The relative leaf water content (RLWC) was estimated as per the method of Barrs and Weatherly (1962). The reduction assay for 2,3,5-triphenyltetrazolium chloride (TTC) was performed for each treatment (control and cold stress) using six replicates (Steponkus and Lanphear 1967). The samples (1 g) collected from control and stressed plants were washed three times with sterile distilled water and blotted with filter paper. Three replicates of each treatment were incubated for 2 min in 1 ml of water at 95 °C (heated samples). All the six replicates were then placed in glass tubes containing 4 ml of 50 mM phosphate buffer (pH 7.4) with 0.4 % (w/v) TTC. These were incubated for 20 h at 28 °C in darkness. The solution was drained, the segments were washed twice, 4 ml of ethanol was added and the tubes were vigorously shaken. The absorbance due to formazan formed *in vivo* was recorded at 485 nm. The TTC reduction was expressed in terms of per cent ability, based upon the following equation:

$$\text{TTC reduction (\%)} = (T_1 - T_2)/(C_1 - C_2) \times 100,$$

where T_1 is for treated samples, T_2 for 'heated' treated samples, C_1 for control samples and C_2 for 'heated 'control samples. The chlorophyll and sucrose contents were measured according to the methods of Arnon (1949) and Liu and van Staden (2001), respectively, elaborated elsewhere (Nayyar et al. 2005).

Assays of enzymes

The enzymes (sucrose synthase, soluble starch synthase and invertase) were assayed from freshly harvested seeds from the pods collected from the upper three nodes of the plants. The activity of the enzymes was assayed by homogenizing samples in the presence of ice-cold 200 mM of HEPES/KOH buffer (pH 7.8) containing 3 mM of ethylenediaminetetraacetic acid (EDTA)-$Na_2{\cdot}2H_2O$, 3 mM of magnesium acetate, 10 mM of dithiothreitol (DTT) and 1 % (w/v) of polyvinyl pyrrolidone (PVP). The homogenate was centrifuged (8400 *g*) for 20 min at 4 °C and the supernatant was used directly as the enzyme and protein source. The activity of invertase (EC 3.2.1.26) was soluble starch synthase (E.C.2.4.1.21) assayed per the method of Xu et al. (1996) while that of sucrose synthase (EC 2.4.1.13) was assayed according to Sung et al. (1989). The assays were performed at 25 °C in a final volume of 1 ml as explained earlier (Nayyar et al. 2005).

Analysis of seed reserves

The seed reserves were analysed from the pods located at branches at the upper three nodes that had been tagged at two stages (early pod-filling and late pod-filling). The mature seeds (including the seed coats) of the control and the stressed plants were subjected to analysis of various seed reserves. The starch and soluble sugars were extracted with 30 % perchloric acid (v/v) and 95 % ethanol (v/v), respectively. Both the components were quantified by the phenol–sulphuric acid method of Dubois et al. (1956) using glucose as a standard. The standard AOAC procedures were used for measuring the ash content, crude protein, crude fat and crude fibre (Helrich 1990). The sucrose content was measured as per 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).

Seed growth rate and filling duration

Twenty plants growing under control and stressed conditions were used for this purpose. The pods at the early-filling stage and those at the late-filling stage (pod size as above) were tagged and followed until physiological maturity of the seeds. The dry weight of the seeds was recorded at 4 days (for early pod-filling stage) and 9 days (for late pod-filling stage) and at physiological maturity. Thereafter, the seeds were oven-dried at 45 °C for 5 days, and the difference in their initial and final weight divided by the number of days indicated the seed growth rate. The duration (days) required to complete seed filling was noted in the tagged pods.

Yield parameters

The number of pods was recorded from 20 control and stressed plants. The seed weight per plant, seed weight and seed size were recorded in 100 pods of each treatment. The observations were replicated thrice and the data were statistically analysed for standard error and by Duncan multiple range test.

Germination and seedling growth

The mature seeds harvested from the plants were used for observations on germination and seedling growth. The seeds were surface-sterilized with 0.1 % mercuric chloride for 2 min and subsequently washed twice with distilled water. The seeds were germinated in glass Petri dishes (9 cm) lined with double-layered blotters. Germination count was taken after 48 h and seedling growth was measured after 7 days. The vigour index was measured as G% (48 h) × root length (cm; 7 days old).

Results

The leaves of the cold-stressed plants showed increase in EL in leaves at both the pod-filling stages without any significant difference between the stages (Table 1). The TTC reduction percentage, RLWC, total chlorophyll and

Table 1 Effects of cold stress on chickpea during early and late pod-filling stages on electrolyte leakage (EL), 2,3,5-triphenyl tetrazolium chloride (TTC) reduction, relative leaf water content (RLWC), total chlorophyll and sucrose content in the leaves

Parameter	Control	Stressed at early pod-filling stage	Stressed at late pod-filling stage
EL (%)	16.8 ± 3.2 b	56.2 ± 4.2 a	53.1 ± 3.6 a
TTC reduction (%)	83.6 ± 3.2 a	68.3 ± 2.4 b	70.6 ± 2.6 b
RLWC (%)	84.8 ± 2.5 a	67.6 ± 3.1 b	70.3 ± 2.4 b
Total chlorophyll (mg g^{-1} FW)	2.1 ± 0.3 a	1.2 ± 0.4 b	1.4 ± 0.4 b
Sucrose (μmol g^{-1} DM)	42.1 ± 2.2 a	29.6 ± 2.8 b	31.3 ± 3.4 b

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

Table 2 Effects of cold stress on chickpea during early and late pod-filling stages on yield contributing traits

Parameter	Unstressed control	Cold-stressed at early pod-filling stage	Cold-stressed at late pod-filling stage
Total dry weight per plant (g)	12.1 ± 2.2 a	10.2 ± 2.1 a	11.4 ± 2.6 a
Seed yield per plant (g)	5.5 ± 0.24 a	4.2 ± 0.11 b	3.1 ± 0.14 c
Seed growth rate (mg per seed per day)	8.9 ± 0.49 a	7.6 ± 0.54 b	6.8 ± 0.46 c
Seed fill duration (days)	19.9 ± 1.2 a	14.5 ± 1.2 b	11.8 ± 1.3 c
Average seed weight (mg)	133 ± 3.1 a	106 ± 3.4 b	92 ± 2.3 c
Average seed size (mm)	6.5 ± 0.21 a	6.1 ± 0.24 b	5.2 ± 0.31 c
Pods per plant	20.2 ± 1.1 a	17.1 ± 1.1 b	15.4 ± 1.3 c

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

sucrose content showed marked decrease in the leaves of the stressed plants because of stress, with little variation between the plants subjected to stress at both the stages.

Total plant weight (without pods) was found to have decreased when compared with the control plants by 17 % and 21 % in the plants stressed at early and late pod-filling stages, respectively; the differences between the stages were insignificant (Table 2). Seed yield per plant decreased by 43 % in plants cold-stressed at late filling stages and by 23 % in those cold stressed at early pod-filling stage when compared with control.

Seed growth rate decreased due to cold stress when compared with control by 14 % in plants stressed at early stage and by 23 % in plants stressed at late pod-filling stage, with a significant difference between the two stages (Table 2). Compared with controls, the seed-filling duration was reduced by 5.4 days in plants stressed at early pod-filling stage and by 8.1 days at late pod-filling stage because of cold stress. The seed number per 100 pods came down from 118 in control plants to 90 and 103 in plants stressed at early- and at-late filling stages, respectively. The average seed weight decreased from the control plants (133 mg) by 27 and 41 mg at early and at late pod-filling stages, respectively. The average seed size decreased to a greater extent in plants stressed at the late pod-filling stage (23 % over control) than those stressed at the early pod-filling stage (10 % over control). The number of pods showed a 23 % and 15 % decrease in plants cold-stressed at early and late pod-filling stages, respectively, when compared with controls. This was due to greater abortion of pods in plants stressed at the early pod-filling stage.

Seed reserves

Compared with control plants, the seeds of the plants cold-stressed at the late pod-filling stage showed greater reduction in contents of starch (32 %), proteins (36 %), soluble sugars (31 %), fat (33 %), fibres (26 %) and ash content (38 %) than the plants stressed at early pod-filling stage, which showed a decrease of 24 %, 11 %, 21 %, 20 %, 14 % and 26 %, respectively (Table 3). The storage proteins like albumins, globulins, glutelins and prolamins experienced a reduction of 31 %, 39 %, 28 % and 35 %, respectively, over controls in seeds of plants stressed at the late pod-filling stage compared with a decrease of 17 %, 22 %, 16 % and 25 %, respectively, in seeds of plants stressed at the early pod-filling stage (Table 4).

Table 3 Effects of cold stress on chickpea during early and late pod-filling stages on various seed reserves (g 100 g^{-1} %)

Parameter	Control	Stressed at early pod-filling stage	Stressed at late pod-filling stage
Starch	40.3 ± 2.1 a	30.3 ± 1.3 b	27.3 ± 1.3 c
Proteins	23.6 ± 1.2 a	16.1 ± 1.2 b	14.6 ± 1.4 c
Soluble sugars	5.4 ± 0.8 a	4.0 ± 0.6 b	3.5 ± 0.52 c
Fat	3.9 ± 0.2 a	3.0 ± 0.3 b	2.6 ± 0.3 c
Crude fibre	5.6 ± 1.6 a	4.8 ± 0.3 b	4.1 ± 0.3 c
Ash	2.6 ± 0.6 a	1.9 ± 0.34 b	1.6 ± 0.31 c

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

The sucrose content (Table 5) in the seeds decreased to a greater extent in seeds of plants cold-stressed at the late pod-filling stage (38 % over control) compared with those stressed at the early pod-filling stage (25 % over

Table 4 Effects of cold stress on chickpea during early and late pod-filling stages on protein fractions (%)

Parameter	Control	Stressed at early pod-filling stage	Stressed at late pod-filling stage
Albumins	10.8 ± 1.1 a	8.9 ± 1.4 b	7.4 ± 1.2 c
Globulins	51.3 ± 1.2 a	40.1 ± 1.4 b	31.3 ± 1.5 c
Glutelins	18.6 ± 1.3 a	15.6 ± 1.4 b	13.4 ± 1.4 c
Prolamins	4.3 ± 0.3 a	3.2 ± 0.5 b	2.8 ± 0.3 c

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

Table 5 Effects of cold stress on chickpea during early and late pod-filling stages on sucrose content and activities of enzymes in seeds

Parameter	Control	Stressed at early pod-filling stage	Stressed at late pod-filling stage
Sucrose (μmol g^{-1} DM)	38.2 ± 3.1	28.3 ± 2.6	23.6 ± 1.8
Sucrose synthase (nmol min^{-1} mg^{-1} protein)	58.4 ± 2.7	47.3 ± 2.9	41.1 ± 3.1
Soluble starch synthase (nmol min^{-1} mg^{-1} protein)	2023 ± 12.4	1806 ± 10.3	1589 ± 11.6
Invertase (nmol min^{-1} mg^{-1} protein)	1856 ± 16.2	1327 ± 14.3	1156 ± 12.9

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

control). The activity of sucrose synthase decreased over control and was observed to be lesser in seeds of plants cold-stressed at the late pod-filling stage than at the early pod-filling stage. The activity of invertase decreased when compared with control by 28 % and 40 % at early and late pod-filling stages, respectively. Soluble starch synthase activity also reduced to a higher extent (22 % over control) in seeds of plants cold-stressed at late pod-filling stage compared with those stressed at early pod-filling stage (11 % over control).

Seed germination and vigour

The germination percentage decreased significantly when compared with control in seeds of plants stressed at both the stages with greater reduction at early pod-filling stage compared with late pod-filling stage (Table 6). Seeds stressed at the early pod-filling stage also showed higher decrease in vigour index when compared with controls. In root length, a decrease of 28 % and 16 % occurred in seeds stressed at early pod-filling and late pod-filling stages when compared with controls, respectively. Shoot growth showed reduction of similar extent.

Discussion

The chickpea plants exposed to low temperature conditions of the field during both early as well as late pod-filling stages resulted in significant increase in electrolyte leakage (EL), which is an indicator of membrane damage to the stressed plants (Murata et al. 1992). These findings are in accordance with the earlier findings reporting the effects of cold stress on membrane injury (Bertin et al. 1996). The decrease in TTC reduction percentage implied loss of mitochondrial stability (Leopold and Musgrave 1979) in cold-stressed plants and is consistent with previous findings (Chen et al. 2000). The RLWC decreased possibly due to reduced water uptake in stressed plants (McWilliam et al. 1982). The chlorophyll loss might have occurred possibly due to cold-induced photo-oxidation of this pigment (Wise 1995), which matches the earlier findings on chilling injury (Nayyar et al. 2007). It was noticeable that compared with controls, the cold stress at both the pod-filling stages caused changes in the above-stated traits almost to the same extent, implying similar cold sensitivity of leaves at these stages but differences existed between the response of seeds, as discussed below.

There was a significant fall in the total plant weight, quantity as well as quality of the seeds of the cold-stressed plants. The decrease in total dry weight of the plant occurred due to reduction in vegetative growth by low temperature, which is in accordance with the earlier studies on chickpea on this aspect (Srinivasan et al. 1998, Nayyar et al. 2007). There was no significant difference between the two stages for plant dry weight, suggesting similar impact of the cold stress on vegetative growth at these stages. On the other hand, differences existed in seed yield in between the plants stressed at two stages. The seed number in our studies declined primarily due to reduction in the number of pods because of their

Table 6 Effects of cold stress on chickpea during early and late pod-filling stages on germination (%), vigour index, root and shoot length (cm) of seeds

Parameter	Control	Stressed at early pod-filling stage	Stressed at late pod-filling stage
G % (after 48 h)	100 ± 8.6 a	66 ± 6.7 c	79 ± 5.8 b
Vigour index (after 7 days)	250 ± 7.3 a	119 ± 6.6 c	166 ± 8.1 b
Root length (cm) (after 7 days)	2.5 ± 0.31 a	1.8 ± 0.21 c	2.1 ± 0.23 b
Shoot length (cm) (after 7 days)	1.2 ± 0.12 a	0.8 ± 0.15 c	1.1 ± 0.18 b

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

abortion and infertility caused by cold stress. Seed weight, a function of rate and duration of filling, reduced in cold-stressed plants because of decrease in both these parameters, resulting in smaller sized seeds. These observations are in line with the previous reports about low temperature effects in chickpea (Nayyar et al. 2007) and other plant species (Judd et al. 1982, Egli et al. 2005). The seed yield was affected to a greater extent in seeds stressed at late pod-filling stage than at early pod-filling stage, which may be related to larger decrease in seed growth rate and seed fill duration at the former stage. This was manifested in reduced seed weight and seed size at late pod-filling stage.

There was a considerable decrease in seed reserves of stressed plants. The decrease in seed reserves due to chilling in chickpea is in accordance with earlier observations on cold stress effects on chickpea (Nayyar et al. 2005) and other plant species (Ying et al. 2000). Some previous findings on the effects of environmental constraints like drought and high temperature on seed development are also indicative of impairment in seed-filling processes causing poor quality of seeds (Triboï et al. 2000, Nayyar and Walia 2004; Larmure et al. 2005).

The seed starch content possibly decreased because of restrictions in assimilate supply to the developing seeds as indicated by lowered sucrose content in the leaves and seeds of the stressed plants. In cereal grains developing under the influence of water stress, the decrease in starch content was attributed to the reduced capacity of the endosperms because of the decline in the number of amyloplasts (Jones et al. 1996); a similar possibility might exist in our case too that needs to be examined. We observed reduced activities of invertase and sucrose synthase (sucrolytic enzymes) that might decrease the availability of glucose precursors for starch synthesis. This probably also decreased the activity of soluble starch synthase in cold-stressed seeds observed here. These findings are similar to those of Hawker and Jenner (1993), who observed a decrease in activities of starch-synthesizing enzymes in wheat grains exposed to heat stress during gain filling.

The reduction in protein and fat contents in the cold-stressed seeds matches with the findings of Triboï et al. (2000) and Champolivier and Merrien (1996), respectively. The seed protein content in our studies might have decreased because of reduction in allocation of nitrogen by the stress to the developing seeds, as reported in peas (Larmure et al. 2005). Carvalho et al. (2005) noticed about 50 % reduction in protein and oil content of lupin seeds developed under water stress conditions.

The sucrose content dropped in the leaves and seeds of cold-stressed plants that was reflective of impairment in its production in leaves and reduced transport to the developing seeds. Previous studies also report decrease in sucrose content because of limitations in the photosynthesis and sucrose biosynthesis in other cases (Perz et al. 2001). Coupled with the decrease in sucrose was the decline in the activities of sucrose synthase and invertase enzymes that might have occurred due to reduced availability of the substrates or effects of cold stress *per se* on enzyme activities. These findings get support from the past studies involving chilling effects on maize that showed decrease in activity of sucrose metabolism enzymes and inhibited transport of sucrose because of its restrictions in loading (Sowiński et al. 1999).

The reduction in the performance of the cold-stressed chickpea seeds in terms of germination potential and vigour of the seedlings may be attributed to impaired embryo growth and decreased accumulation of seed reserves. The germination, vigour and growth were inhibited to a larger extent in plants cold-stressed at the early pod-filling stage. It was expected since the embryo growth is active during early stage of seed filling. These findings are in accordance with the previous ones on soybean that reported the adverse effects of abiotic stresses on seed quality in terms of nutritional components and growth potential of seeds (Dornbos and Mullen 1991; Egli et al. 2005).

The present studies indicated that although membrane damage, chlorophyll loss, and decrease in RLWC and sucrose levels in the leaves of cold-stressed plants occurred to a similar extent at both the stages, variations existed in the quality and quantity of the seeds produced by the plants stressed at early and late pod-filling stages. The larger decrease in the quantity and quality of seeds at the late pod-filling stage might have occurred because of the greater effect of cold stress on seed-filling processes at this stage. This was indicated by the significantly low content of sucrose and activities of enzymes pertaining to starch synthesis and sucrose hydrolysis in the seeds stressed at the late pod-filling stage than at the early pod-filling stage. This implied that these variations might arise from the greater effects of stress on metabolism of the seeds rather than that of leaves. Our findings also indicated that cold stress at the early pod-filling stage had a higher inhibitory effect on the germination and growth of the seeds. These observations suggested that sucrose mobilization and its utilization in the seeds rather than the sucrose production in the leaves might be a key determinant in deciding the sensitivity of the seed development phases in cold-stressed plants.

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