Low temperature stress has deleterious effects on chickpea at various growth stages. It affects the plant survival, growth and biological yield of the plant. Information about response of Desi and Kabuli Chickpea genotypes to cold stress is not available. Hence, the present studies were conducted with aims (i) to examine the cold stress induced damage at various growth stages (ii) to compare Desi and Kabuli chickpea genotypes for their relative sensitivity at different developmental phases towards cold stress (iii) to probe the involvement of cryoprotectants like polyamines, proline, glycine betaine and ABA in cold response.

Three main field trials were carried out in the winter season of 2003-04, 2004-05 and 2005-06 in the department of Botany, Panjab University, Chandigarh. Simultaneously, the germination experiments were carried at 5°C, 10°C, 15°C, 20°C and room temperature under controlled conditions. The work was divided into four major experiments. The findings are summarized as follows:
EXPERIMENT – I

5.1. ASSESSMENT OF RELATIVE SENSITIVITY OF THE DESI AND KABULI GENOTYPES TO CHILING STRESS (5-20°C) AT GERMINATION AND SEEDLING STAGE

The experiment was carried out under laboratory conditions in the year 2003-04. The seeds of chickpea genotypes, both Desi and Kabuli were subjected to cold stress of varying temperature levels to assess the relative sensitivity of both these genotypes during germination and seedling stage. In order to mitigate the cold stress damage to germination and seedling growth, we evaluated some of the cryoprotectants namely polyamines-putrescine (PUT) and spermine (SPM)), compatible solutes-proline (PRO), glycine betaine (Glybet) and Abscisic acid (ABA). These cryoprotective solutes were applied exogenously in the germination medium at various concentrations. The observations are summarized as follows:

5.1.1. DAYS TO 50% AND 100% GERMINATION

At room temperature, SPM, PRO, PUT, Glybet and ABA treated seeds took less time to reach the 100% germination (G) in both Desi and Kabuli genotypes in comparison to untreated controls. At 20°C, PUT, Glybet and ABA reduced the number to days to 2 to reach 100% germination in both Desi and Kabuli genotypes in comparison to control which took 5 days for 100% germination. Similarly at 15°C and 10°C all the treatments were observed to be effective in attaining 50 and 100% germination in 2-3 days in both Desi and Kabuli genotypes while the control plants were observed to take 2-3 extra days to attain 100% G. At 5°C temperature, after 15 days, PRO elevated G (60-75%) in comparison to control (40%) and after 20 days PUT enhanced G (80-95%) in comparison to control (74%) in Desi genotype. In Kabuli, PRO and Glybet stimulated the germination (87%) in comparison to control (80%).

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5.1.2. SHOOT LENGTH AND SHOOT GROWTH RATE/DAY

At room temperature (25-28°C), PUT and PRO observed to have most pronounced effect in Desi genotype and ABA in case of Kabuli genotypes. All other treatments were also observed to be effective in comparison to control plants in increasing the shoot length and shoot growth rate. At 20°C, PUT, SPM, Glybet, PRO and ABA all were observed to promitory to shoot length and shoot growth rate in Desi genotype while in Kabuli genotype, only PUT was observed to effective in comparison to control plants. At 15°C, PUT and SPM increased the shoot length and growth rate/day in Desi genotype while in Kabuli genotypes PUT, ABA, PRO and Glybet treatments showed an increase in shoot length and growth rate/day. At 10°C, all the treatments were effective in both Desi and Kabuli genotypes in comparison to control plants in enhancing shoot length and shoot growth rate/day.

5.1.3. ROOT LENGTH AND ROOT GROWTH RATE/DAY

At room temperature (25-28°C) a few concentrations of PUT, SPM, PRO and ABA were observed to be promitory to root length and root growth rate/day in Desi genotype while all the concentrations of PUT, Glybet, PRO and ABA significantly enhanced the root length and the root growth rate in Kabuli genotype. At 20°C, a few concentrations of PUT, SPM, PRO, Glybet and ABA were observed to be effective in enhancing the root length and root growth rate/day in Desi genotype in comparison to control plants while in Kabuli genotype, PUT, PRO and SPM were observed to be promitory. Similarly at 15°C, all the treatments were observed to effective in Desi and Kabuli genotypes. PRO and Glybet had a marked increase in root length and growth rate/day in Kabuli genotype. At 10°C in comparison to control, PUT, SPM and PRO slightly increased the root length and growth rate in Desi genotype while in Kabuli genotype ABA and glybet were also observed
to be promotory. At 15°C all the concentrations of PUT and Glybet significantly increased the root length and root growth rate/day in both Desi and Kabuli genotypes in comparison to control plants.

5.1.4. MOISTURE CONTENT

At 20°C, in Desi genotype, SPM, PRO and Glybet caused a significant rise in moisture content while in Kabuli genotype PUT, SPM and PRO resulted in an increase in moisture content in comparison to control plants. At 15°C, all the treatments were significantly effective in increasing the moisture content in Kabuli genotype in comparison to control plants. At 10°C, almost all the treatments were observed to be effective in enhancing the moisture content in comparison to control plants in both Desi and Kabuli genotypes. At 5°C at the end of 25th day, all the treatments were observed to be effective in increasing the moisture content in Desi genotype while in Kabuli, SPM was observed to be promotory in raising the water content in comparison to control.

5.1.5. TTC ACTIVITY (Indicator of Cellular viability)

At room temperature, SPM, PRO and Glybet effectively increased the TTC reduction in Desi genotype while all the treatments were observed to be significantly effective over control in Kabuli genotype. At 20°C, SPM, PRO, Glybet and ABA increased TTC reduction significantly in Desi genotype while in Kabuli genotype, PUT and SPM showed an increase at 15°C and 10°C. All the treatments were observed to be effective in both Desi and Kabuli genotypes over control. At 5°C, all the treatments significantly increased the TTC reduction in comparison to control in Desi genotype while in Kabuli genotype PUT and ABA were observed to be promotory over control.
5.1.6. ELECTROLYTE LEAKAGE (Indicator of stress injury to membranes)

At room temperature, PUT, SPM and PRO caused decline in the electrolyte leakage effectively in Desi genotype. A few concentrations of PUT, PRO, Glybet and ABA appeared to decrease the electrolyte leakage in comparison to control plants in Kabuli genotype. At 20°C, PUT, Glybet and ABA were effective in declining the electrolyte leakage in both Desi and Kabuli genotypes along with SPM and PRO that were effective in Kabuli genotype. At 15°C all the treatments were effective in reducing the electrolyte leakage in Desi genotype. At 10°C, PUT, SPM, PRO and Glybet in Desi genotype and all the treatments in Kabuli genotypes were observed to be effective against control in declining the electrolyte leakage. At 5°C, all the treatments were observed to be inhibitory to electrolyte leakage in comparison to control conditions.

5.1.7. TOTAL CHLOROPHYLL AND CAROTENOID CONTENT

Temperature < 15°C showed decrease in chlorophyll and carotenoids content in comparison to other temperatures in both Desi and Kabuli genotypes. At room temperature, Glybet and ABA increased the chlorophyll and carotenoid content in Desi while PUT, SPM and PRO significantly improved the chlorophyll and carotenoid content in Kabuli genotype in comparison to untreated plants. At 20°C all the treatments had pronounced effect in increasing chlorophyll and carotenoid content in Desi genotype while in Kabuli genotype Glybet and ABA observed to be effective over control. At 15°C, SPM, PRO, Glybet and ABA were promotory in Desi genotype while in Kabuli SPM observed to be effective in comparison to control. At 10°C, in Desi genotype, PRO and ABA effectively increased total chlorophyll content and carotenoid content while in Kabuli genotype all the treatments were observed to be effective in comparison to control.
5.2. COMPARATIVE EVALUATION OF DESI AND KABULI GENOTYPES UNDER CONTRASTING TEMPERATURE CONDITIONS AND EFFECTS ON REPRODUCTIVE BIOLOGY

Both the genotypes were compared under contrasting growth conditions with differences their temperature regimes. A set of plant was grown continuously in the glass house under warm conditions (25/28°C; GH-plants). The second set was grown continuously low temperature conditions of the field (FD-plants). The third set of plants comprised those ones which were grown till flowering in the glass house and then subjected to cold stress conditions of the field (GH-FD plants). The observations were recorded during vegetative and reproductive stage.

5.2.1. GROWTH, PHENOLOGY AND YIELD PARAMETERS

The plants were observed in relation to growth, phenology, flowering response, podding response and yield parameters. GH plants took significantly less days to emergence (4 days) for attaining 100% germination while FD-plants took more days (9-10) in both the genotypes. Significant reduction in height and number of primary branches was also observed in FD-grown plants. More secondary branching was common under FD-condition. Root and shoot length was observed to be more under GH-condition but the FD-plants possessed more root and shoot weight due to increased branching.

FD plants had a prolonged vegetative phase so they took more days to bud initiation, flowering, pod initiation, pod filling and pod maturity. GH-FD plants had insignificant difference with FD-plants while GH-plants had comparatively less days to attain the reproductive maturity. Kabuli genotype appeared to be more sensitive for its phenology towards cold stress than the Desi genotype. Flowering began
in Desi genotype earlier than Kabuli genotype. During the month of December and January, GH-plants began to produce flowers in Desi genotypes while Kabuli produced flowers in January even under GH conditions. However, the FD-plants and GH-FD plants flowered during February in both the genotypes. The flower retention percentage was also more in Desi genotypes than Kabuli genotype. The GH-grown plants produced significantly greater number of pods than plants growing under field conditions in Desi genotype. The total number of pods produced was greatest in case of GH-FD plants in Kabuli genotype during the month of March.

Observations revealed that GH-FD plants and FD plants had considerably greater total number of pods including 1-, 2- and 3-seeded pods than the GH grown plants in Desi genotype consequently greater pod and seed weight was recorded. Likewise the Kabuli genotype also possessed greater number of pods, pod and seed weight under FD conditions but the number of empty pods was also higher. Pod dimensions in terms of length and width was more in Kabuli genotype under FD-grown plants in comparison to Desi genotype under similar conditions.

5.2.2. FLOWER DEVELOPMENT

Low temperature stress (< 10°C) resulted in accumulation of anthocyanin pigment in the stem branches and leaves of field grown plants. This pigmentation or colouration is sustained till the low temperature prevails and disappears slowly when plants come under normal temperature (> 15°C). Plants grown under warm conditions do not show such pigmentation. In some of the cold-stressed plants floral dimorphism, reduced leaf size, shrieveled leaves, chlorosis, burning of leaf tips, shrinkage and curling of whole leaf, abnormal androecium occurs. Abnormal floral reproductive organs resulted in either floral abortion or infertile pods.
5.2.3. FLORAL BIOLOGY

The comparison of gamete performance was made on the basis of pollen functioning (pollen viability, pollen load, *in vitro* pollen germination, *in vivo* pollen germination, pollen morphology), stigma receptivity, ovule viability. The maximum pollen viability and pollen load was observed under the GH condition in comparison to GH-FD plants and FD plants in both Desi and Kabuli genotypes. GH-plants of both the genotypes were found to be more receptive in comparison to FD-plants and GH-FD plants. Similarly the *in vitro* pollen germination was observed to be more in control over stress plants. Ovule viability under control condition was observed to be maximum in comparison to GH-FD plants and FD-plants. Similarly, the *in vivo* pollen germination was also affected due to cold condition in both Desi and Kabuli genotypes.

5.2.4. ANATOMICAL CHANGES (MICROSPOROGENESIS AND MEGASPOROGENESIS)

Serial transverse sections of anthers of Desi genotype under cold stress conditions showed poor cytoplasmic content, poorly developed and irregular callose layer and uneven tapetal cells. The wall layers and vascular tissue was also observed to be affected. Likewise the cold stressed Kabuli genotypes were also observed to have damaged wall layers, severely affected pollens and appressed callose sheath. Serial longitudinal section of ovules of stressed Desi buds showed normal egg like structure but polar nuclei or secondary nucleus was not visible indicating the cold conditions affected development process. Some of the 3-day open flowers showed less endosperm nuclei and delayed division of zygote as compared to normal ovules. Likewise the serial longitudinal sections of Kabuli genotype showed well developed embryosac in closed flower antipodals. But the 1 day open flowers
showed no further development from closed flower stage indicating the sensitivity of Kabuli genotype. Under stress conditions, disorganized zygote, no clear nuclear membrane around primary endosperm nuclei was observed in Kabuli genotype.

EXPERIMENT – III

5.3. INVESTIGATING THE ROLE OF CRYOPROTECANTS (POLYAMINES – SPERMINE AND PUTRESCINE), COMPATIBLE SOLUTES (GLYCINE BETAINES AND PROLINE) AND GROWTH HORMONE (ABA) AT REPRODUCTIVE STAGE

In order to reduce the cold damage to reproductive growth, both Desi and Kabuli chickpea genotypes were treated exogenously with cryoprotectants, compatible solutes and ABA at the reproductive stage during December to March. In the month of January, GH-plants, GH-FD plants and FD-plants were compared after the exogenous application of growth regulators for various stress injury markers such as electrolyte leakage, TTC reduction, relative leaf water content, total chlorophyll content and carotenoid content at the interval of 7-10 days. Second application of growth regulators was also followed by the above-mentioned parameters.

5.3.1. EXAMINATION OF CHILLING INJURY WITH APPLICATION OF GROWTH REGULATORS

The exogenous application of these chemicals improved the performance of plants growing under all the situations though their effectiveness varied. Electrolyte leakage (EL) was greatly reduced with the application of PUT, SPM and ABA in Desi and Kabuli genotype. PRO also significantly reduced the electrolyte leakage in Kabuli after the first application of chemicals. After the second application PUT and
PRO effectively reduced the EL in Desi genotype but EL did not vary appreciably in any growth conditions in Kabuli genotype. Kabuli genotype is relatively more sensitive to EL under all the growth conditions. PRO and ABA treatments were found to be effective in increasing the relative leaf water content (RLWC) under cold stress conditions in Desi genotype along with Glybet in Kabuli genotype after the first spraying. After the second application of chemicals PUT and PRO were significantly effective in Desi genotype and PUT and ABA in Kabuli genotype enhanced the water status in plants. Chemical treatment was observed to be more effective in Kabuli genotype under field transferred plants and field plants than Desi genotype. TTC activity under cold stress condition was significantly reduced by SPM, PRO, Glybet and ABA in Desi genotype. Likewise in field transferred plants, all the treatments were observed to be effective in reducing the TTC activity while ABA reduced the activity in field plants in Kabuli genotype after first spraying. Second application of chemicals especially PUT, PRO and Glybet reduced the activity among Desi genotype plants. Kabuli plants showed the greater reduction with PUT and Glybet treatments. Under cold stress conditions TTC activity was greater in Kabuli genotype than Desi genotype. PUT, SPM and ABA were effective under GH-condition in improving chlorophyll content in Desi genotype and PUT, PRO, SPM and ABA showed elevation in chlorophyll content under cold stress condition. PUT, SPM and Glybet in Desi and SPM in Kabuli genotype significantly enhanced the carotenoid content under cold stress conditions. After the second application of treatments SPM and Glybet in Desi genotype and PUT and ABA in Kabuli genotype had higher chlorophyll content.

5.3.2. EFFECT OF CHEMICAL TREATMENTS ON FLOWERING, PODDING AND YIELD PARAMETERS

Desi and Kabuli varieties of chickpea were examined for the effect
Summary and Conclusions

of polyamines, compatible solutes and ABA on flowering and podding particularly during the months of December, January and February. During December Glybet was effective in retaining more number of flowers in Desi genotype. In January all the treatments were effective in increasing the flower retention under cold stress conditions. Likewise in February all the treatments were proved to be effective for flower retention in field transferred plants of Desi genotype. Kabuli being sensitive was not much affected in retaining flowers by the chemical treatments except with PUT, SPM and ABA. Exogenous application of cryoprotectants especially PUT, Glybet and ABA were effective in increasing the total flower production in both the genotypes during the low temperature extremes of temperatures in the month of January. Flower abortion reduced to a minor extent during January and February by the application of growth regulators in both Desi and Kabuli genotype under GH-FD plants and FD-plants.

Total number of pods also increased under control conditions in Desi genotype by all the treatments. Even during the month of January and February the pod production was comparatively more by the treatments of all growth regulators in both Desi and Kabuli genotypes under cold stress conditions. Likewise the pod retention and retention percentage was significantly more under cold stress condition due to polyamines and other growth regulators in both Desi and Kabuli genotypes. There was not much impact of treatments in reducing the pod abortion. Glybet, PRO and PUT were observed to be effective overall in improving the pod production and retention.

The yield of both the genotypes was more in terms of total number of pods produced under GH-FD condition and FD-condition in comparison to plants without treatment. The root weight under FD-condition was observed to be maximum in both the genotypes in comparison to others. Similarly, the shoot weight was also observed to be significantly more in treated GH-FD plants and FD-plants in
comparison to untreated plants. There was not much difference observed in root length and shoot length under all the growth conditions, but it was higher in growth regulator plants to some extent in comparison to untreated controls. The field transferred plants had significantly more pod and seed weight. Maximum number of 3-seeded pods were observed in field condition in Desi and Kabuli genotypes while 2-seeded pods had an increase in number due to various treatments especially PUT, SPM, ABA and Glybet in Desi and Kabuli genotype. There was not much effect on 1-seeded pods, however, it was more in watered plants. Under cold stress conditions, SPM, PRO and Glybet reduced the number of infertile pods in Desi genotype under GH-FD plants. Likewise PUT and ABA reduced the number of infertile pods under GH-FD condition in Kabuli genotype. There was not much impact of treatments on pod dimensions. There was slight improvement in the length of pod under field transferred plants by the application of PUT, Glybet, ABA and PRO in Desi genotype. PRO improved the pod length of Kabuli genotype to significant extent under GH-FD conditions. Similarly slight improvement in pod width of Desi and Kabuli genotype was observed under GH-FD and FD-condition.

EXPERIMENT – IV

5.4. PROBING THE BIOCHEMICAL BASIS OF COLD SENSITIVITY UNDER CONTRASTING CONDITIONS AT VARIOUS STAGES IN BOTH THE GENOTYPES

The plants were subjected to cold stress of the field at flowering stage and podding stage (GH-FD plants) in order to see the relative sensitivity of these stages to cold conditions in both Desi and Kabuli genotypes. Simultaneously a set of plants of both the genotypes were also kept in warm conditions of glass house (GH plants) and in the field
On the basis of yield contributing parameters, the possible reasons of cold sensitivity at metabolic levels were evaluated.

GH-grown plants showed reduced yields than the FD-grown ones. The vegetable growth was also comparatively lesser in GH conditions than under FD-conditions and GH-FD plants. The total pod weight decreased to a greater extent in GH-FD plants at podding stage. Total pod weight and total seed weight decreased to a greater magnitude in Kabuli plants transferred at podding stage over the flowering stage compared to Desi genotype. The number of single and double seeded pods were also lower in Kabuli than in Desi genotype under stressed conditions.

To evaluate the reasons of cold sensitivity, at metabolic levels, the plants were investigated at following two reproductive stages:-
- Flowering
- Pod development

Observations were recorded on electrolyte leakage, chlorophyll content, carotenoid content, relative leaf water content, anthocyanin content, TTC reduction activity, carbohydrate metabolism (Total sugars, reducing sugars, starch), α-amylase, β-amylase, invertase, sucrose, synthase, nitrogen metabolism (nitrate reductase, free amino acids, protease), oxidative metabolism (H$_2$O$_2$, malondaldehyde content, ascorbic acid, superoxide dismutase, catalase, ascorbate peroxidase) and a cryoprotectant (proline). Since flowering and pod development stage coincided with chilling conditions, the metabolic changes occurring with regard to above said parameters were expected to yield some information about their ability to deal with cold stress.

The electrolyte leakage, which is reflected as a stress injury marker to membranes was higher in cold stress conditions at both Flowering and Podding stages in both the genotypes while the relative...
leaf water content was reduced under stress environment. Increased total chlorophyll and carotenoid was observed under GH-FD plants while it was reduced under FD-conditions. Desi genotype showed more anthocyanin content under stress condition in comparison to Kabuli genotype. TTC reduction activity decreased at both Flowering and Podding stages in both the genotypes under cold stress condition. A reduction in content of starch, total sugars and reducing sugars was observed under stress environments in Kabuli as well as in Desi genotypes. The α-amylase, β-amylase and invertase activity was reduced more in Kabuli than in Desi genotypes under cold stress conditions. Higher proline contents under stress conditions in Kabuli genotype with the marginal difference from Desi genotype, higher contents of ascorbic acid, malondialdehyde content, hydrogen peroxide activity, superoxide dismutase activity, catalase activity, ascorbate peroxidase activity was greater under stress conditions in Desi and Kabuli genotypes. Marginally increased activity of sucrose synthase and nitrate reductase was observed but protease activity and free amino acid content was reduced under cold stress condition.

**CONCLUSIONS**

- The germination was inhibited markedly below 20°C in both the genotypes.
- Kabuli genotypes showed greater sensitivity to temperatures below 20°C in terms of germination and growth.
- The stress injury in terms of electrolyte leakage, TTC reduction ability was greater in the Kabuli genotype than in Desi genotype.
- The damage to germination by cold conditions could be minimized effectively by application of Polyamines, Proline, Glycine betaine and ABA. Particularly, Glycine betaine was more effective and offers a potential seed treatment in for germination in cold soils.
The plants growing under warmer conditions of the glass house showed rapid phenology but had lesser over all biomass and yield due to less vegetative growth and number of pod bearing branches.

The flower production was greater in December and January in the warm conditions of the glass house while no flowering and pod set occurred in the field grown plants corroborating the sensitivity of flowering and podding phase to cold stress.

The plants transferred to field at flowering stage showed less yield than the plants growing in the field conditions throughout their growth. This possibly occurred due to cold shock experienced in field transferred plants.

Cold conditions caused several abnormalities at morphological level such as accumulation of anthocyanins, loss of chlorophyll, distorted leaves, reduced size of the flowers.

A considerable reduction occurred in pollen viability, pollen load, *in vitro* and *in vivo* pollen germination, stigma receptivity, ovule viability. The male components showed greater damage in Kabuli than in Desi genotype while the reverse was true for female components.

The development of male and female gametophyte was inhibited severely in cold stressed plants which were noticed in terms of abnormal pollen development, abnormal egg and embryo, delay in formation of proembryo and unhealthy zygote. No specific differences could be marked out for these traits between Desi and Kabuli genotypes suggesting similar extent of damage in both the genotypes.

Exogenous application of Cryoprotectants like polyamines, Glycine betaine, Praline and ABA mitigated the adverse effects of
cold stress and improved the retention of flowers and pods, increased pod set and enhanced yield contributing parameters.

- Kabuli genotype responded more effectively to the treatments.
- The plants cold stressed at flowering and podding stage showed marked reduction in growth and yield. The former stage showed greater stress sensitivity than the latter.
- The metabolic dysfunction occurred in terms of inhibited activities of amylases, invertases, sucrose synthase, nitrate reductase, protease with greater inhibition in Kabuli genotype.
- The oxidative stress was greater in Kabuli than in Desi genotype with latter genotype having relatively stronger expression of antioxidant like ascorbic acid.