INFLUENCE OF PRECONDITIONING ON WATER RELATIONS AND PIGMENT SYSTEM DURING REPRODUCTIVE STAGE IN WINTER SOWN CHICKPEA

Dissertation Submitted to the Central University of Punjab

For the award of
Master of Science
In
Life Sciences
BY
Poonam Jarial

Supervisor
Dr. Sanjeev K. Thakur

Centre for Biosciences
School of Basic and Applied Sciences
Central University of Punjab, Bathinda
November, 2016

CERTIFICATE

I declare that the dissertation entitled "INFLUENCE OF PRECONDITIONING ON WATER RELATIONS AND PIGMENT SYSTEM DURING REPRODUCTIVE STAGE IN WINTER SOWN CHICKPEA" has been prepared by me under the guidance of Dr. Sanjeev K. Thakur, Associate Professor, Centre for Plant Sciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this dissertation/thesis has formed the basis for the award of any degree or fellowship previously.

Poonam Jarial
Reg.No. : CUPB/M.Sc.-BIO/SBAS/BIO/2014-15/09
Centre for Biosciences
School of Basic and Applied Sciences
Central University of Punjab,
Bathinda-151001.
Date:

CERTIFICATE

I certify that POONAM has prepared her dissertation entitled “INFLUENCE OF PRECONDITIONING ON WATER RELATIONS AND PIGMENT SYSTEM DURING REPRODUCTIVE STAGE IN WINTER SOWN CHICKPEA ", for the award of M.Sc. degree of the Central University of Punjab, under my guidance. She has carried out this work at the Centre for Biosciences, School of Basic and Applied Sciences, Central University of Punjab.

Dr. Sanjeev K. Thakur
Centre for Plant Sciences
School of Basic and Applied Sciences,
Central University of Punjab,
Bathinda-151001.
Date:

ABSTRACT

INFLUENCE OF PRECONDITIONING ON WATER RELATIONS AND PIGMENT SYSTEM DURING REPRODUCTIVE STAGE IN WINTER SOWN CHICKPEA

Name of student:	Poonam Jarial
Registration number:	CUPB/M.Sc.-BIO/SBAS/BIO/2014-15/09
Degree for which submitted:	Master of Science
Name of supervisor:	Dr. Sanjeev K. Thakur
Name of Centre:	Centre for Biosciences
Name of School:	School of Basic & Applied sciences

Key words: Preconditioned, RLWC, ELI, chilling stress.

Chickpea is self –pollinated legume crop which was believed firstly originated in South-Eastern Turkey and parts of Syria. Chickpea is the second most important pulse crop in the world. In Northern India, chickpea is grown in winter season. Preconditioning the plant with mild drought stress may trigger the various signaling pathways which will prepare the plants to conquer lethal cold stress. In this study, PBG1, PBG5 and GPF2 variety are more sensitive to the chilling stress, which were preconditioned with mild drought stress and then exposed to lethal cold stress. To see the effect of preconditioning various cell responses were monitored by measuring ELI, Relative Water Content and chlorophyll content at different stages of chickpea. All genotypes generated a variable response. Overall, relative water content and chlorophyll content were significantly higher in preconditioned PBG1, GPF2 and PBG5, which are sensitive to chilling stress. The study showed increase tolerance capacity in preconditioned plant towards chilling temperature and improve tolerance against chickpea.

Poonam Jarial   Dr. Sanjeev K. Thakur

ACKNOWLEDGEMENTS

The first and foremost person to acknowledge in this study is my supervisor Dr. Sanjeev K. Thakur, Associate Professor and Coordinator of Centre, Centre for Plant Sciences, who guided me in the finest way during the course of this piece of research. His immensely knowledgeable and experienced supervision helped me a lot in pursuing this work.

I would like to express my sense of gratitude to Prof. R. G. Saini, Former Coordinator of Centre, and Centre for Biosciences and Prof. A. K. Dhawan, Coordinator of Centre, Centre for Biosciences, Prof. P. Ramarao, Dean, Academic Affairs, for their valuable words to me. I am absolutely thankful to Prof. R. K. Kohli, Vice Chancellor, Central University of Punjab, Bathinda for his fabulous leadership in growth and development of Research infrastructure in the University.

I am really indebted to my lab mates Rashmi Saini, Arindam Adhikary, Renu Yadav, Rashpal Kumar, Sumandeep Juneja, Ranjana, Ram Kumar and my entire lab mate. I am especially thankful to my friends Damini, Rangman, Soumyadeep, Ravneet, Nilanjan and Mansi garg for help during writing of this dissertation. I would like to thank my parents and my family members who worked hard to make me capable to complete my work due to their support and endless affection.

Poonam

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Sr.No.	Full Form	Abbreviation
1	Abscisic acid	ABA
2	Analysis of variance	ANOVA
3	Carbon Dioxide	CO_2
4	Celsius	°C
5	Centimeter	cm
6	Chilling Stress	CS
7	Chlorophyll	Chl
8	Days After Sowing	DAS
9	Electrolyte Leakage	EL
10	Electrolyte Leakage Index	ELI
11	Electron Transport Chain	ETC
12	Feet	f
13	Figure	Fig
14	Food and Agriculture Organization of the United Nation	FAOSTAT
15	Fresh Mass	FM
16	Fresh Weight	FW
17	Gram	g
18	Hactare	ha
19	Hours	H
20	Kilo Dalton	kDa
21	Kilogram	Kg
22	Maximum	Max^m
23	Mega Base pair	Mbp
24	Microgram	µg
25	Microliter	µL
26	Milliliter	mL
27	Millimeter	mm
28	Minimum	Min^m
29	Nanometer	nm
30	Non-Preconditions	NP

31	Preconditions	P
32	Punjab Agricultural University	PAU
33	Random Block Design	RBD
34	Reactive Oxygen Species	ROS
35	Tones	t
36	Transcription Factors	TFs
37	Turgid Weight	TW

Chapter 1

Introduction

1.1 Origin of problem

Chickpea is self-pollinated legume crop belonging to family Fabaceae. It was firstly originated in South-Eastern Turkey and the parts of Syria. Chickpea is the second most essential pulse crop in the world and is grown in at least in 33 countries (including South Asia, West Asia, North Africa, East Africa, North and South America) and covers 15% (10.2 million hectares) of area worldwide. In the cereal dominated diet area of South East and West Asia and North Africa, it is widely used and is a rich source of high-quality proteins (Singh, 1997).

In nature, plants are exposed to various environmental conditions that may pose several biotic (including animals, bacteria, viruses or fungi) and abiotic stress to the plants (Anjum et al., 2011). Stress can be defined as any change in the growing conditions of a plant within its natural habitat that causes disruption of metabolic homeostasis in a plant. In order to avoid such stressful conditions, plants adjust themselves with the changing environment which is referred to acclimatization. Cold and drought stress when combined with high light conditions, it results in the production of Reactive Oxygen Species (ROS) by the photosynthetic apparatus. It mainly limits the availability of CO_2 for dark reactions and leaving oxygen which is the main reductive product of photosynthesis (Mittler, 2006).

Various biotic and abiotic stresses adversely affect the plant growth and productivity. Plants are constantly exposed to stress conditions like chilling and drought stress. These stress factors are hazardous for plant growth as plant could not grow up to its full genetic potential and crop productivity. Abiotic stress is the major factor for the crop failure (Mahajan and Tuteja 2005). Out of all, chilling stress poses a severe threat to the crop production (Anjum et al., 2011). Mainly in Northern India, chickpea is grown in the winter season and requires an optimum temperature of 23°C to 30°C (Beck et al., 2007). Drought and cold stress are the major factors that affect the reproductive phase of chickpea. Most of the plants possess various mechanisms in response to cold and drought stress. In both stresses, it causes ABA (abscisic acid) production which plays a major role in defense mechanism (Liu et al., 1998). In the reproductive stage, plant experience abnormally the cold and drought stress at low temperatures (at -1.5°C to 15°C) which harshly affects the germination of seeds, photosynthesis, respiration, the stability of membranes and seeds quality (Leport et al., 2006; Croser et al., 2003).

In such adverse conditions, the establishment of seedlings is a critical process for plant growth (Bohnert et al., 1995). Cold stress leads to the dehydration of the cells which reduces the water conduction by roots and ultimately leads to osmotic stress. It may result in less water uptake by the roots which hinder the closure of stomata (Aroca et al., 2003 ; Chinnusamy et al., 2007). Relative water content mainly helps to assess the capability of a cell to tolerate the scarcity of water in its liquid form and has thus been tracked often as a parameter to study the ability of the cell to tolerate cold stress and it was also reported that the level of water stress and the extent of the consequent injuries under cold temperature conditions which depends on the sensitivity of genotype under chilled conditions (Janowiak and Markowski, 2008). Chickpea is cultivated in the residual soil moisture which often subjected to the drought stress. High seed emergence and seed establishment contribute directly the crop yield. Thus, preconditioning the plants with mild drought stress might trigger various signaling pathways that will help the plants to overcome from the lethal cold stress. This could be achieved by monitoring the various cell responses at different phases and making the strategies which help to improve cold stress resistance in plants.

1.2 Hypothesis

Mild drought (non-lethal) stress exposure during vegetative stage may strengthen the chickpea plants to tolerate the incoming chilling stress during the reproductive stage.

Chapter 2

Review of Literature

2.1. Origin and importance of chickpea

Chickpea (Cicer arietinum L.) is a self-pollinating diploid species (2n=2x=16) having 740 Mbp genome size (Garg et al., 2011). It belongs to family Fabaceae and subfamily Papilionaceae. It was originated in south eastern Turkey and adjoining part of the Syria from its wild progenitor C. reticulatum (Ladizinsky and Adler, 1976). Chickpea is a rich source of proteins and carbohydrates. Starch is the main source of carbohydrate followed by dietary fibers, oligosaccharides and simpler sugars including glucose and sucrose. Chickpea is also rich source of vitamins such as riboflavin, thiamin and vitamin A (precursor of β-carotene) (Jukanti, 2012). Chickpea seeds contain 23% of digestible protein, carbohydrate content 64%, starch 47%, fats primarily linoleic acid and oleic acid 5%, soluble sugar 6% and 3% ash. Chickpea also contains high mineral components i.e., Phosphorus, Calcium, Magnesium, Iron, and Zinc (Singh et al., 1991). It also provides nutritional values; their seeds contain crude protein (12.6 – 30.5%) (Singh,1985). Chickpea shows antioxidant and anti-mutagenic, apoptosis-related and anti-proliferative effects which are incorporated with phenolic compounds in the seeds of chickpea (Hirdyani, 2014).

2.2. Morphology

It is an annual cool season crop that ranges in height from1-3 feets. It has a long tap root system and also fixes a higher amount of atmospheric nitrogen. Its growth habit is indeterminate, i.e. it can continue vegetative growth even after initiation of flowering. Chickpea has two main varieties: Desi (local), which is characterized by small, angular and variously colored seeds. It bears 3-4 seeds/pod. It comprises of irregularly shaped seed coat. The plants are of small size and flowers are mostly purple in color. The other variety is Kabuli (it was considered that it had been originated in Afghani capital, Kabul) is characterized by large sized seeds (>25g/100 seeds), ram-head shaped, beige-colored seeds. It bears 1 or 2 seeds or pods and large size leaflets (10-20 mm) and lacks anthocyanin (Auckland and Maesen 1980).

2.3. Area and production

Chickpea is the world's 3rd ranking food legume, and is grown on about 10 million ha. In India, chickpea is grown as in rainy fed, post rainy season crop on 6-7 million ha across the country. In Punjab, it is an important winter season crop which was cultivated in about 13,000 ha with 10,000 tones (t) annual production

(Singh et al., 1990). The production of chickpea has been expanded over past few years from 6.5 million t to 9.5 million t. Such a huge increase is the result of increased grain yields. Asian farmers contribute about 81% of global chickpea production including India as the principle chickpea producing country (84% of its share). In India, the area for production has been increased from 9.1 million to 9.5 million ha. Several drought conditions had led to declining the chickpea production area. Over the past few decades, chickpea production has been shifted from cooler climatic conditions (Northern India) to warmer conditions (Southern India). In the states of Andhra Pradesh, Karnataka and Maharashtra, there was a significant increase in the area, production and productivity of chickpea due to the adoption of Fusarium Wilt resistant, short duration varieties. India is the largest producing country which producing 67% of world chickpea and its annual global production was around 13.1 million t from 13.5 Mha (FAOSTAT, 2014). Contrastingly, the production had been increased to 88,00,000 t per annum in 2012-2013 ("All India Coordinated Research Project on Chickpea," 2016). India achieved the top position in chickpea production worldwide with a production quantity of 9,880,00.00 t with productivity 9199.00 hg/ha in the year 2014 (FAOSTAT, 2015).

2.4. Major factors affecting growth and yield of plant

Plant experience many stresses like as salinity stress, drought stress, cold stress and high temperature stress (Hossain et al., 2013). Plant does not always have the favorable condition for their survival therefore, plants have to adapt the new condition or die because they have no ability to escape from external stresses. Cold stress during the reproductive phase causes many reproductive functional abnormalities within the reproductive organs of plants which may lead to the failure of fertilization or premature abortion of seed or flower. Low-temperature stress during the development of reproductive organs induces the flower abscission, pollen tube distortion, ovule distortion, pollen sterility and reduces the fruit production which leads to the lowering of yield and production of the crop. In chickpea (Cicer arietinum L.), during the reproductive phase, the temperature goes down below 15°C, and it causes the flower abscission, and pod set reduced in Northern India and Australia. Chilling sensitivity is greater at its reproductive phase of chickpea plant primarily greater at its reproductive stage, which leads to abortion of flowers, pods and poor pot set and limits the yield of seed (Shrinivasan et al., 1998; Clarke and Siddiqui , 2004 and Nayyar et al., 2005). Cold stress has important key value component of economic yield during the reproductive phase. In the entire humanity, it is the principle source of food (Thakur et al., 2009).

2.5. Chilling stress

Chilling stress is limited to some plants which were grown in the tropical or subtropical region of the world. In chilling stress condition, the temperature for such plants ranges from 15 to 20°C in chilling -sensitive plants. It can vary with the variety and growth condition of plants. Chickpea is sensitive to the chilling temperature (<10°C), mainly in the reproductive phase which further leads to the abortion of flowers. The Low temperature of field environment restricted the growth of plants and delayed the phonological stages in comparison to the control (Kumar et al., 2010). Chilling stress (CS) mainly cause a large amount of responses in plants which includes physio-biochemical responses. (Heidarvand and Amiri, 2010). Chilling stress results from cool the temperature enough to make injury in the plant tissue by without making the ice crystals in plant tissue. Whereas, freezing stress make the ice crystals within the plant tissue. Both freezing and chilling causes low temperature stress. Various phenotypic symptoms could be observed under chilling stress condition which includes the reduction in the leaf expansion, wilting, discoloration, chlorosis (yellowing of leaves) and it also leads to necrosis (death of tissue) (Ahmad et al., 2011). Exposure of chilling temperature is the major environmental signal in the process of flowering through the vernalization and to break the seed dormancy (Boyer, 1982).

2.6. Membrane damage

Membrane damage is one of the major factors which cause injury in the plant cell and it is also responsible for other dysfunction in the plant. Membrane damage mainly happens due to the lipid per-oxidation and cause the production of ROS. The cell membrane de-stability is caused by inappropriate functioning of protein compounds which hinder the biochemical responses such as, denaturation of protein and the solutes leakage and energy dependent metabolisms (Kim et al., 2013). During the low temperature stress the fatty acids become unsaturated and alter the lipid-protein ratio of the membrane which ultimately it affects the fluidity and structure of cell membrane. In cold stress, the fluidity of membrane changes due to the rigidity of membrane which causes electrolyte leakage (Shahandashti et al., 2013; Hossain et al., 2013). In the plants, stress mainly damages the cell membrane which inhibits the Intra and extracellular nutrients and water movement. Chilling stress induces the cellular dehydration and reduces the water uptake. Dehydration during the cold also occurs due to the hindrance to closing of stomata which may also lead to freezing of cell constituents, water and solutes. In plant tissues, different strains are produced by cold and drought stress. These two forms of abiotic stress affect the water relations on the cellular level as well as whole plant level which causing the specific as well as unspecific reactions and causing the damage and adaptation reactions (Beck et al., 2007).

2.7. Physiological and Biochemical effects of chilling stress

Each plant has its own temperature requirements, which is required for its proper growth and development. A set of optimum temperature required for one plant may be stressful for another plant. In some cases, chilling stress help in the tolerance of freezing temperature (cold acclimation). Chilling stress was defined as the low temperature within the range of (0-20°C) which has been recognized as a stress for many years and having a major impact on plant growth (Boyer, 1982; Heide and Prestrud, 2005). During the chilling stress there are modifications on the cellular components in the plant which includes swelling of mitochondria, plastids and thylakoids lamellae, thylakoid vesiculation and lipid droplets accumulation. It also disrupt whole plastid, condensed the nuclear chromatin, disrupt the many system in the plants including electron transport, carbon cycle metabolism which lead to senescence and then ultimately plant death (Hasanuzzaman et al., 2013). There are various biochemical responses could be observe under chilling stress condition which affects the water relation, membrane damage and chlorophyll content in plants.

2.7.1. Impact of chilling stress on water status

Percentage relative water content, leaf water potential, stomatal resistance, leaf temperature, transpiration rate and temperature of canopy they are the major characteristics that influence the plant water relations. The Relative Water Content (RLWC) in chickpea leaves was higher initially during leaf development and decreased when the leaf matured (Farooq et al., 2008). Cell membranes are the one of the first target of various plant stresses and it is generally believed that this is the major component of drought stress for the maintenance of their integrity and stability under water stress conditions in plants. The RWC related with the uptake of water by roots as well as loss of water by transpiration. Decrease in RWC under cold and drought stress has been noted in many varieties of plants as reported by Nayyar and Gupta (2006) that when under cold and drought condition leaves exhibit the large amount of decrease in the RWC. Exposure of plant under abiotic stress decreased the RWC and transpiration rate (Siddique et al., 2001).

2.7.2. Impact of chilling stress on membrane permeability

Electrolyte leakage essentially used to assess the permeability of membrane (Javanmardi et al., 2013). Electrolytes are mainly present in the membrane of plant cell, and these are sensitive to the environment such as chilling and freezing conditions (Wilson, 2004). The degree of cell membrane injury is induced by the water stress which may be easily measured by the electrolyte leakage from the cells. Such type of technique has also been applied to quantify damages in the cell membranes under various abiotic stress conditions. Chilling stress mainly increased the level of electrolyte leakage in stressed plants (Farooq et al., 2008). ELI measurements may also drives to correlated with various physiological and biochemical parameter conditioning the plant responses to environmental conditioning (Bajji et al., 2001).

2.7.3. Impact of chilling stress on photosynthesis

A severe effect of abiotic stresses is reduction in the leaf pigmentation, which arises by the reduction in leaf expansion, impaired photosynthetic machinery, premature leaf senescence which is associated with the reduction in food production. Cold stress damaged the CO_2 uptake; due to stress- induce stomata closure, which limits the CO_2 uptake through leaves. When CO_2 uptake is restricted it could possibly leads to increased susceptibility to photo-damage. Stress produced changes in the photosynthetic pigments and their components, also damage the photosynthetic apparatus, which cause reduction in the crop yield. Various experiments have described that stomatal responses are often more closely linked with the leaf water content (Anjum et al., 2011). Environment conditions mainly enhance the rate of transpiration which also enhance the pH of leaf sap, and diminished the stomata concentration (Tylor et al., 1982). Photosynthetic pigments are imperative for plant mostly for harvesting of light and both chlorophyll a and chlorophyll b are mainly prone to soil dehydration (Farooq et al., 2009).

2.8. Defense mechanism

Plants especially respond and adapt for survive under the stress conditions which inducing various morphological responses and physiological and biochemical responses to the cold and drought stress. Plants involve changes at whole plant level, tissue, physiological level and molecular level. Various type of morphological mechanisms respond against the abiotic stresses is described below.

The primary function of cold acclimation is to stabilize the membrane against the chilling and freezing injury in the plant. Acclimation results in an increase in the proportion of unsaturated fatty acids and therefore decreases the level of transition temperature. Cold acclimation result physiological and biochemical responses restricting the cell membrane by causing changes in the lipid composition and it also induces the other non- enzymatic proteins which alter the freezing point of water and due to the addition of solutes it helps in decreasing the freezing point of water and prevent the formation of ice. Low -temperature induce the various alteration in the cellular components which including the extent of unsaturated fatty acids and also cause changes in the carbohydrate and protein composition and also activate the ion channels. Accumulation of sucrose and other sugars also help in the cold acclimation and helps in the stabilizing the cell membrane. Low temperature stress inhibit the growth of plant by disintegration of membrane, solute leakage which induces the cold- inducible genes such as, dehydrins, lipid transferase proteins and late-embryogenesis-abundant protein and other transcription factors which helps in cold acclimation by stabilizes the integrity of cellular membrane with the help of osmolytes and also enhances the antioxidant mechanism and increase the sugar level which protect the intracellular protein by inducing the gene which encodes the molecular chaperones (Mahajan et. al., 2005; Pirzadah et al., 2014) (Fig.2.8).

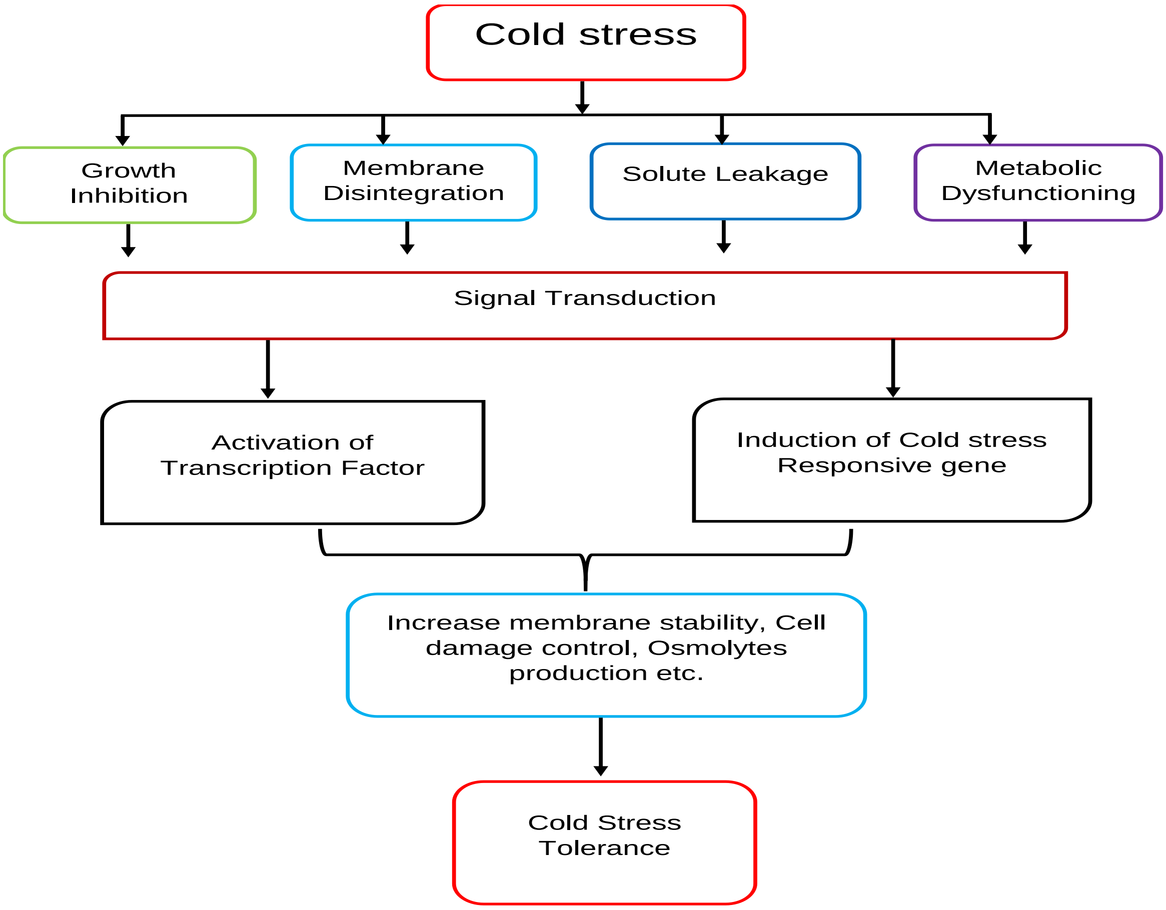

Figure 2.8: Defense mechanism under cold stress condition.

2.9 Preconditioning

Preconditioning is defined as the process in which the one abiotic stress is used to make the plant tolerant to another abiotic stress which would be lethal for plants. Preconditioning is well accepted way which avoids the abiotic stress and activated similar kind of mechanism with different abiotic stress. Cold and drought stress had the sever environment conditions which affect the water relation of plant at cellular level and whole plant level which leads to cause specific and unspecific responses in plants (Bakht et al., 2006; Beck et al., 2007). Preconditioning is the most widely accepted way to avoids the abiotic stress and activate the similar kind of mechanism under different stresses (Ladjal et al., 2000). Preconditioning with low temperature mainly cause, increase in the electrolyte leakage content and the opposite was observed in the relative water content and chlorophyll content (Javanmardi et al., 2013).

2.10 Objective

- Influence of pre-conditioning on water relation during reproductive stage of winter sown chickpea.

Chapter 3

Materials and Methods

3.1 Collection of sample

In this study, five varieties of the chickpea (Cicer arietinum L.) were employed. Seeds of five cultivars namely PDG3, PDG4, PBG1, PBG5 and GPF2, were procured from Plant Breeding and Genetics Department, Punjab Agriculture University, Ludhiana, Punjab (PAU).

3.2. Experimental design, plant material, and growth conditions

Experiment was carried out in fields at Central University of Punjab, Bathinda (30.17°N 76.45°E). Healthy seeds were sown on 24 Oct, 2015. Entire area of work was divided into two fields, one for growing plants normally (16 x 26 f^2) and other for preconditioning (24 x 25 f^2). Each field was divided into three blocks equally and each block was divided into five plots of equal dimensions. For experiment, Randomized Block Design (RBD) was followed. Five varieties of chickpea i.e., PDG3, PDG4, PBG1, PBG5 and GPF2 were sown in their respective labeled plots. Seeds of chickpea were sown in two rows at interval of 10 cm in each plot. 60 days after sowing (DAS), plant reached to their maximum vegetative potential and they were designated as preconditioned and non-preconditioned plants. In non-preconditioned irrigation was done once in a week and on other hand irrigation was stopped completely. The plant preconditioned with mild drought showed tolerance to forthcoming low-temperature stress. Temperature ranges between (>10 to <20°C), precipitation was also noted at particular weeks of months from the day of sowing (October 24, 2015) to last day of experiment (March 16, 2016).

When the experiment was completed the data was generated and subjected to stastical analysis for ANOVA and multiple comparisons by using sigma plot and Tukey's test.

3.3. Biophysical parameters

3.3.1. Relative Leaf Water Content (%RLWC)

Fresh leaves were collected, and the fresh weight (FW) was measured. To obtain the turgid weight (TW), leaves were floated in distilled water and incubated at room temperature for 2 hours. After incubation leaf samples were gently wiped with tissue paper to remove excess water and weight. The leaf samples were then placed in a pre- heated oven at 80˚C, for 24 hours. The dried samples were weighed and %RLWC was calculated, using the formula (Gonzalez and Gonzalez-Vilar, 2001).

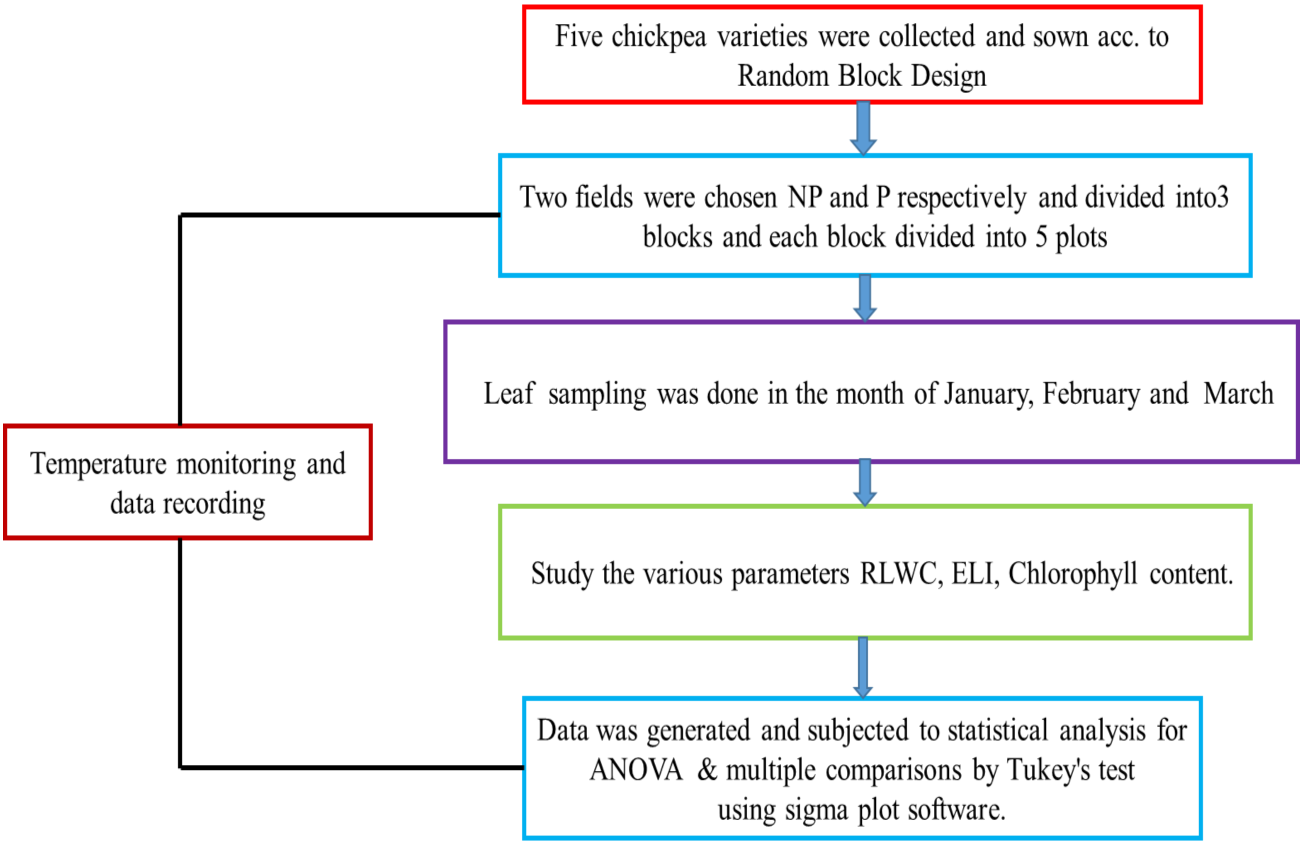

Figure 3.2.1: Plan of work

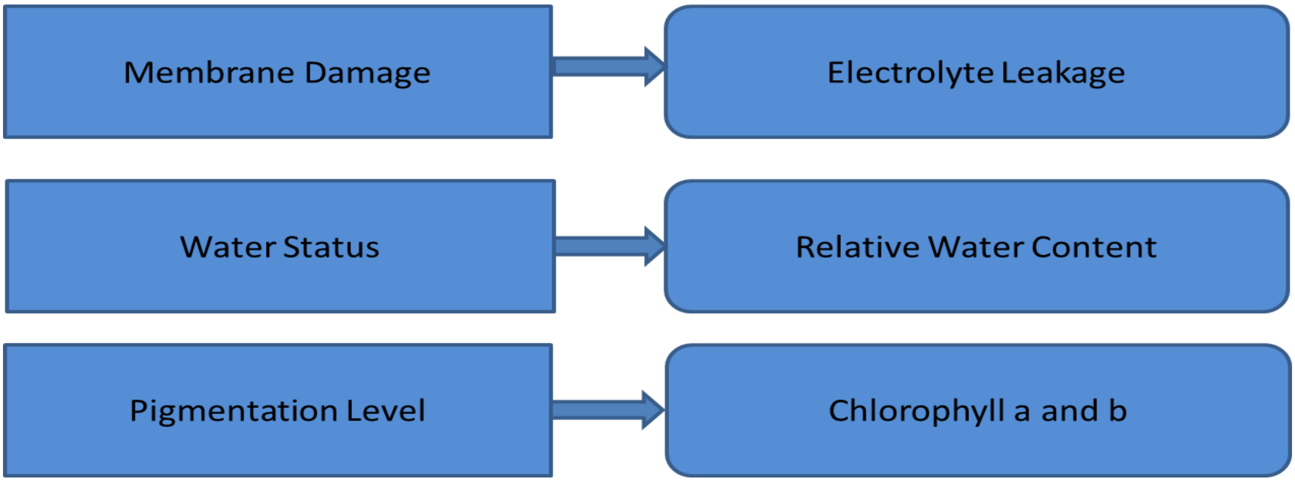

Figure 3.2.2: Setup of the experiment.

%RLWC was calculated, by using this formula.

$$\% \text{ RLWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

3.3.2. Electrolyte Leakage Index (%ELI)

To check the membrane injury, fourth leaf from top of the plant was sampled for electrolyte leakage. 50 mg of fresh mass weighed, washed and kept in 10 ml of distilled water for 24 hours and conductivity (L1) was recorded. After this, the sample was completely damaged by boiling it for 20 minutes or autoclaving it at 120˚C for 20 minutes. Final conductivity (L2) was recorded with conductivity meter and electrolyte leakage was calculated as (Lutts et al., 1996).

$$\%ELI = (L1/L2)*100$$

3.3.3. Chlorophyll estimation

The 50 mg of fresh leaves were cut into small pieces and put in the test tubes containing 4 ml of 80% acetone in 4˚C for 48 hours, and the absorbance was measured at 663 and 646 nm. The chlorophyll content (chlorophyll a, b) was calculated using following standard formula (Kannahi and Kowsalya,2013),

mg of chlorophyll ‘a’/ml of = 0.0127 (A663) – 0.00269 (A646) (Fresh leaves)

mg of chlorophyll ‘b’/ml of = 0.0229 (A646) – 0.00468 (A663) (Fresh leaves)

Chlorophyll content was calculated as mg/g FW.

3.4. Statistical analysis

A randomized design with three replicates was followed. Experimental data of physiological sections of this research were subjected to a Two Way ANOVA (analysis of variance) using Sigma Plot 11.0 software. All pairwise comparison of the mean responses to the different treatment groups were performed by Tukey Test at $p<0.05$.

Chapter 4

Results

4.1 Temperature profile and chickpea reproductive growth during the season

In the field, five genotypes of chickpea were grown to check the response of preconditioning with drought stress during reproductive growth. During the entire experimental period from (January to April), the field temperature was closely monitored and its coincidence was made with the physiology of the growing chickpea. It was observed that from the 1st week of January to the 2nd week of February, when most of the chickpea plants genotypes started flowering, the average minimum temperature lowered to <10˚C, which is detrimental for reproductive growth of chickpea's. Thereafter, the temperature started rising every week but the maximum temperature remain <15˚C during flowering and pod maturation stage (Fig. 4.1).

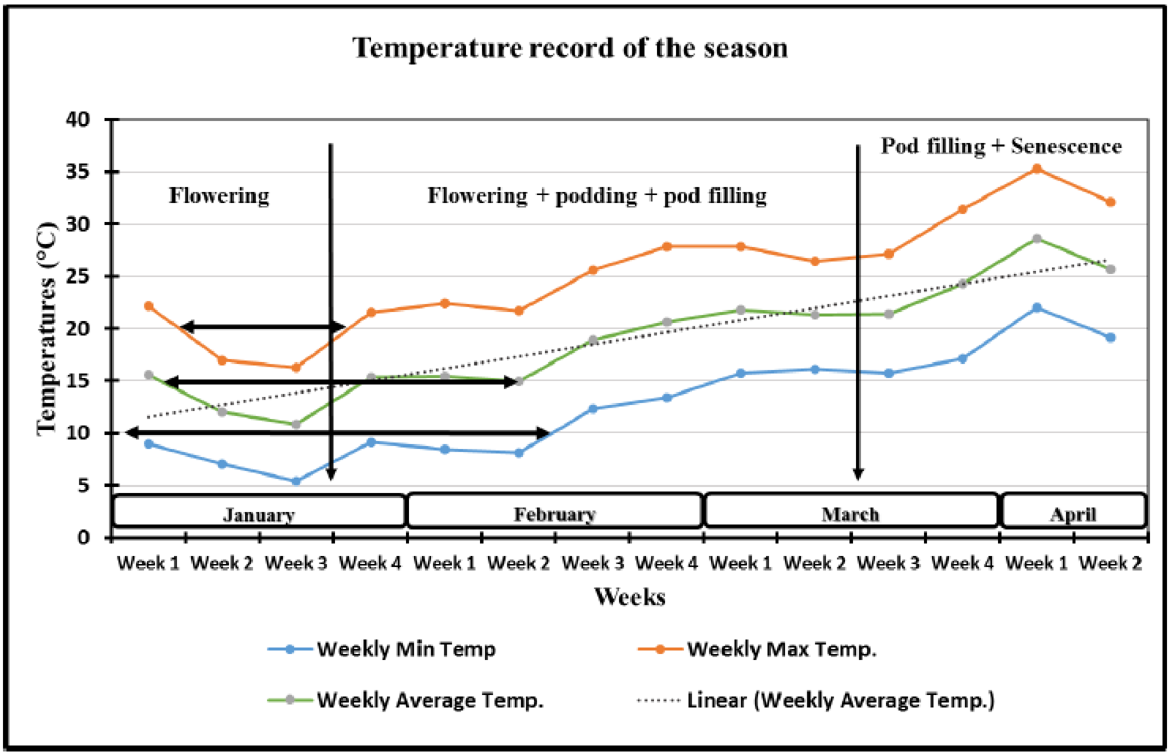

Fig 4.1 Weekly Temperature record (minimum average, maximum average and mean temperature) of the season starting from 01/01/2016 to 14/04/2016 ("AccuWeather.com for Bathinda, India," 2016) and reproductive physiology of chickpea.

The present study was conducted with aim to achieve the objective i.e. influence of preconditioning on water relation and chlorophyll level during reproductive stage of winter sown chickpea. Different parameters including RLWC, ELI, and chl. a and b were related with temperature condition.

Table 4.1 Temperature index and corresponding reproductive stages of plant.

Date	Plant Stage	Temperature (Max)	Temperature (Min)
23-1-2016	Stage-1 (Flowering)	14 °C	6 °C
5-2-2016	Satge-2 (Early Podding)	24 °C	8 °C
3-3-2016	Stage-3 (Pod filling)	28 °C	17 °C
16-3-2016	Stage-4 (Terminal reproductive stage)	28 °C	14 °C

4.2. Water status

4.2.1. Relative water content (%RLWC)

Relative water content was analyzed by two ways ANOVA with all pair wise multiple comparisons using Tukey's test. The analysis showed that there was statistically significant interaction between Treatment, treatment x Variety ($p< 0.05$). There was significant difference observed between preconditioned (P) and non- preconditioned (NP) plant which was denoted with (*).

At stage1(Flowering): At flowering stage, non-preconditioned (NP), no significantly change in RLWC level in PDG4, PDG4, PBG1, PBG5 and GPF2 varieties was observed (Table 4.2.1). In preconditioned plants also, there was no significant change observed in all varieties. There was no significant difference observed between preconditioned and non-preconditioned plant at stage 1 (Fig 4.2.1).

At stage 2 (Podding): There was no significant difference among all the varieties in both P and NP plants. While comparing the treatments (P and NP), again no significant difference was observed between P and NP.

At stage 3 (pod filling): At this stage, RLWC level was same in all the varieties. Same trend was observed while analyzing preconditioned and non-preconditioned plants.

At stage 4 (Terminal reproductive stage): At this stage, huge variations were observed in preconditioned plants when compared with non-preconditioned plants. In preconditioned plants, the RLWC was significantly higher in PDG4 when compared with other varieties. In non-preconditioned plants, RLWC level did not show any significant change. In preconditioned GPF2, RLWC was significantly higher than non-preconditioned GPF2 variety.

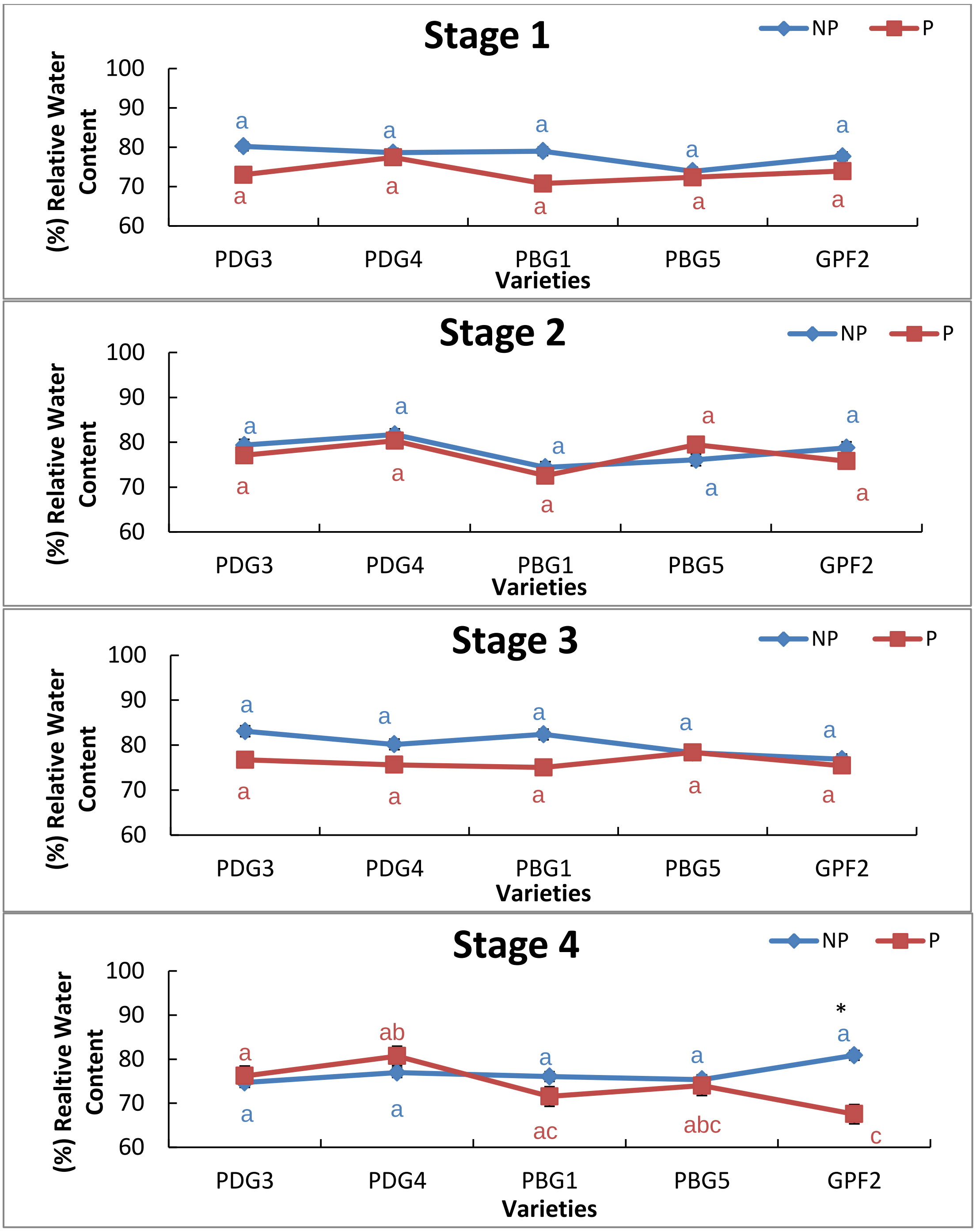

Fig. 4.2.1. Relative Water Content (%) in five chickpea varieties. Graph showing comparison between the non-preconditioned and preconditioned plants at different stages. Alphabet a-c represents level of significance among different varieties. * represents level of significance between P and NP at $P < 0.05$ based on Tukey's test. NP: non-preconditioning, P: Preconditioning.

Table 4.2.1. Relative Water content (%), in preconditioned, non-preconditioned plants of five chickpea varieties.

	Stage 1		**Stage 2**		**Stage 3**		**Stage 4**	
	NP	**P**	**NP**	**P**	**NP**	**P**	**NP**	**P**
PDG3	80.239±2.068 a	72.986±2.194 a	79.362±2.068 a	77.088±2.068 a	83.102±2.068 a	76.734±2.068 a	74.694±2.194 a	76.202±2.068 a
PDG4	78.610±2.068 a	77.436±2.068 a	81.716±2.194 a	80.335±2.345 a	80.177±2.068 a	75.623±2.068 a	76.953±2.068 a	80.711±2.068 ab
PBG1	78.996±2.068 a	70.555±2.194 a	74.399±2.068 a	72.542±2.068 a	82.391±2.068 a	75.040±2.068 a	76.052±2.068 a	71.554±2.194 ac
PBG5	73.893±2.068 a	72.357±2.068 a	76.068±2.068 a	79.463±2.194 a	78.302±2.068 a	78.357±2.068 a	75.386±2.068 a	73.996±2.068 abc
GPF2	77.68±2.068 a	73.921±2.194 a	78.767±2.068 a	75.786±2.068 a	76.851±2.068 a	75.441±2.068 a	80.8776±2.068a *	67.521±2.068c *

4.3. Membrane damage

4.3.1. Electrolyte Leakage Index (%ELI)

Percentage electrolyte leakage content was measured to check the extent of membrane damage due to cold. During the cold stress, membrane transition from liquid crystalline phase to solid gel phase which improving the membrane permeability causing solute leakage and disrupted ion balance. To measure the extent of solute leakage, %ELI was determined. Data collected in the form of %ELI was analysed by two-way ANOVA with all pair wise multiple comparisons using Tukey's test. The analysis showed statistically significant interactions between the treatments and variety X treatment ($p<0.05$). Whereas, there was no change noticed in the various varieties.

At stage 1 (Flowering): At flowering stage, in non-preconditioned (NP) plants, there was no change in ELI level in PDG3, PDG4, PBG1, PBG5 and GPF2 variety (Table 4.3.1). In preconditioned plants also, there was no significant change observed in all varieties. Between the treatments, no significant difference was observed between preconditioned and non-preconditioned plant at stage 1 (Fig 4.3.1).

At stage 2 (Podding): Non preconditioned PBG 5 showed significantly lower ELI as compared to rest of the varieties**.** There was no significant difference among all the varieties in both Preconditioned plants. But, while comparing the treatments (P and NP), there was significant difference observed in PBG1.

At stage 3 (pod filling): At this stage, ELI level showed similar trend in all the varieties especially in non-preconditioned conditions. In preconditioned plants, PDG4 showed significantly low ELI level as compared to PDG3 and GPF2 variety. No significant change was observed for ELI value between P and NP.

At stage 4 (Terminal reproductive stage): At this stage, huge variations were observed in non-preconditioned plants. ELI was significantly high in PDF4, PBG 1, PBG 5 and GPF 2. Whereas, In preconditioned plants, no significant difference was noticed. In preconditioned PDG3, ELI was significantly higher than non-preconditioned PDG3.

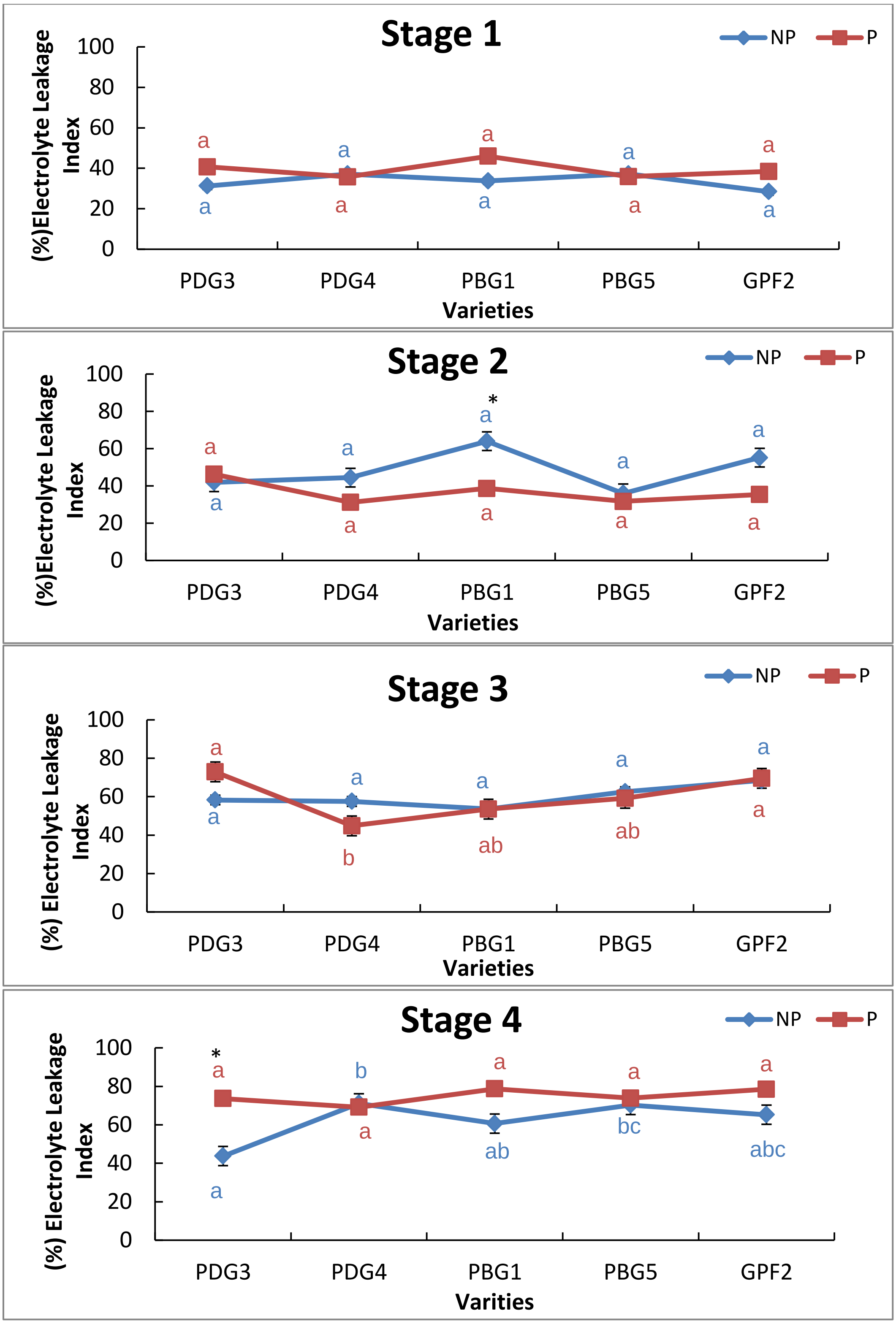

Fig.4.3.1. **Electrolyte Leakage Index (%) in five chickpea varieties. Graph showing comparison between the non-preconditioned and preconditioned plants at different stages. Alphabet a-c represents level of significance among different varieties. * represents level of significance between P and NP at $P < 0.05$ based on Tukey's test. NP: non-preconditioning, P: Preconditioning.**

Table 4.3.1. Electrolyte Leakage Index (%), in preconditioned, non-preconditioned plants of five chickpea varieties.

	Stage 1		**Stage 2**		**Stage 3**		**Stage 4**	
	NP	**P**	**NP**	**P**	**NP**	**P**	**NP**	**P**
PDG3	31.215±5.844 a	40.655±6.247 a	41.904±5.844 a	46.263±6.247 a	58.300±5.844 a	72.889±6.247 a	43.687±5.84a *	73.643±5.84a *
PDG4	37.145±5.509 a	35.749±5.509 a	44.424±5.844 a	31.172±5.844 a	57.568±5.509 a	44.863±5.844 b	71.118±5.509 b	69.147±5.844 a
PBG1	33.737±5.844 a	45.937±5.509 a	63.96±5.844a *	38.661±5.50a *	53.633±5.509 a	53.549±5.844 ab	60.678±5.844 ab	78.707±6.247 a
PBG5	37.223±5.844 a	35.866±5.509 a	35.996±5.844 a	31.703±6.247 a	62.565±5.844 a	59.151±5.509 ab	70.259±5.509 bc	73.839±5.509 a
GPF2	28.419±5.509 a	38.381±6.247 a	55.169±5.509 a	35.385±6.247 a	68.458±5.509 a	69.502±5.844 a	65.187±5.509 abc	78.514±5.509 a

4.4. Leaf pigment content

4.4.1. Chlorophyll a

Chlorophyll ‘a’ content was analyzed by two-way ANOVA with all pair wise multiple comparisons using Tukey's test. ‘There were statistically significant interactions between treatments and variety x treatment ($p<0.05$). Here also no difference was noticed in varieties (Figure 4.4.1).

At stage 1 (Flowering): At flowering stage, under non-preconditioned (NP) conditions, there was significantly low chlorophyll content in GPF2 variety as compared to PDG3 variety (Table 4.4.1). In preconditioned plants, there was no significant change in all varieties of chickpea. GPF2 showed high Chl a content in preconditioned plants (Fig 4.4.1).

At stage 2 (Podding): At this stage, there was no difference among the varieties in NP plants. In preconditioned plants, PDG3 variety showed significantly low chlorophyll a content as compared to PDG4, PBG1 and PBG5. Also, no significant difference was observed between P and NP plants.

At stage 3 (pod filling): At this stage, chlorophyll ‘a’ level was higher in PDG3 variety as compared to PDG4 and PBG1 in non-preconditioned plants. In preconditioned plants, higher chlorophyll content was observed for GPF2 variety than PBG5. Also, significantly higher chlorophyll content was observed in NP PBG5.

At stage 4 (Terminal reproductive stage): At this stage, the chlorophyll ‘a’ content was similar in all non-preconditioned plants. In preconditioned plants, the chlorophyll ‘a’ content was significantly low in PBG1 when compared with PDG3, PDG4 and PBG5. While comparing P and NP plants, significantly less Chlorophyll a was observed in GPF2 variety.

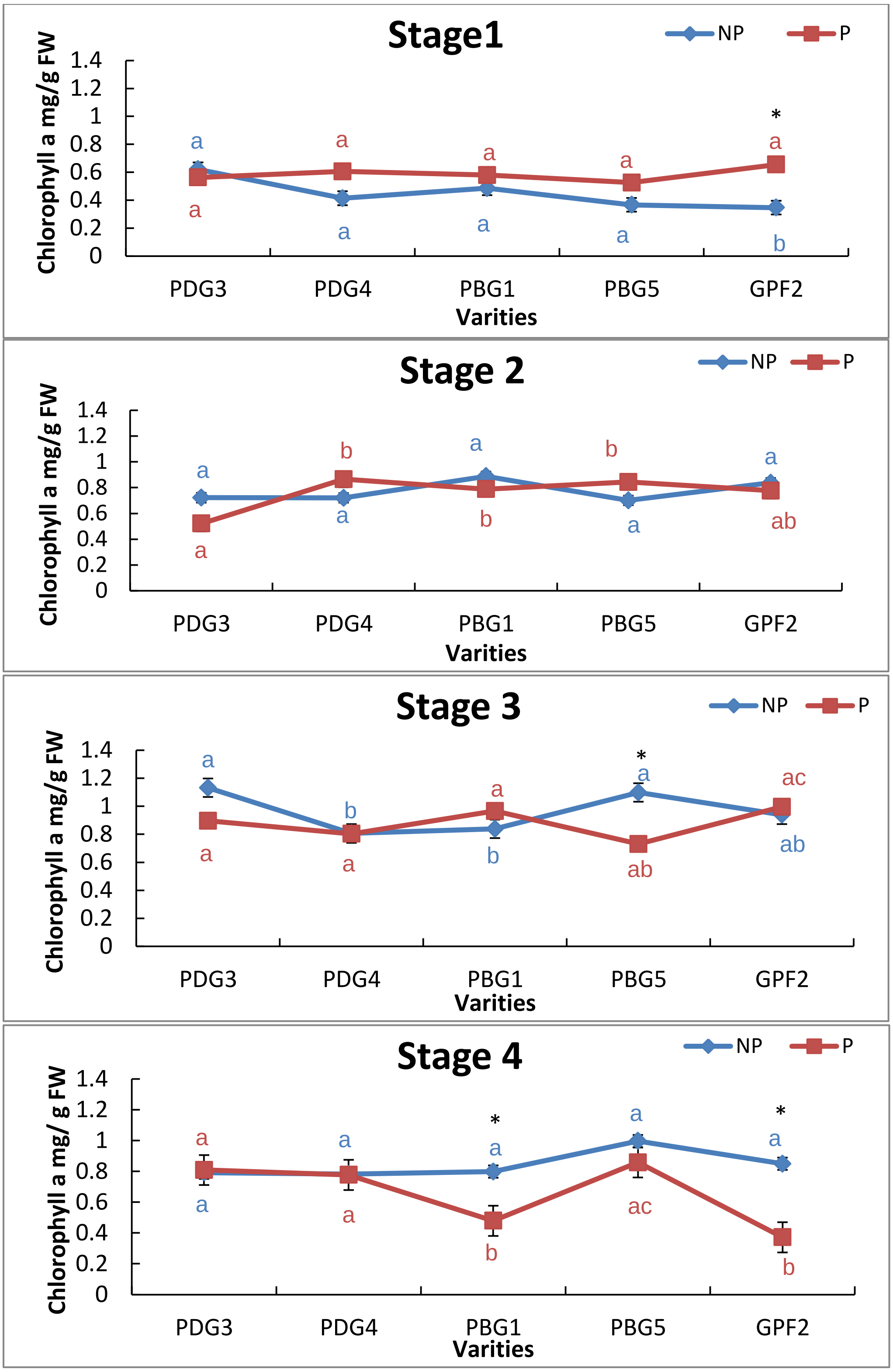

Fig. 4.4.1. Chlorophyll 'a' content in five chickpea varieties. Graph showing comparison between the non-preconditioned and preconditioned plants at different stages. Alphabet a-c represents level of significance among different varieties. * represents level of significance between P and NP at $P < 0.05$ based on Tukey's test. NP: non-preconditioning, P: Preconditioning.

Table 4.4.1. Chlorophyll a content (%), in preconditioned, non-preconditioned plants of five chickpea varieties.

	Stage 1		**Stage 2**		**Stage 3**		**Stage 4**	
	NP	**P**	**NP**	**P**	**NP**	**P**	**NP**	**P**
PDG3	0.621±0.066 a	0.563±0.066 a	0.722±0.066 a	0.521±0.066 a	1.132±0.066 a	0.896±0.066 a	0.792±0.066 a	0.809±0.066 a
PDG4	0.414±0.066 a	0.606±0.066 a	0.720±0.066 a	0.866±0.066 b	0.806±0.066 b	0.803±0.066 a	0.783±0.066 a	0.777±0.066 a
PBG1	0.486±0.066 a	0.580±0.066 a	0.888±0.066 a	0.788±0.066 b	0.838±0.066 b	0.966±0.066 a	0.798±0.066a *	0.479±0.066b *
PBG5	0.367±0.066 a	0.526±0.066 a	0.701±0.066 a	0.844±0.066 b	1.098±0.06a *	0.730±0.06ab *	0.997±0.066 a	0.858±0.066 ac
GPF2	0.347±0.06b *	0.655±0.06a *	0.837±0.066 a	0.777±0.066 ab	0.939±0.066 ab	0.996±0.066 ac	0.849±0.066a *	0.372±0.066b *

4.4.2. Chlorophyll b

Chlorophyll b content was analysed by two-way ANOVA with all pair wise multiple comparisons using Tukey's test. showed statistically significant interaction between varieties, treatments and variety x treatment ($p < 0.05$) (Figure 4.4.2).

At stage 1 (Flowering): At flowering stage, there was no significant change in chlorophyll b content in PDG3, PDG4, PBG1, PBG5 and GPF2 variety (Table 4.4.2) under non-preconditioned (NP) conditions. Similar trend was observed in preconditioned plants. Also, no significant difference was observed between preconditioned and non-preconditioned plant at stage 1 (Fig 4.4.2).

At stage 2 (Podding): At this stage, there was higher chlorophyll b content in PBG1 variety as compared to others varieties in NP plants. In preconditioned plants, PDG3 variety showed significantly less chlorophyll b content as compared to PDG3, PDG4, PBG1, PBG5 and GPF2. While comparing the treatments (P and NP), no significant difference was observed between P and NP.

At stage 3 (pod filling): At this stage, chlorophyll b level was similar in all the varieties. The same trend was observed while analyzing preconditioned and non-preconditioned plants. While comparing P and NP plants, significant difference was observed in PBG5 variety only.

At stage 4 (Terminal reproductive stage): At this stage, no significant difference was observed in non-preconditioned plants. In preconditioned plants, the chlorophyll b level was significantly lower in PBG1 when compared with rest of the varieties. While comparing P and NP, there was no significant difference observed at stage 4. It can be summarized from the findings that there were significant variations in the observed parameters but nothing conclusive was obtained during course of study. P reduced the water content especially at stage 4, especially in GPF 2. Otherwise no difference was recorded in the water content of the plants. As far as membrane damage is concerned PBG1 and PDG 3 showed variations w.r.t. pre and NP treatments. Stage 2 (Pod filling) and Stage 4 (terminal reproductive stage) was found to be more sensitive w.r.t. the behavior of these varieties. GPF 2 was responsive towards P as it improved the Chlorophyll content in P plants, whereas, rest of the varieties did not show any positive interaction w.r.t. preconditioned.

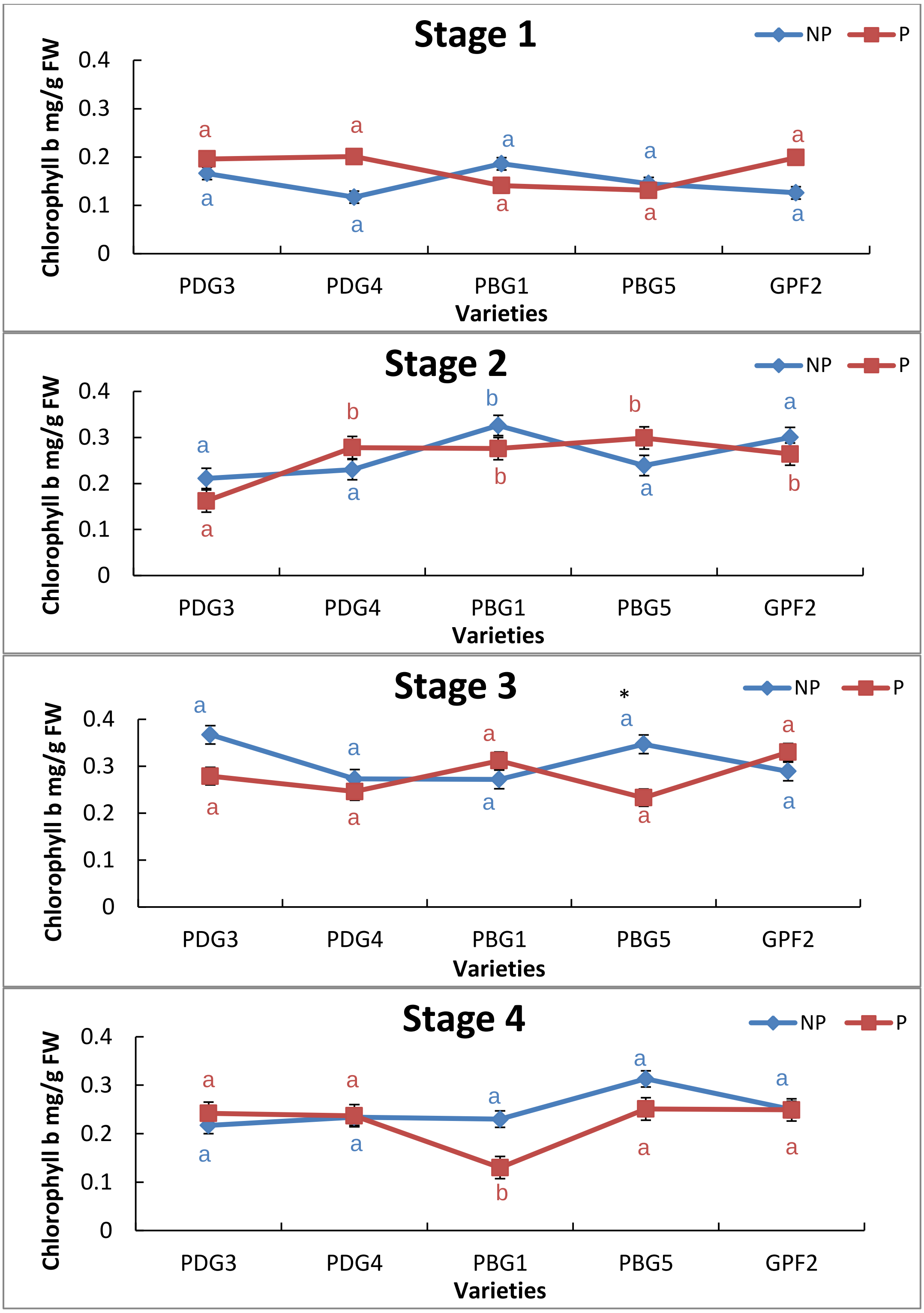

Fig. 4.4.2. Chlorophyll 'b' content in five chickpea varieties. Graph showing comparison between the non-preconditioned and preconditioned plants at different stages. Alphabet a-c represents level of significance among different varieties. * represents level of significance between P and NP at P **< 0.05 based on Tukey's test. NP: non-preconditioning, P: Preconditioning.**

Table 4.4.2. Chlorophyll b (%), in preconditioned, non-preconditioned plants of five chickpea varieties.

	Stage 1		**Stage 2**		**Stage 3**		**Stage 4**	
	NP	**P**	**NP**	**P**	**NP**	**P**	**NP**	**P**
PDG3	0.160±0.026 a	0.19±0.026 a	0.210±0.020 a	0.160±0.026 a	0.360±0.026 a	0.270±0.026 a	0.210±0.026 a	0.242±0.026 a
PDG4	0.117±0.026 a	0.201±0.026 a	0.230±0.026 a	0.278±0.026 b	0.273±0.026 a	0.246±0.026 a	0.234±0.026 a	0.237±0.026 a
PBG1	0.186±0.02 a	0.141±0.026 a	0.326±0.026 b	0.276±0.02 b	0.272±0.026 a	0.312±0.026 a	0.230±0.026 a	0.130±0.026 b
PBG5	0.145±0.026 a	0.131±0.026 a	0.239±0.026 a	0.299±0.026 b	0.347±0.02a *	0.233±0.06a *	0.313±0.026 a	0.251±0.026 a
GPF2	0.126±0.026 a	0.199±0.026 a	0.300±0.026 a	0.264±0.026 b	0.289±0.026 a	0.330±0.06 a	0.250±0.026 a	0.249±0.026 a

Chapter 5

Discussion

The focus of present study was to observe the effect of preconditioning on plant water relations, cold induced damage and pigment system. Relative Water Content, Electrolyte Leakage Content, Chlorophyll a and b content were measured during lethal chilling stress in field grown chickpea. It was previously observed that the low temperature below 10°C (min temperature) and below 20°C (average max temperature) is lethal for reproductive growth (Kumar et al., 2010). There are enough evidences of membrane damage in term of ELI in Cucumber (Lee et al., 2005) during chilling stress. In present study ELI was higher in preconditioned (P) plant than non-preconditioned (NP) plants. However, PBG1 behaved differently as ELI content was high in NP plant than P plant during pod filling stage. The chickpea plants were exposed to low temperature condition of field during both early as well as late pod formation in reproductive stage resulted in significant increase in electrolyte leakage Index (ELI), which is an indicator of membrane damage to stressed plants (Murata et al., 1992).

Relative Water Content was observed higher in PDG4 variety of preconditioned (P) plant than other varieties during terminal reproductive stage of chickpea. Previous studies also reported that chilling stress reduced the RLWC in chickpea (Kaur et. al 2011). The RLWC decrease possibly due to reduced water uptake in cold stressed plants. GPF2 behaved differently where it had higher RLWC in non-preconditioned plant than preconditioned plants.

Chlorophyll content is a good criterion to estimate the effects of stress factor on photosynthetic apparatus. The decline in chlorophyll content is well known response of chilling sensitive plant. In present study the chlorophyll a content was significantly high in preconditioned GPF2 variety as compared to NP plant during flowering stage. PBG5 behaved differently where it had higher Chlorophyll a content in NP plant than P plants during pod filling stage of chickpea. PBG1 and GPF2 also, showed higher Chlorophyll a content in NP as compared to P during terminal reproductive stage. At this plant reaches the maturity stage and undergoes senescence.

Chlorophyll b content was significantly high in GPF2 variety of P plant as compared to other varieties during podding stage. PBG5 behaved differently where it had higher Chlorophyll b content NP plant than P plants during pod filling stage. There was a significant fall of chlorophyll a and b content due to cold in stressed plants. The decrease in chlorophyll is directly related to reduction in vegetative growth and failure in the reproductive organs due to cold. This is accordance with earlier studies on chickpea on this aspect (Srinivasan et al. 1998; Nayyar et al. 2007).

Overall, it can be inferred from the present study that plant water relations are influenced by cold yielding significant damage in terms of degradation of pigment system and membrane damage.

Since it was a field study, more conclusive inference can be made after repletion of the trial and correlating it with other physio-biochemical studies.

Summary

Chickpea is the second most important legume in the world grown during winter season in northern parts of India. Due to low temperature at reproductive stage during winter season results in a severe decrease in the crop yield of chickpea. Therefore, there is an urgent need of appropriate measures to improve the crop yield. This could be possible, if we precondition the plants with alternate stress which can be implemented in the field conditions. Drought stress is more feasible and can be used easily in the fields by withholding the water given to crop before onset of period of chilling injury to chickpea. The present study evaluates the effect of preconditioning (P) with mild drought stress on plants during lethal cold stress (CS) by examining various biophysical parameters responsible for membrane stability, photosynthesis. From the results we summarized that, ELI was higher in preconditioned (P) plant than non-preconditioned (NP) plants at temperature range (>10 and <20°C). ELI content showed that preconditioning maintains the stability of the membrane during cold stress. As far as membrane damage is concerned PBG1 and PDG3 showed variations w.r.t. pre and non-preconditioned treatments. Overall, RLWC, preconditioning reduced the water content especially at stage 4(terminal reproductive stage), mainly in GPF 2. Difference in RLWC at stage 4 is may be due to temperature change from low to moderately high which is unrelated with cold and RLWC. It can be summarized from the findings that under the low temperature range, Stage 2(Pod filling) and Stage 4 (terminal reproductive stage) was found to be more sensitive w.r.t. the behavior of these varieties. GPF2 was responsive towards preconditioning as it improved the chlorophyll content in preconditioned plant. That study showed the preconditioning increases tolerance capacity towards chilling stress and used to improve tolerance against chilling stress in chickpea.

References

AccuWeather.com for Bathinda, India. (2016). <http://www.accuweather.com/en/in/bathinda/190068/hourly-weatherforecast/190068>. Accessed 2016 Jan 01 to 2016 April 14.

Anjum, S. A., Xie, X. Y., Wang, L. C., Saleem, M. F., Man, C., and Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research, **6:** 2026-2032**.**

Aroca, R., Vernieri, P., Irigoyen, J.J., Sanchez-Dıaz, M., Tognoni, F. and Pardossi, A. (2003). Involvement of abscisic acid in leaf and root of maize (Zea mays L.) in avoiding chilling-induced water stress. Plant Science **165**: 671-679**.**

Auckland, A. K., and van Der Maesen, L. J. G. (1980). Chickpea. Hybridization of Crop Plants, **667**: 249-259.

Bakht, J., Bano, A., and Dominy, P. (2006). The role of abscisic acid and low temperature in chickpea (Cicer arietinum L.) cold tolerance. II. Effects on plasma membrane structure and function. Journal of Experimental Botany **57**: 3707-3715.

Beck, E. H., Fettig, S., Knake, C., Hartig, K., and Bhattarai, T. (2007). Specific and unspecific responses of plants to cold and drought stress. Journal of Biosciences **32:** 501-510**.**

Bohnert, H. J., Nelson, D. E., and Jensen, R. G. (1995). Adaptations to environmental stresses. The Plant Cell **7:** 1099**.**

Boyer, J.S. (1982). Plant productivity and environment. Science **218**: 443-448**.**

Chinnusamy, V., Zhu, J., and Zhu, J. K. (2007). Cold stress regulation of gene expression in plants. Trends in Plant Science **12:** 444-451**.**

Croser, J., Clarke, H., Siddique, K. and Khan, T. (2003). Low-temperature stress: implications for chickpea (Cicer arietinum L.) improvement. Critical Reviews in Plant Sciences **22**: 185-219**.**

FAOSTAT. (2015). 2014 Oct, 23. .<http://faostat3.fao.org/browse/Q/QC/E>. Accessed 2016 July 01.

Farooq, M., Aziz, T., Hussain, M., Rehman, H., Jabran, K., and Khan, M. B. (2008). Glycinebetaine improves chilling tolerance in hybrid maize. Journal of Agronomy and Crop Science **194:** 152-160**.**

Farooq, M., Aziz, T., Wahid, A., Lee, D. J., and Siddique, K. H. (2009). Chilling tolerance in maize: agronomic and physiological approaches. Crop and Pasture Science **60:** 501-516**.**

Garg, R., Patel, R. K., Tyagi, A. K. and Jain, M. (2011). De novo assembly of chickpea transcriptome using short reads for gene discovery and marker identification. DNA Research **18**: 53-63.

Gonzalez, L. and Gonzalez-Vilar, M. (2001). Determination of relative water content. In: Reigosa Roger, M.J. (Ed.). Handbook of Plant Ecophysiology Techniques (pp. 207-212.). Springer, Netherlands**.**

Hasanuzzaman, M., Nahar, K., and Fujita, M. (2013). Extreme temperature responses, oxidative stress and antioxidant defense in plants. INTECH Open Science Open Minds.

Heidarvand, L., and Amiri, R. M. (2010). What happens in plant molecular responses to cold stress. Acta Physiologiae Plantarum **32:** 419-431**.**

Hirdyani, H. (2014). Nutritional composition of Chickpea (Cicer arietinum L.) and value added products-a review. Indian Journal of Community Health **26:** 102-106**.**

Hossain, M. A., Mostofa, M. G., and Fujita, M. (2013). Cross protection by cold-shock to salinity and drought stress-induced oxidative stress in mustard (Brassica campestris L.) seedlings. Molecular Plant Breeding **4.**

Janowiak, F and Markowski, A., 2008. Changes in leaf water relations and injuries in maize seedlings induced by different chilling conditions. Journal of Agronomy and Crop Science **172**: 19–28**.**

Javanmardi, J., Rahemi, M., and Nasirzadeh, M. (2013). Physiological and reproductive responses of tomato and pepper transplants to low-temperature conditioning. International Journal of Vegetable Science **19:** 294-310**.**

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., and Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (Cicer arietinum L.): a review. British Journal of Nutrition **108:** 11-26**.**

Kannahi, M. and Kowsalya, M. (2013). Efficiency of plant growth promoting rhizobacteria for the enhancementof Vigna mungo growth. Journal of Chemical and Pharmaceutical Research **5**: 46-52**.**

Kaur, G., Kumar, S., Thakur, P., Malik, J. A., Bhandhari, K., Sharma, K. D., and Nayyar, H. (2011). Involvement of proline in response of chickpea (Cicer arietinum L.) to chilling stress at reproductive stage. Scientia Horticulturae **128:** 174-181**.**

Kumar, S., Nayyar, H., Bhanwara, R. K., and Upadhyaya, H. D. (2010). Chilling stress effects on reproductive biology of chickpea. Journal of SAT Agricultural Research **8:** 1-14**.**

Ladizinsky, G. and Adler, A. (1976). The origin of chickpea Cicer arietinum L. Euphytica **25**: 211-217.

Ladjal, M., Epron, D., and Ducrey, M. (2000). Effects of drought preconditioning on thermotolerance of photosystem II and susceptibility of photosynthesis to heat stress in cedar seedlings. Tree Physiology **20:** 1235-1241**.**

Leport, L., Turner, N. C., Davies, S. L., and Siddique, K. H. M. (2006). Variation in pod production and abortion among chickpea cultivar under terminal drought. European Journal of Agronomy **24:** 236-246**.**

Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought-and low-temperature-responsive gene expression, respectively, in Arabidopsis. The Plant Cell Online **10:** 1391-1406**.**

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

Mahajan, S. and Tuteja, N. (2005). Cold, salinity and drought stresses: an overview. Archives of biochemistry and biophysics **444**: 139-158.

McWilliam, J. R., P. J. Kramer, and R. L. Musser, 1982: Temperature induced water stress in chilling sensitive plants. Australian Journal of Plant Physiology **9:** 343–352**.**

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. Trends in plant science 11**:** 15-19**.**

Murata, N., Ishizaki-Nishizawa, O., Higashi, S., Hayashi, H., Tasaka, Y., and Nishida, I. (1992). Genetically engineered alteration in the chilling sensitivity of plants. Nature **356**:710-713.

Nayyar, H., and Walia, D. P. (2004). Genotypic Variation in Wheat in Response to Water Stress and Abscisic Acid-Induced Accumulation of Osmolytes in Developing Grains. Journal of Agronomy and Crop Science **190:** 39-45**.**

Nayyar, H., Bains, T and Kumar, S., (2005). Low temperature induced floral abortion in chickpea: relationship to abscisic acid and cryoprotectants in reproductive organs. Environmental and Experimentally Botany. **53**: 39–48**.**

Nayyar, H., Kaur, G., Kumar, S., and Upadhyaya, H. D. (2007). Low temperature effects during seed filling on chickpea genotypes (Cicer arietinum L.): probing mechanisms affecting seed reserves and yield. Journal of Agronomy and Crop Science **193:** 336-344**.**

Pirzadah, T.B., Malik, B., Rehman, R.U., Hakeem, K.R. and Qureshi, M.I. (2014). Signaling in Response to Cold Stress Plant signaling: Understanding the molecular crosstalk (pp. 193-226): Springer.

Shahandashti, S. S. K., Amiri, R. M., Zeinali, H., and Ramezanpour, S. S. (2013). Change in membrane fatty acid compositions and cold-induced responses in chickpea. Molecular Biology Reports **40:** 893-903**.**

Singh, K. B. (1997). Chickpea (Cicer arietinum. L.). Field Crops Research 53**:** 161-170**.**

Singh, K.B., L. Holly and G. Bejiga, 1991. A catalog of kabuli chickpea germplasm. International Centre for Agricultural Research in Dry Areas, Aleppo, Syria. 398

Singh, U. (1985). Nutritional quality of chickpea (Cicer arietinum L.): current status and future research needs. Plant Foods for Human Nutrition **35:** 339-351**.**

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

Wilson, B. C., and Jacobs, D. F. (2004, March). Electrolyte leakage from stem tissue as an indicator of hardwood seedling physiological status and hardiness. In Proceedings of the Fourteenth Central Hardwood Forest Conference. Wooster, OH 16-19**.**