ANALYZING REPRODUCTIVE CAPABILITIES OF CHICKPEA IN COLD ENVIRONMENT

Project Report submitted to the Central University of Punjab

For the Award of

Master of Science

In

Life Science (Specialization in Plant Sciences)

BY

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May, 2018

CERTIFICATE

I declare that the project report entitled “ANALYZING REPRODUCTIVE CAPABILITIES OF CHICKPEA IN COLD ENVIRONMENT” has been prepared by me under the guidance of Dr. Sanjeev Kumar, Associate Professor, Department of Plant Sciences, School of Basic & Applied Sciences, Central University of Punjab. No part of this dissertation has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

I certify that SHWETA SELPAIR has prepared her project report entitled "ANALYZING REPRODUCTIVE CAPABILITIES OF CHICKPEA IN COLD ENVIRONMENT" for the award of M.Sc. Degree of the Central University of Punjab, under my guidance. She has carried out this work at the Department of Plant Sciences, School of Basic & Applied Sciences, Central University of Punjab.

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ABSTRACT

Analyzing reproductive capabilities of chickpea in cold environment

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Key Words: Chilling stress, preconditioning, reproductive stages, abortion, retention, pod setting, seed yield, microscopy

Chickpea is winter sown legume crop. In northern India, crop faces chilling stress during the month of January. Chilling stress causes detrimental effects on the reproductive stages of the crop which leads to the abortion of flowers, pod setting and seed yield. Preconditioning is a process in which plants are treated with mild drought stress to induce plant defense system against chilling stress. Effect of preconditioning was studied on five genotypes PBG1, GPF2, PDG3, PDG4 and PBG5. In this experiment, performance was evaluated on the basis of their seed yield. Reproductive structures were studied with the help of compound, scanning electron and confocal microscopy. Results have shown that PBG5 has performed best while PBG1 worst on the basis of seed yield among the five genotypes.

(Shweta Selpair) (Dr. Sanjeev Kumar)

Student's signature Supervisor's signature

ACKNOWLEDGEMENT

First and above all, I praise God, the Almighty for providing me this opportunity and granting me the capability to proceed successfully. This M.Sc. Dissertation appears in its current form due to the assistance and guidance of several people. I would therefore like to offer my sincere thanks to all of them.

I would like to express my gratitude to my supervisor **Dr. Sanjeev Kumar** for their excellent guidance and constant supervision of my M.Sc. Dissertation and also for his encouragement and support throughout the course of this study. He has motivated me very much and this M.Sc. work would not have been completed without his help.

I would also like to thankfully acknowledge again Dr. Sanjeev Kumar, Head of Department, Department of Plant Sciences and Dr. R.K. Kohli, Vice Chancellor, Central University of Punjab, Bathinda for providing me research facilities to carry out my work.

I would also like to thank my seniors and lab mates Rashmi Saini, Arindam Adhikary, Renu Yadav, Sumandeep Juneja, Rashpal Kumar and Agrah Pradhan for their support, co-operation and help during my M.Sc project.

I am thankful to Mrs. Gagandeep Kaur to help and assist in lab during experimental work.

Last but not the least; I would like to thank my family for their support in all aspects of my life.

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LIST OF ABBREVIATION

Sr. No	Full form	Abbreviation
1	Analysis of variance	ANOVA
2	Celsius	C
3	Centimeter	Cm
4	Days after sowing	DAS
5	Food and Agriculture Organization of the United Nations	FAOSTAT
6	February	Feb
7	Field Emission Scanning Electron Microscope	FESEM
8	Gram	G
9	January	Jan
10	Kilogram	Kg
11	March	Mar
12	Milligram	Mg
13	Punjab Agricultural University	PAU
14	Replication	Rep
15	Temperature	Temp

Chapter – 1

Introduction

1. INTRODUCTION

Chickpea is a winter legume as it can withstand temperature range from 8° to 22°C. Optimum temperature needed for proper reproductive phase lies between 10°C to 14°C (as minimum average) and 25°C to 31°C (as maximum average) (Singh *et al*., 1997).In India, chickpea is grown during winter season. Therefore, it faces severe cold temperature during reproductive stage in the month of January i.e., below 15^0C as maximum temperature. These results in plant damage and loss of yield (Croser *et al*., 2003).

70% of crop production is limited by environmental factors (Boyer and John, 1983). Abiotic stress is defined as any environmental condition that affects the growth, development and productivity of plant. Plant responses to abiotic stresses are dynamic and complex (Shinozaki *et al*., 2000).They can be both reversible and irreversible. The plant responses towards stress are dependent on the part of plant affected by the stress (Dinneny *et al*., 2008; Cramer *et al*., 2011).

Chilling stress is one of the abiotic stresses which cause many injuries to the plant. These temperatures are experienced in the northern India and southern Australia during its reproductive phase. Chilling stress shows detrimental effect on the growth of the plant as it can cause reduction in the growth of the plant. On cellular level, it causes production of Reactive Oxygen Species (ROS), membrane damage, accumulation of toxic compounds and denaturation of proteins. Chilling stress impairs the absorption mechanism of nutrients in the roots (Kim *et al*., 2013). Low temperature is also unfavorable for the flowering and pod setting as it induces the floral abortion, formation of undeveloped pods in chickpea which ultimately leads to low yield. Floral abortion has added to improper pollen tube formation which led to poor fertilization process (Kumar *et al*., 2011). Before anthesis, low pollen viability or high ovule sterility during anthesis and fertilization may leads to poor pod setting. This condition may lead to failure of pollen to reach or germinate on the stigma, or the failure of the pollen tube to penetrate the stigma and grow in style (Singh *et al.*, 1997).

Similar responses are induced at molecular and cellular level by various abiotic stresses. These stresses may cause similar downstream signal

transduction chains (Beck *et al*., 2007). Drought and cold stress have many similar features. They induces same kinds of genes indicating towards the involvement of similar biochemical processes (Shinozaki *et al*., 2000).Plant does have similar mechanisms of their physical responses against drought and cold stress. In both cases, ABA is produced in order to tackle the stress (Liu *et al*., 1998). Preconditioning is a process in which plants are treated with mild abiotic stress to induce the plant defense system against other abiotic stress. This can be determine by the observing the various responses of the preconditioned and non-preconditioned plants.

1.2. HYPOTHESIS

Analysis of floral biology may lead to identification of a physiological marker of cold tolerance in chickpea.

1.3. POSSIBLE OUTCOME

Plants treated with preconditioning may show increase in rate of flower retention and decrease in rate of flower abortion relative to plants treated with non-preconditioning which could lead to greater pod retention and yield/plant.

Chapter - 2

Review of literature

2.1. REVIEW OF LITERATURE

Chickpea (*Cicer arietinum* L.) is 3rd major winter crop in world after dry bean and field pea. Many botanical, genetic and archeological evidences point its origination in Fertile Crescent, Turkey (Abbo *et al*., 2009). Now, it has flourished between latitude 20° and 40° including west and central Asia, the Indian subcontinent, southern Europe, Africa (northern parts), Latin America, and more recently North America and Australia. India is largest producer of chickpea i.e., 7818984 tones/hectare (FAOSTAT2016). Cultivation has also spread along the equator. Chickpea has reached India two centuries ago, through Afghanistan. Hence, in Hindi it is called as *kabuli chana*. From India, Ramanujam has summarized the history of chickpea in India. The earliest occurrence of chickpea in India was observed at Atranjikhera in Uttar Pradesh in 2000 BC (Chowdury *et al*., 1971).

*Cicer arietinum.*L belongs to Fabaceae family and Faboidae subfamily. Cicer is now categorized in its own monogeneric tribe, Cicereae Alef on the bases of its pollen morphology and vascular anatomy. Cicer is divided into two subgenera and comprises 43 species. It is divided into four sections Monocicer, Chamaecicer, Polycicer, and Acanthocicer on the basis of morphology and growth habitat.Perennial progenitor of chickpea is identified as *Cicer anatolicum* and *C. reticulatum* is proposed as annual proginator. Chickpea can be found in cultivated weedy, open dry rubble slopes etc. Plant is generally drought resistant but genetic variations do exist in them. Taproot may be 1m long and its branches die off above the ground after the dispersal of seeds (Tayyer *et al*., 1996).

Chickpea is an important source of carbohydrates and proteins among Asian and African countries. Its nutritional quality is better than pulses. It contains all essential amino acid except amino acids which contain sulphur. It has starch followed by dietary fibre, oligosaccharides, glucose and sucrose. Lipids are in low amount especially it contains unsaturated fatty acids like oleic and linoleic acid. Chickpea oil contains important sterols like stigmasterol, β-Sitosteroland campesterol. It also has many vitamins like niacin, riboflavin, thiamin, precursor of

vitamin A β- carotene and folate. It has some beneficial effects on diseases like type 2 diabetes, CVD, digestive problems and cancer (Maesen *et al*. 1987).

Any disturbance that adversely influences the plant growth is termed as stress. Cold stress can be divided into chilling range and freezing range. For chickpea, freezing temperature lies below -1.5°C while chilling temperature lies between -1.5° C and 15°C (Jukanti *et al*., 2012).Chilling sensitivity is a characteristic of tropical and sub-tropical climate. Early sown crops or early flowering genotypes is largely limited by the abortion of flowers and pod in late winter and early spring, which in turn leads to low harvest index. Although late sowing can reduce the flower and pod abortion associated with low temperature. Early flowering would benefit the yield if flowers were fertile, because pod production can start earlier (Graham *et al*., 1982). There is a positive correlation between the numbers of seedlings emerged and the average soil temperature and air temperature (Leport *et al*., 1999). The days between sowing and emergence are negatively correlated with the average soil temperature. There is considerable variation on the recommended optimum temperatures for chickpea germination (Ellies *et al*., 1986).

In chickpea, as the plant progresses from germination to flowering sensitivity to freezing and chilling range temperatures increases. During germination, temperature lying under chilling range leads to poor crop establishment, enhances susceptibility to soil-borne pathogens, and reduces seedling vigor. At seedling stage, long period of chilling stress can retard the plant growth, production of anthocyanin pigments, undeveloped leaves, browning of coleoptiles (Wery *et al*., 1993). Some symptoms can be non-visible like alteration in membrane properties, proteins, lipids, solute leakage and carbohydrate metabolism. Major effects observed are membrane injury, reduced respiration and photosynthesis, loss of turgor which leads to wilting and cold induced water stress. Macroscopic effects are also seen on leaf shape and size, development of root, plant height and floral initiation (Prasad, 2001).In severe cases; it can lead to the death of the plant. At the vegetative stage also, it shows negative effect on the growth of the plant and dry matter production. Less dry matter production reduces

the reproductive sink which results into reduction of potential yield (Croser *et al*., 2003)

Reproductive events in chickpea are generally influenced by air temperature and photoperiod. Non-optimum temperatures constrain reproductive development in higher plants at various stages, including pollen development, transfer of viable pollen to the stigma, pollen germination and tube growth, and ovule fertilization and seed development (Croser *et al*., 2003). Chilling temperature causes the floral abortion and poor seed filling that leads to reduced seed yield (Nayyer *et al*., 2005). In plants, the process of male reproductive development is extremely sensitive to adverse climatic environments and biotic stress. Cold stress, during reproductive phase produces structural and functional abnormalities which lead to the failure in fertilization or premature abortion of fruit or seed (Farooq *et al*., 2009). Chilling stress in male gametophytic organs often shows morphological, metabolic and structural defects which leads to meiotic malfunctioning or premature spore abortion and male reproductive sterility. Cellular defects vary depending on the type of stress and exposure duration. It may cause tapetal irregularities cytoskeletal alterations, aberrations in auxin metabolism, altered sugar utilization, induction of programmed cell death (PCD) or accumulation of reactive oxygen species (ROS) (Storme *et al*., 2014). In cold treated rice plants, both histological and cytological abnormalities were found to be greater in the anthers than other organs of the flower. Cold damage can also be improved by pollinating the female flowers with artificial pollens collected from the non-stressed plant (satake *et al*., 1970). In rice plant, chilling stress can cause degradation of spikelets, incomplete panicle extension and an increase in spikelet sterility (Terres *et al*., 1991).

Scarcity of water is a major environmental factor which negatively affects the plant productivity. Drought stress affects plant metabolism as it reduces leaf size, stems extension and root proliferation. It disturbs plant water relations and reduces water-use efficiency. Mechanisms like stomatal closure, membrane damage and disturbed activity of various enzymes are also seen. CO_2 assimilation is reduced by CO_2 fixation and adenosine triphosphate synthesis. Increased metabolite flux

through electron transport chain enhances the oxidative load on the tissue which leads to production reactive oxygen species. Injury caused by reactive oxygen species (oxidative stress) to biological macromolecules under drought stress is among the major threat to growth. Plants have certain range of mechanisms to tolerate drought stress. The major mechanisms include enhanced water uptake, water loss by increased diffusive resistance, deep root systems with its proper use and smaller leaves with succulent property reduces the transpirational loss. Among the nutrients, potassium ions help in osmotic adjustment; silicon increases root endodermal silicification and improves the cell water balance. Plant growth regulators like salicylic acid, auxins, gibberellins, cytokinin and abscisic acid modulate the plant responses towards drought.Low molecular weight osmolytes, including glycinebetaine, proline and other amino acids, organic acids, and polyols, play crucial role in sustaining the proper cellular functions under drought. Polyamines, citrulline and many enzymes act as antioxidants and reduce the adverse effects of water deficieny. At molecular levels large numbers of drought-responsive genes and transcription factors have been identified like dehydration-responsive element-binding gene, aquaporin, dehydrins and late embryogenesis abundant proteins. Plant drought tolerance can be enhanced by adopting strategies such as mass screening and breeding, marker-assisted selection and exogenous application of hormones and osmoprotectants to seed or growing plants (Farooq *et al*., 2009).

Experiments have established that exposure to a range of different types of stress alters subsequent plant responses. The process of priming or hardening involves prior exposure to a biotic or an abiotic stress factor making a plant more resistant to future exposure. This feature generates “memory” in higher plants. However, the molecular mechanism(s) of this plant memory is entirely different from memory of animals, as their memory system is dependent on nervous system. Therefore, we use the term “stress imprint” for plant memory. An explanation for how plant metabolism is altered by exposure to various stresses can be given by sustained alteration in levels of key signaling metabolites or transcription factors. Epigenetic changes could play a role as they cause long-term

changes in gene expression. Exposure to a priming agent could activate a gene or set of genes which can tackle effects caused by stress. Hence, it helps in generation of defense mechanism against another stress (Bruce *et al*., 2007). Plant can be preconditioned by exposing to the temperature slightly above the chilling range. Preconditioning or hardening does not result into the increase in the amount of fatty acids or unsaturated lipids in leaves, but priming the seedlings with low temperature prevents the ATP loss which occurs due to chilling stress (Wolk *et al*., 1982).The amount of unsaturated fatty acid and lipid content starts decreasing with the age of the leaf at 25°C (Wilson *et al*., 1974). Drought priming has improved tolerance efficiency of plants against various stresses. Wheat (*Triticum aestivum* L. cv. Vinjett) was exposed to moderate water-deficit conditions during its vegetative stage. It was done in order to investigate drought priming effects on tolerance to drought and heat stress events occurring during the grain filling stage. Drought priming has decreased the photoinhibition in leaves caused due to heat and drought stress (Wang *et al*., 2015). In wheat plant, drought priming at vegetative stage improves the antioxidant capacity and photosynthetic efficiency when exposed to low temperature stress (Li *et al*., 2015). Pre-drought priming at vegetative stages in drought tolerant and sensitive wheat cultivars has improved drought tolerance to post-anthesis drought stress (Abid *et al*., 2016). Drought priming in wild type and abscisic acid deficient mutant barley has enhanced the melatonin production. It generates cold tolerance in plants (Li *et al*., 2016).

Future research is necessary to establish the molecular mechanism which enables the plants to store information on stress exposure. It is needed because biotic and abiotic stresses limit agricultural production.

2.2. OBJECTIVES

To study the effect of pretreatment (minor drought stress) on the reproductive phase of CHICKPEA during chilling stress.

Chapter – 3

Materials and methods

3. METHODOLOGY

3.1. Collection of sample

Chickpea (*Cicer arietinum* L.), released genotypes PBG1, GPF2, PDG3, PDG4 and PBG5 were employed to study the preconditioning response on reproductive phase of chickpea during chilling stress. The germplasm consisting of five released varieties PBG1, GPF2, PDG3, PDG4 and PBG5 was procured from Punjab Agricultural University, Ludhiana.

3.2. Experimental design, Plant material, growth conditions

The experiment was conducted in two separate sites. Healthy chickpea seeds treated with *Rhizobium* were sown on15thOctober 2017 in two different fields 45 x 15 f^{2}each respectively in Randomize Block Design with three replications. Row to row and plant to plant distance was maintained as per Package of Practices, Rabi crops (PAU). A total of 40 number of plants were maintained in every single block. 30 days after sowing (DAS), when the plants reached their maximum vegetative potential, they were designated as normal and preconditioned by keeping the irrigation normal (weekly) in one site and stopping the irrigation for 30 days completely in the other site. The latter one was preconditioned with non-lethal drought to develop tolerance in the plants to forthcoming low-temperature stress during the months of December and January. The temperature (minimum and maximum) and relative humidity (minimum and maximum) in every 24 hour were recorded throughout the experiment from 15th October 2017 to 31st March 2018.Three plants from each replication were chosen and tagged for observations total flowers, pods and yield parameters. Data pertaining to reproductive/ floral biology was collected from another three plants/replicates/cultivar. After the completion of the experiment the primary data was subjected to analysis and secondary data was further analyzed using statistical tool (Sigma Plot 11.0).

3.3. Floral studies

Following parameters were recorded from the tagged plants in triple replicates from each plot of five varieties in both non-preconditioned and preconditioned fields.

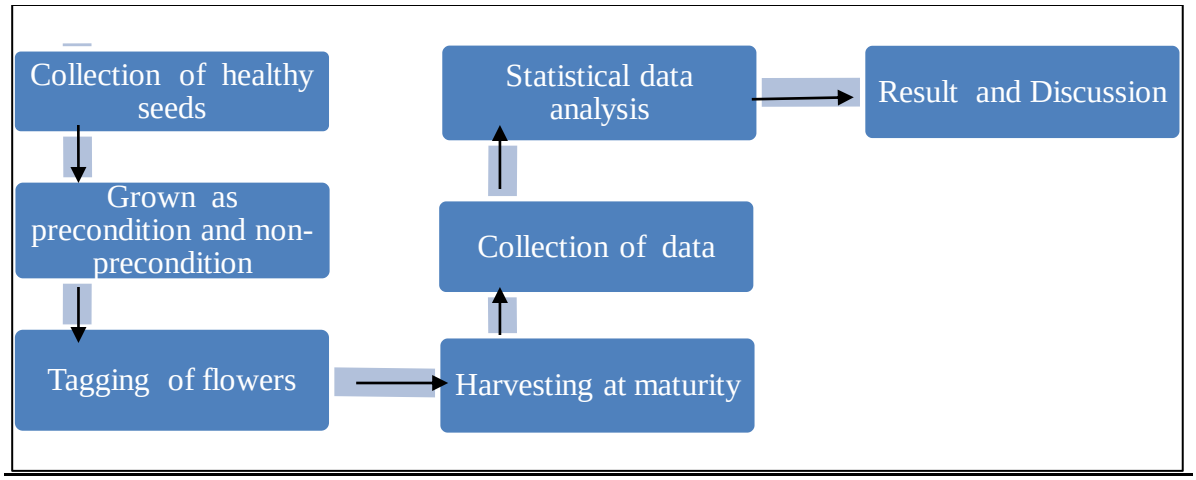


Figure 3.1 The Experimental Plan

- Number of flowers/plant
- Number of total pods/plant
- Number of undeveloped pods/plant
- Number of developed pods/plant
- Number of total seeds/plant
- Number of undeveloped seeds/plant
- Number of developed seeds/plant

3.3.1 Flower abortion/ retention

To calculate % retention and % abortion of flowers, each and every flower in three tagged plants were tagged and observed till pod formation.

No. of aborted flower produced/plant

=Total No. of flowers/plant- Total No. of pods produced/plant

$$\%\ abortion\ of\ flowers = \frac{No.\ of\ flowers\ aborted/plant}{Total\ no.\ of\ flowers\ produced/plant} \times 100$$

$$\%\ retention\ of\ flowers = \frac{No.\ of\ pods\ produced/plant}{Total\ No.\ of\ flower\ produced/plant} \times 100$$

3.3.2 Pod abortion/ retention

Harvesting of tagged plants were done and total number of pods were counted on the plant and collected in separate envelops. After that, developed pods and undeveloped pods were counted separately. Size of the pods was measured and photographed.

$$\% \text{ abortion of pods} = \frac{\text{No. of undeveloped pods produced /plant}}{\text{Total No. of pods produced /plant}} \times 100$$

$$\% \text{ retention of pods} = \frac{\text{No. of developed pods produced /plant}}{\text{Total No. of pods produced/plant}} \times 100$$

3.3.3 Seed abortion/ retention

Pods were taken out of envelops and seeds were collected from them. Some pods had seeds and some were deprived of seeds. Many pods had single seed, while some had two or three seeds. Total seed were counted and then segregated into developed and undeveloped seeds. Both categories were counted. Size of the seed was measured and photographs were taken.

$$\% \text{ abortion of seeds} = \frac{\text{No. of undeveloped seeds/plant}}{\text{Total No. of seeds produced/plant}} \times 100$$

$$\% \text{ retention of seeds} = \frac{\text{No. of developed seeds/plant}}{\text{Total No. of seeds produced/plant}} \times 100$$

3.3.4. Final yield

Both undeveloped and developed seeds were collected and weighed to get yield/plant of each genotype, measured by analytical balance.

3.5 Reproductive biology

3.5.1 Pollen viability

Viability of pollens is suppressed by the chilling stress as they affect their size and shapes. To check this, closed flowers (day before opening of the flower) were collected and anthers were separated from the flower and tapped on the slide. After that, they were stained with potassium iodide and observed under compound microscope at 20X and 40X. 200 pollens per replicates were counted in which viable and non-viable pollens were counted on the basis of the size, shape and the intensity of the stain taken by the pollen.

3.5.2 *In vivo*-pollen tube growth

Pollen germination and vigor is affected by chilling range temperatures. Sensitive and tolerant cultivars can be ascertained under chilling range temperatures from the relative germination of pollen and growth of pollen tubes *in-vivo* (Clarke, 2001).In order to check this, flowers were collected of different days like first day

open, second day open and third day open. They were treated with fixative made up of glacial acetic acid and ethanol in the ratio of 1:3 and preserved. Flower samples were taken out from the fixative and their gynoecium part was removed by forceps and placed in the sodium hydroxide for 6 hours in order to clear the extra tissues present. Then, samples were washed with distilled water and placed in 0.1% aniline blue overnight. Next day, samples were taken out from dye and placed over clean slide having drop of 10% glycerol. Cover slip was placed and samples were observed under confocal microscope.

3.5.3. *In vitro* pollen tube germination

Closed flowers were collected from the field. Pollens were incubated in the growth medium (pH 6.5) containing-

a. 10% sucrose
b. 100 ppm boric acid
c. 300 ppm calcium nitrate
d. 200 ppm magnesium sulphate and
e. 100 ppm potassium nitrate

Incubation was done at 25°C for overnight in growth medium. Then, drop of potassium iodide solution was added in the medium in order to stop the growth. Drop of medium was put on the slide and pollen tube germination was observed under compound microscope. 200 pollens per replicates were counted in which viable and non-viable pollens were estimated on the basis of the pollen tube germination.

3.5.4 Pollen load and *in vivo* pollen tube germination

One day open flowers were collected from the field. Their gynoecium part were taken out and placed on the slide. Samples were treated with few drops of potassium iodide solution. Coverslips were placed and observation done under compound microscope. No. of pollens and no. pollens with pollen tube were counted.

3.5.5 Pollen morphology

First day open flowers were collected from each variety and treatment. To check the pollen load, stigma was separated out with the help of pointed fine forceps and

teased on the metallic stubs. Samples were coated with gold palladium and observed under Field Emission Scanning Electron Microscope (FESEM, Carl Zeiss Merlin Compact 6073).

3.6 Statistical analysis

A random block design with nine biological replicated (three replications × three plants) per genotype in both the field was followed. Data collected from the experiment throughout the season were subjected to Two Way ANOVA (Analysis of Variance).

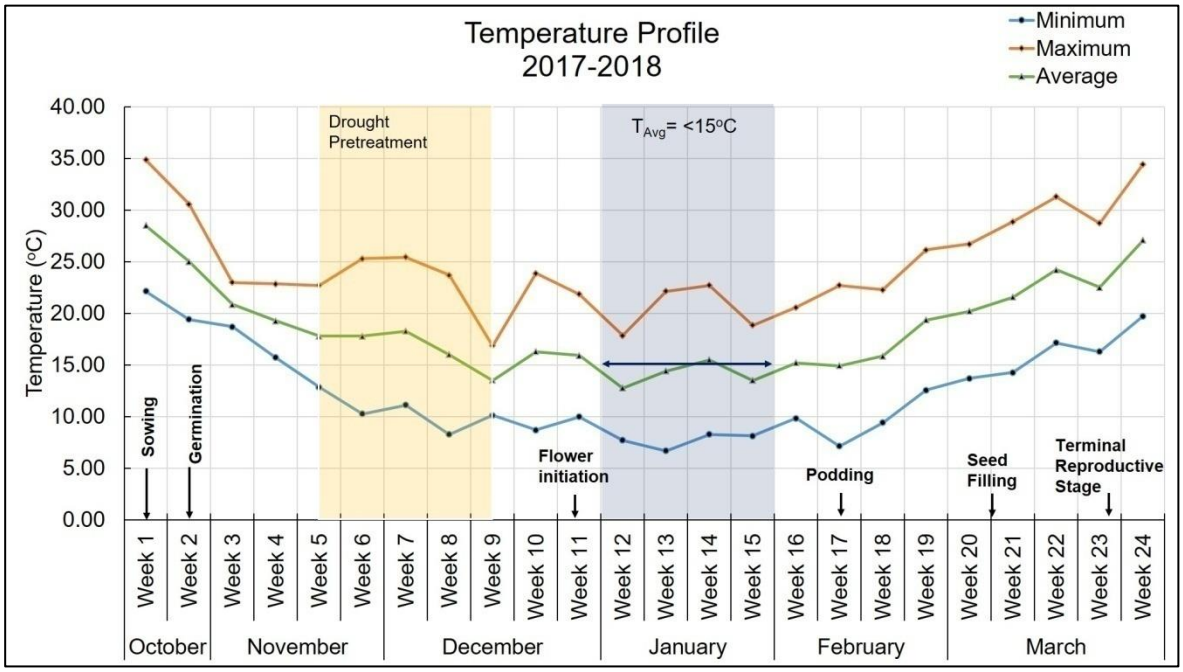
Chapter - 4

Results

4. RESULT

4.1 TEMPERATURE PROFILE AND REPRODUCTIVE GROWTH OF THE CHICKPEA DURING THE SEASON

Five genotypes of chickpea were grown in sowing season i.e. October in order to check the response of crop against mild drought stress. For the study, coolest period of the season was chosen. Temperature was recorded along experimental period i.e. from sowing to terminal reproductive stage (October 2017- March 2018). Emergence of flowering started from 4th week of December among all genotypes when average temperature went down to < 15°C. This temperature is harmful for the reproductive phase of chickpea (Figure 4.1).



.Figure 4.1 Weekly temperature record (minimum average, maximum average and mean temperature) of the season starting from 15/10/2017 to 31/03/2018 ("AccuWeather.com for Bathinda, India,"2018) for reproductive phenophases of chickpea.

Our observation mainly focused the coolest period of season because it causes detrimental effect on reproductive growth of plant. Hence, we can determine the effect of preconditioning on the parameters like flowering, pod set, seed set, pollen

viability, *in vivo* and *in vitro* pollen germination, pollen load etc. from tagged plants, number of flowers and pods were counted in order to generate secondary data like retention and abortion percentage etc.

4.2 NUMBER OF FLOWERS PRODUCED PER PLANT UNDER PRECONDTION AND NON-PRECONDITION TREATMENT

No significant difference was found among genotype and genotype × treatments, which were recorded in terms of flowers grown in a season; whereas, significant difference was observed between preconditioned and non-preconditioned. Numbers of flowers were more in preconditioned plants. In genotypes PBG1 (283.78 P/176.78 NP), GPF2 (202.22 P/97.00 NP) and PBG5 (295.11 P/100.89 NP) no. of flowers/plant were observed significantly more in preconditioned plants (Figure 4.2).

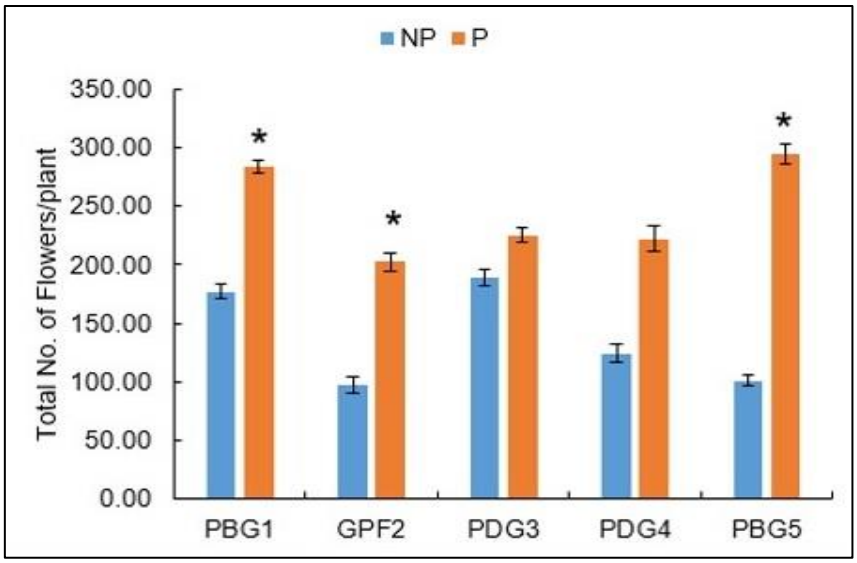


Figure 4.2 Number of flowers/plant in five chickpea genotypes in both non-precondition and precondition. Level of significance was measured at p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

4.2.1 FLOWER ABORTION/ RETENTION PER PLANT

In case of flower abortion and flower retention, there was no significant among genotype, genotype × treatment and between precondition and non-precondition treatments (Fig 4.3)

Table 4.1 Reproductive potential in terms of total flowers, flower abortion and retention in Chickpea genotypes to preconditioning (Mean±S.E).

	Preconditioned			Non-preconditioned		
Genotypes	**Total flower**	**%abortion**	**%retention**	**Total flower**	**%abortion**	**%retention**
PBG1	283.78±5.86	70.49±1.01	29.51±1.44	176.78±6.21	58.61±4.10	41.84±3.59
GPF2	202.22±7.18	60.61±4.06	39.39±3.29	97.00±6.81	57.73±5.19	42.27±4.00
PDG3	225.17±6.38	58.01±2.69	41.99±3.48	189.22±7.24	74.41±2.67	25.59±4.60
PDG4	222.11±10.95	66.19±3.41	33.81±4.58	124.33±7.80	71.84±2.23	28.16±2.68
PBG5	295.11±8.45	53.39±2.80	46.61±4.36	100.89±4.83	48.82±3.21	51.18±3.23

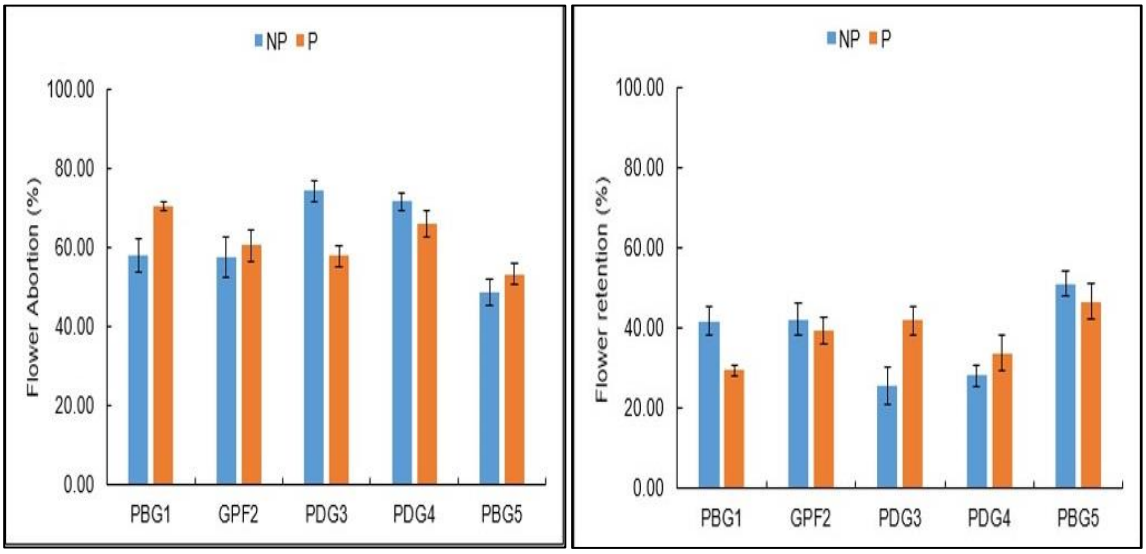


Figure 4.3 Percent of flower abortion and retention per plant in five chickpea genotypes in both non-precondition and precondition. Level of significance was measured at p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

4.3. NUMBER OF PODS PRODUCED BY THE PLANTS UNDER PRECONDITION AND NON PRECONDITION TREATMENT

In this case, there was no significance among genotype and genotype × treatment. Overall significance was observed between the treatments. Preconditioned PBG5 (127.33 P/49.11 NP) showed significant difference (Figure 4.4).

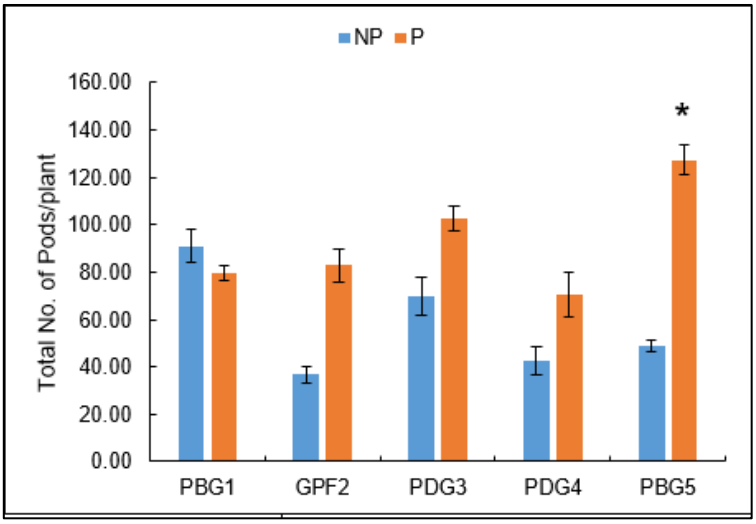


Figure 4.4 Number of pods/plant in five chickpea genotypes in both non-precondition and precondition. Level of significance was measured at p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

4.3.1 POD ABORTION/RETENTION PER PLANT

Data collected was analyzed by Two Way ANOVA with all pair wise multiple comparison using Tukey's test. There was no significance among genotype, genotype × treatment and between the treatments in terms of pod abortion/retention (Figure 4.5).

Table 4.2 Reproductive potential in terms of total pods, pod abortion and retention in Chickpea genotypes to preconditioning (Mean±S.E).

	Preconditioned			Non-preconditioned		
Genotypes	Total pods	% abortion	% retention	Total pods	% abortion	%retention
PBG1	79.56±2.87	34.97±7.33	65.03±3.57	90.89±7.10	26.75±4.96	51.02±4.04
GPF2	82.78±7.20	30.91±7.44	57.98±3.71	36.67±3.19	26.75±2.99	51.03±3.98
PDG3	102.50±5.13	45.14±3.55	54.86±10.61	69.67±8.17	7.60±1.68	59.06±5.25
PDG4	70.56±9.16	31.83±16.84	57.06±3.99	42.67±5.87	36.21±8.99	52.68±4.32
PBG5	127.33±6.35	6.10±1.87	92.77±0.54	49.11±2.48	16.30±14.44	72.59±3.48

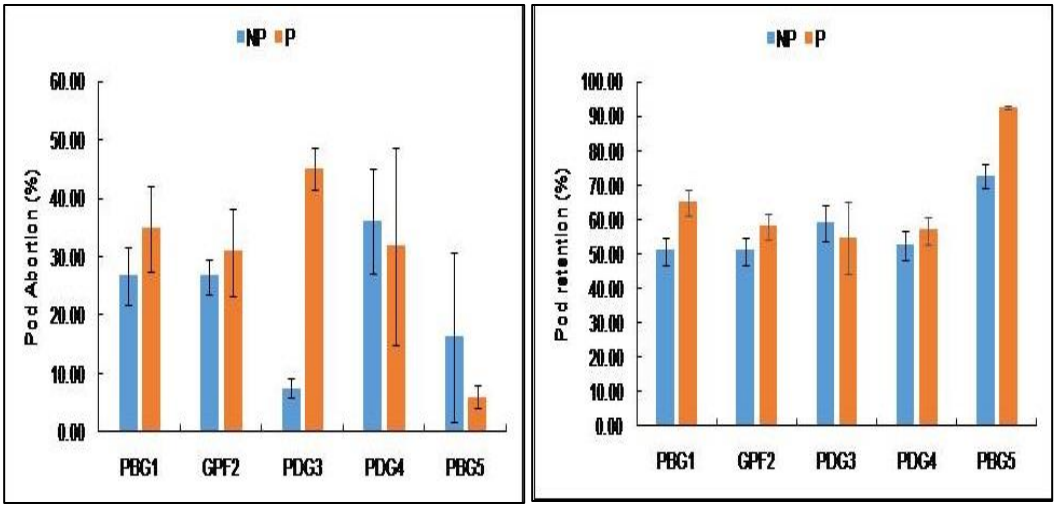


Figure 4.5 Percent of pod abortion and retention per plant in five chickpea genotypes in both non-precondition and precondition. Level of significance was measured at p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

Pods of all five genotypes were collected. Pods which had seeds were termed as developed seeds and pods without seeds as undeveloped seeds. Photographs were taken, in which undeveloped pods are placed at left side and developed ones at right side (Figure 4.6).

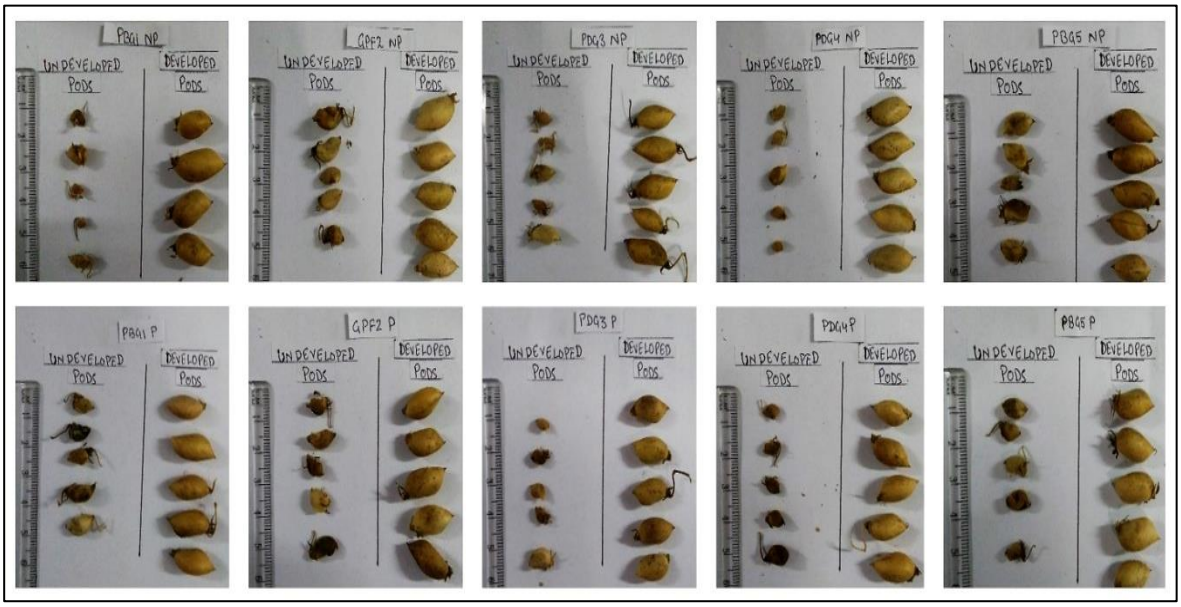


Figure 4.6 Undeveloped and developed pods showing difference in their size and shape in precondition and non-precondition chickpea genotype.

4.4 NUMBER OF SEEDS PRODUCED BY THE PLANTS UNDER PRECONDITION AND NON PRECONDITION TREATMENT

There was no significance among genotype, treatments and genotype × treatment in terms of seed produced per plant (Figure 4.7).

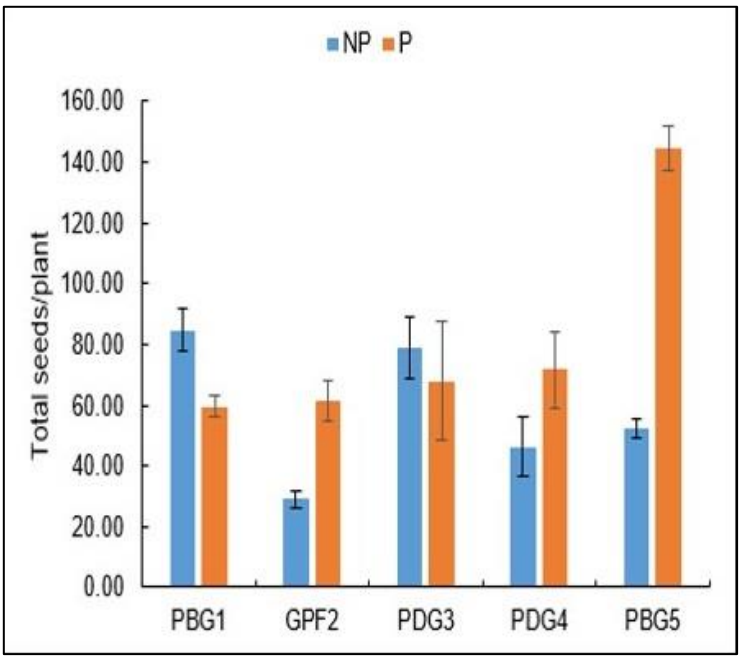


Figure 4.7 Number of seeds/plant in five chickpea genotypes in both non-precondition and precondition. Level of significance was measured at p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

4.4.1 SEED ABORTION/RETENTION PER PLANT

Data collected was analyzed by Two Way ANOVA with all pair wise multiple comparison using Tukey's test.

In case of seed abortion, there was no significant among genotypes and genotype × treatment. Significant difference was observed between precondition and non-precondition treatments in seeds abortion. Seed abortion was significantly more in PBG1 (2.93% P/52.44%NP) preconditioned plants than non-preconditioned plants.

In case of seed retention, there was no significant among genotype, genotype × treatment and between treatments (Figure 4.8).

4.5 FINAL SEED YIELD/PLANT

In this case, there was no significance among genotype and genotype × treatment. Overall significant difference was observed between the treatments, seed yield

was more in preconditioned plants. Seed yield were observed more in preconditioned plants of GPF2, PDG3, PDG4 and PBG5. But only PBG5showed significant increase in seed yield on preconditioning (Figure 4.9).

Table 4.3 Reproductive potential in terms of total seeds, seed abortion and retention in Chickpea genotypes to preconditioning (Mean±S.E).

	Preconditioned			Non-preconditioned		
Genotypes	Total seeds	% abortion	% retention	Total seeds	% abortion	%retention
PBG1	59.67±3.34	37.41±9.33	62.59±3.24	84.78±7.12	11.61±4.84	66.16±4.34
GPF2	61.44±6.82	17.42±6.66	71.47±3.33	29.11±2.82	7.39±3.37	70.39±4.16
PDG3	68.00±19.80	12.85±6.86	87.15±1.90	79.11±10.19	10.88±8.11	55.79±4.50
PDG4	71.56±12.44	21.34±11.34	67.55±4.28	46.33±9.74	9.94±4.00	67.84±4.17
PBG5	144.56±7.45	2.93±4.26	97.07±0.51	52.44±2.95	9.10±4.49	79.79±3.26

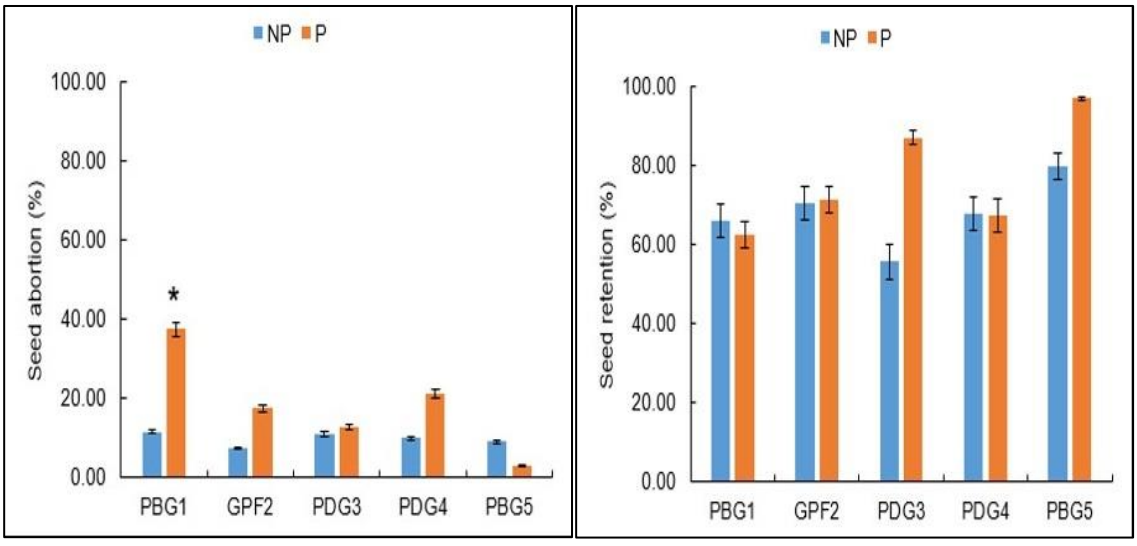


Figure 4.8 Percent of seed abortion and retention per plant in five chickpea genotypes in both non-precondition and precondition. Level of significance was measured at p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

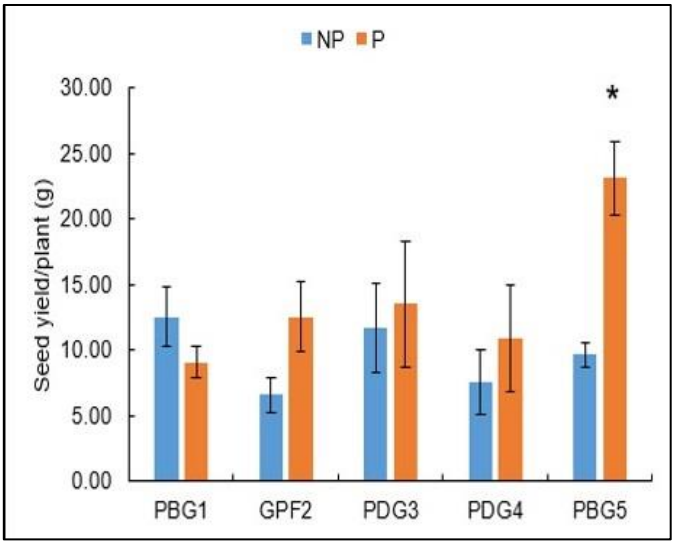


Figure 4.9 Seed yield (g)/plant in five chickpea genotypes in both non-precondition and precondition. Level of significance was measured at ±p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

Seeds of all five genotypes were collected. Seeds which had deformed structure were called as undeveloped seeds while round and healthy ones were termed as developed seeds. Photographs were taken by placing undeveloped seeds at left side and developed seeds at right side (Figure 4.10)

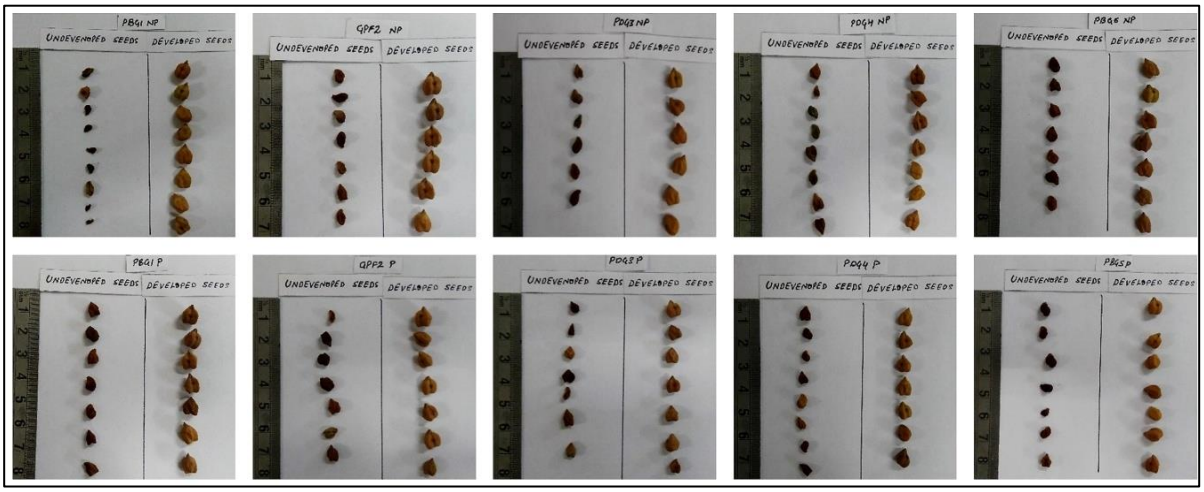


Figure 4.10 Undeveloped and developed seeds showing difference in their size and shape in precondition and non-precondition chickpea genotype.

4.6. % POLLEN VIABILITY

Pollen viability was observed during the period of chilling stress in non-preconditioned and preconditioned plants of five genotypes. Pollen viability was

significantly increased in preconditioned plants of two genotypes PBG1 (92.75 P/82.75 NP) and PBG5 (93.42 P/83.42 NP) (Figure 4.11). Pollen viability was improved by preconditioning.

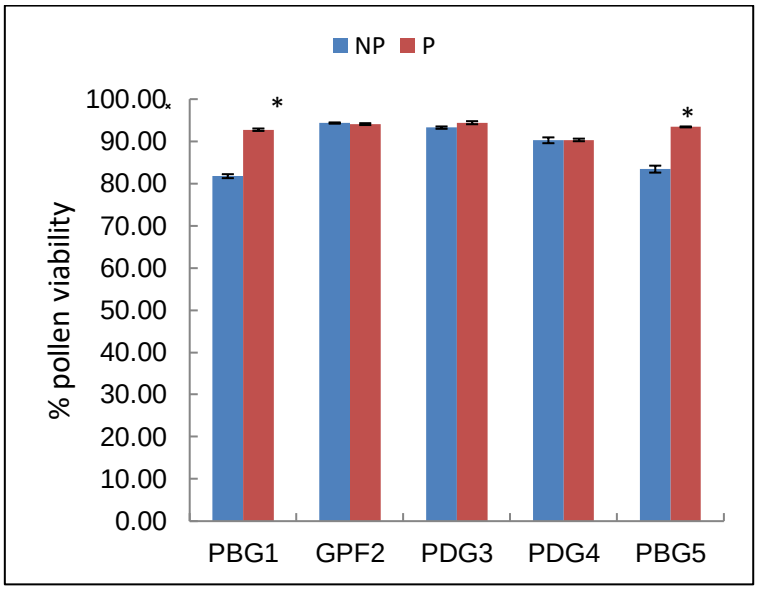


Figure 4.11 Percent pollen viability in five chickpea genotypes in both non-precondition and precondition during chilling stress. Level of significance was measured at p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

The pollens with round shape and deep stained while those with deformed structures and without stains were termed as unviable pollens. Photographs were taken and deformed pollens are indicated with the help of black arrow (Figure 4.12).

4.7. *IN-VITRO* POLLEN TUBE GERMINATION

Significant difference was observed among genotype, genotype × treatment and between precondition and non-precondition treatments during the duration of chilling stress. In-vitro pollen germination observed was more in preconditioned Plants of PBG5 (92.42 P/45.42 NP) while significantly low in preconditioned PBG1 (60.00 P/87.75 NP) (Figure 4.13). PBG1 is sensitive to cold stress.

In vitro pollen tube germination was observed in all five genotypes of chickpea. Pollen tube germination is counted when pollen tube is equal to the size of pollen.

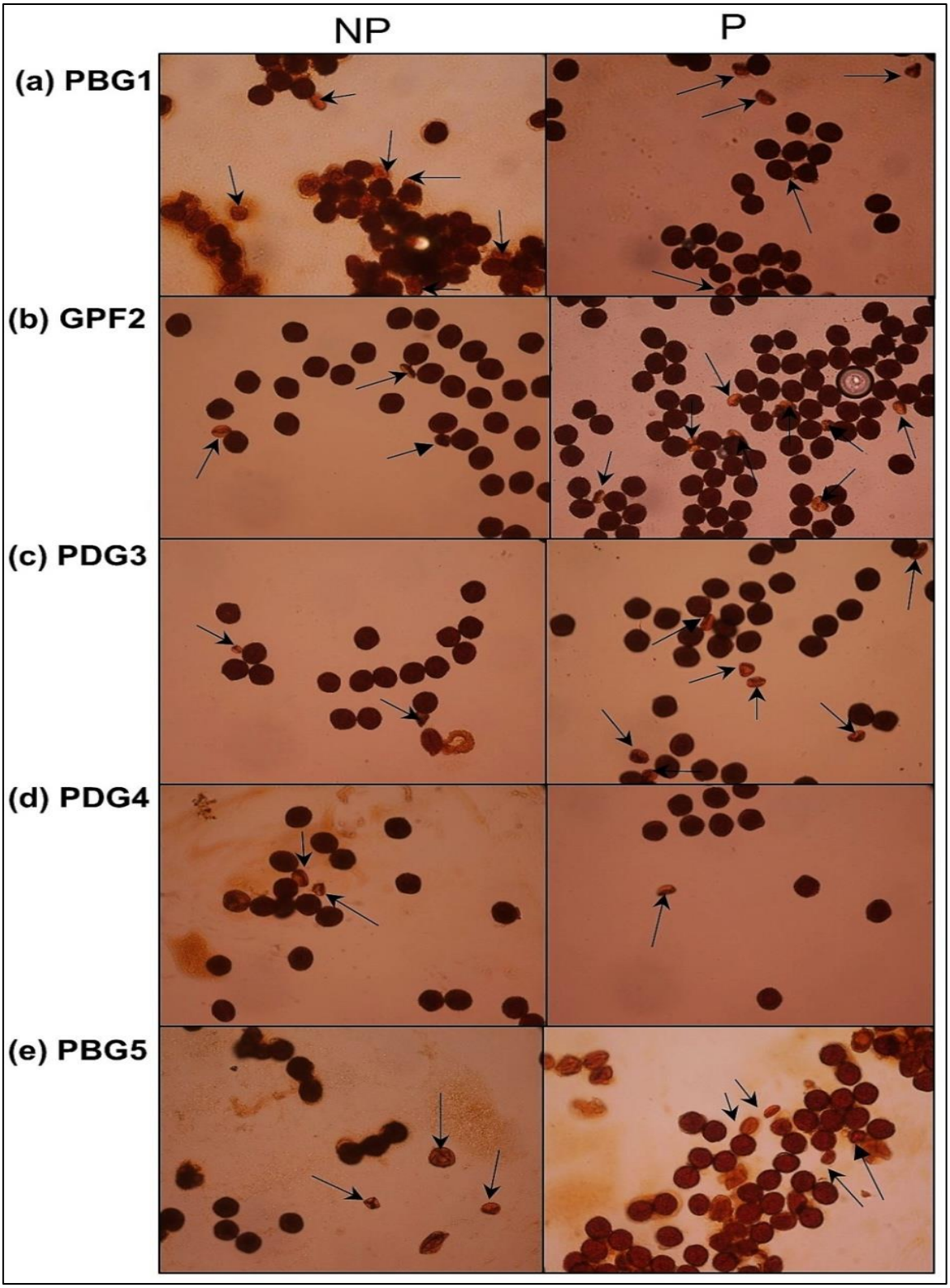


Figure 4.12 Pollen viability of precondition and non-precondition of five genotypes of chickpea during cold stress (Magnification at 40X).

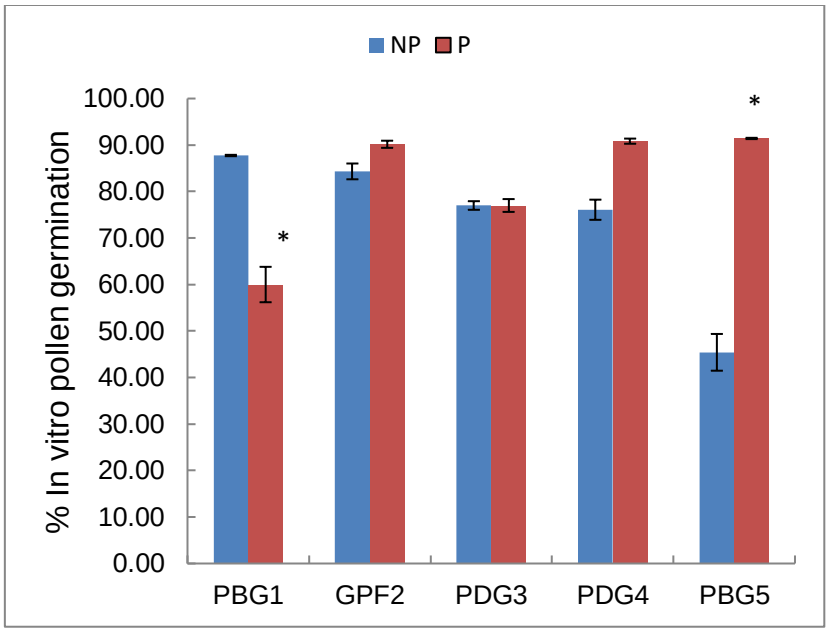


Figure 4.13 Percent *In-vitro* pollen germination in five chickpea genotypes during chilling stress in both non-precondition and precondition. Level of significance was measured at p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

Photographs were taken in which pollens of non-preconditioned are placed on left hand side and preconditioned one at right hand side (Figure 4.15).

4.8. POLLEN LOAD

Pollen load is number of pollen grains on the stigma.

IN VIVO POLLEN GERMINATION

No significant difference was observed among genotype, genotype × treatment and between precondition and non-precondition treatments (Figure 4.14).

Photographs of *in vivo* pollen germination were taken. Flat substrate like structure is stigma while small tubules present on it are pollens. Non-preconditioned are placed at left side while preconditioned on the right side (Figure 4.16).

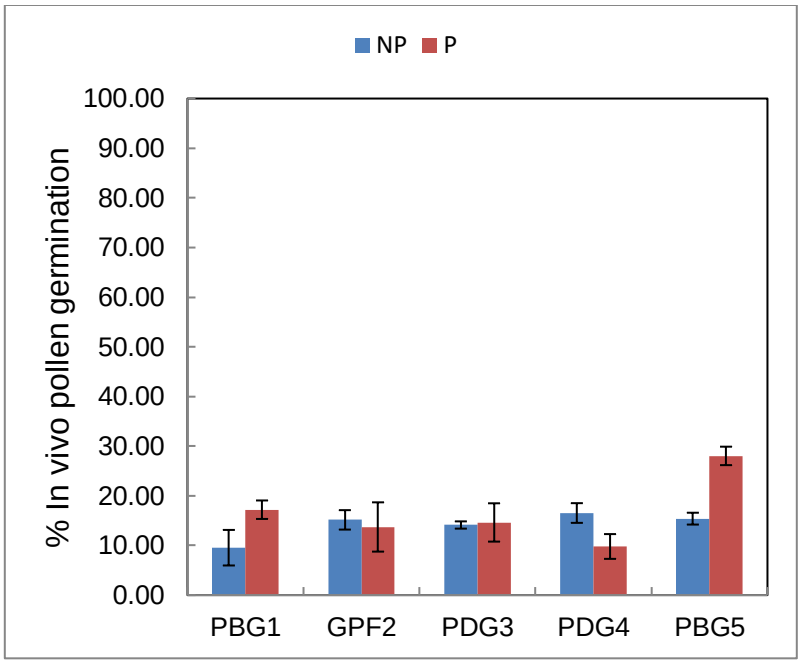


Figure 4.14 Percent *In-vivo* pollen germination in five chickpea genotypes during chilling stress in both non-precondition and precondition. Level of significance was measured at p<0.050 using pair-wise multiple comparison procedure using Tukey's test.

4.9. Pollen morphology

Pollen surface was observed with the help of Scanning electron microscope. Shriveled structures of pollens are indicated with the white arrows. PBG1 didn't show any shriveled pollen in neither preconditioning nor non-preconditioning treatments (Figure 4.17). In case of GPF2, deformed pollens were observed in non-preconditioned plants (Figure 4.18). Chilling stress caused formation of shriveled pollens in both treatments of PDG3 and PDG4 (Figure 4.19) and (Figure 4.20) respectively. In PBG5, chilling stress caused injury in preconditioned plant (Figure 4.21).

In vivo pollen tube growth was observed with confocal microscope. No pollen tube was observed in 1st day open flowers (Figure 4.22). In 2nd day open flowers, pollen tube was seen in non-preconditioned PBG1 and PDG3. In preconditioning, it was observed in PDG4 (Figure 4.23). Most number of pollen tube germination was

seen in 3rd day open flower. In non-preconditioning, it was present in PDG3, PDG4 and PBG5 while preconditioned PBG5 (Figure 4.24)

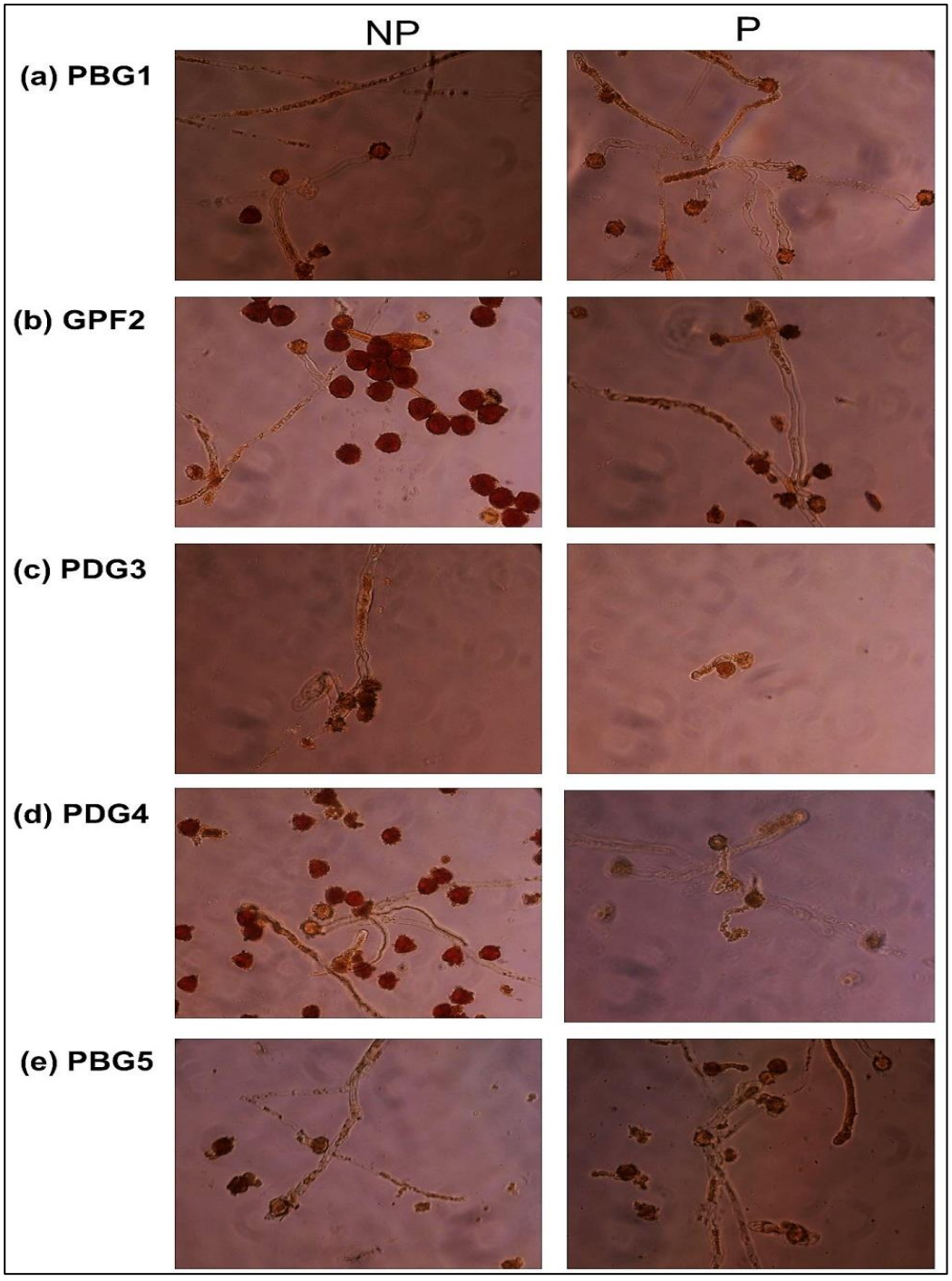


Figure 4.15 *In vitro* pollen tube germination of precondition and non-precondition of five genotypes of chickpea during cold stress.

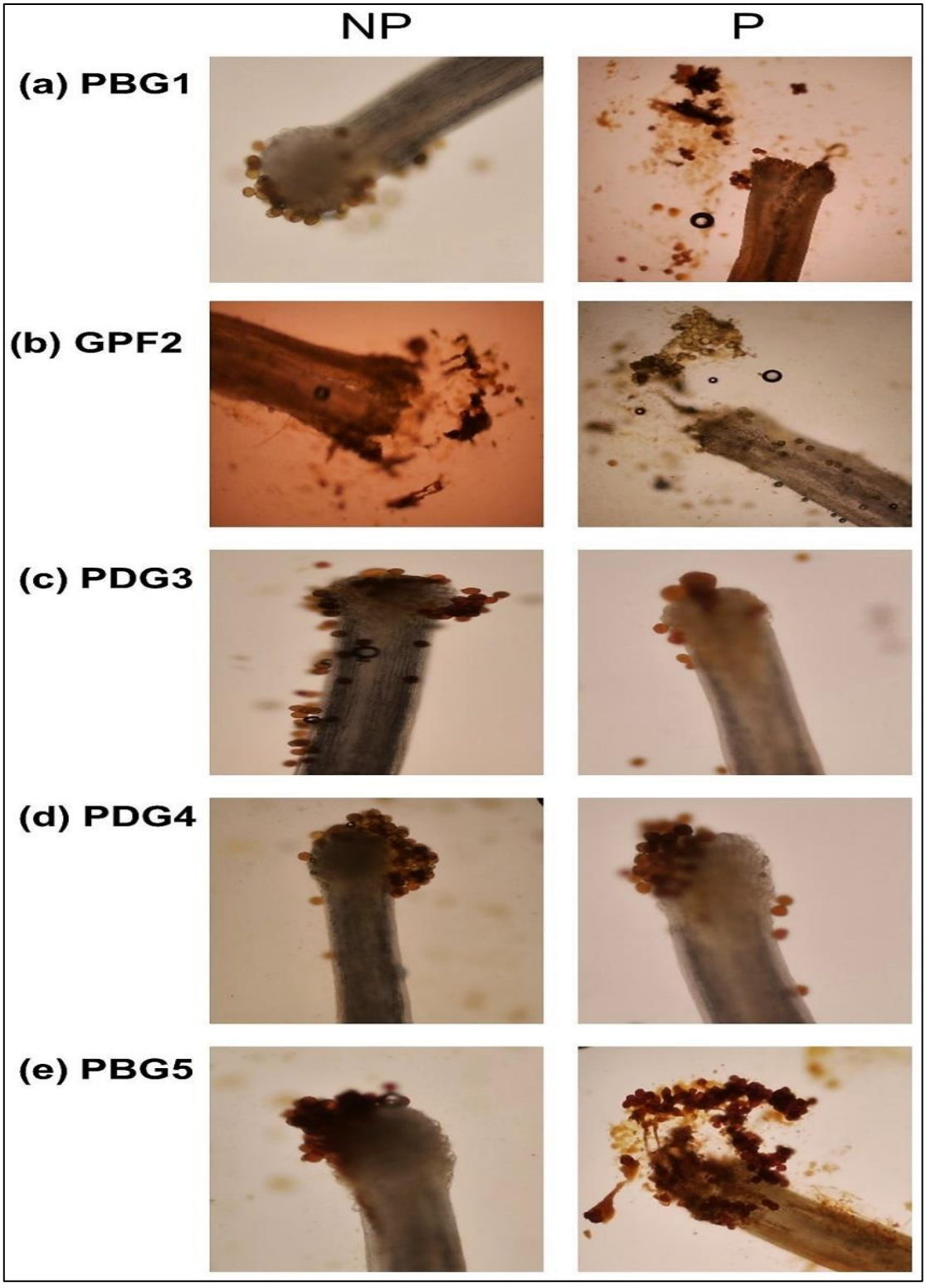


Figure 4.16 Pollen load of precondition and non-precondition of five genotypes of chickpea during cold stress.

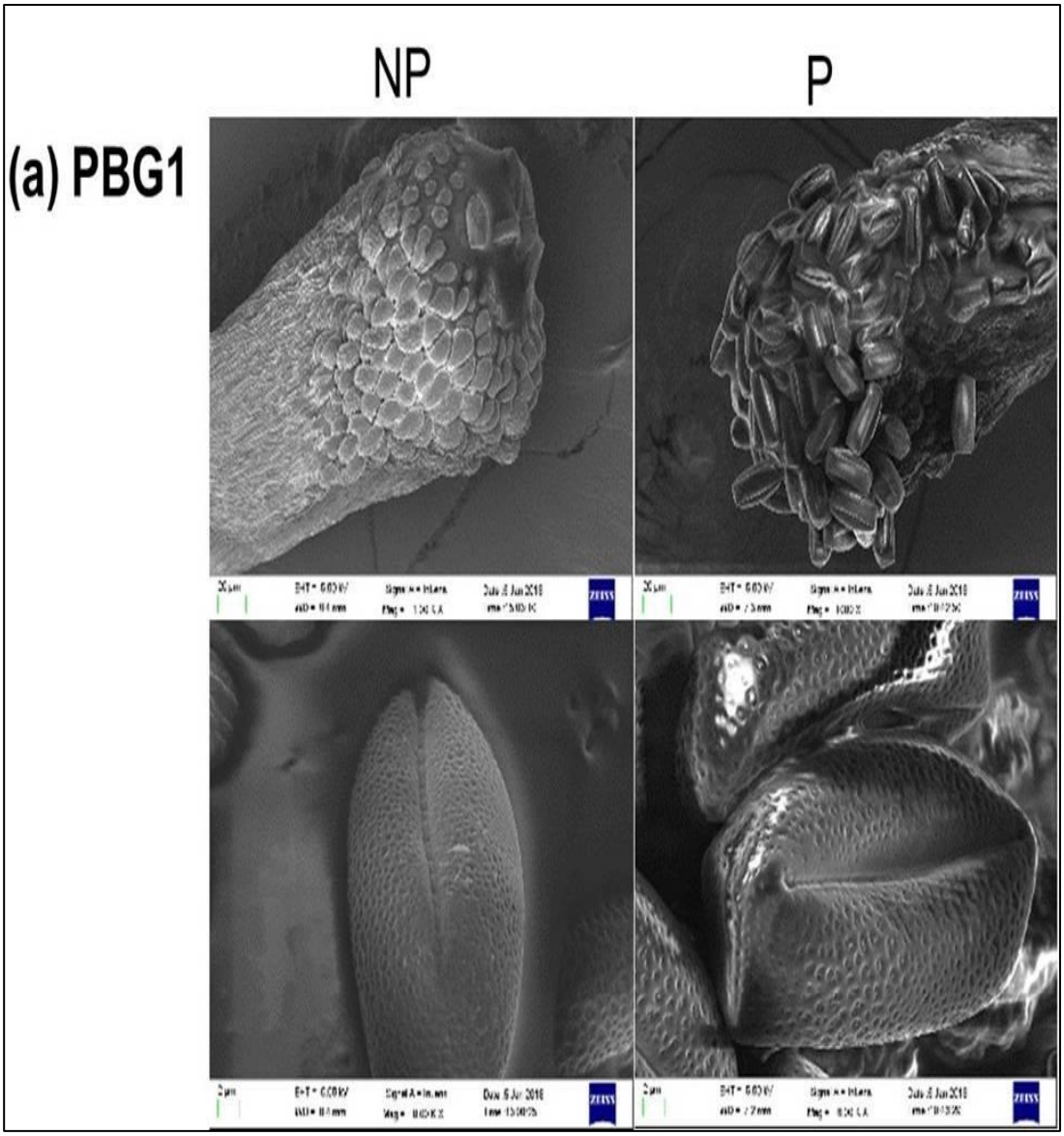


Figure 4.17 Field Emission Scanning Electron Micrographs of chickpea stigma showing formed and deformed (white arrows) pollens during cold stress in genotype PBG1.

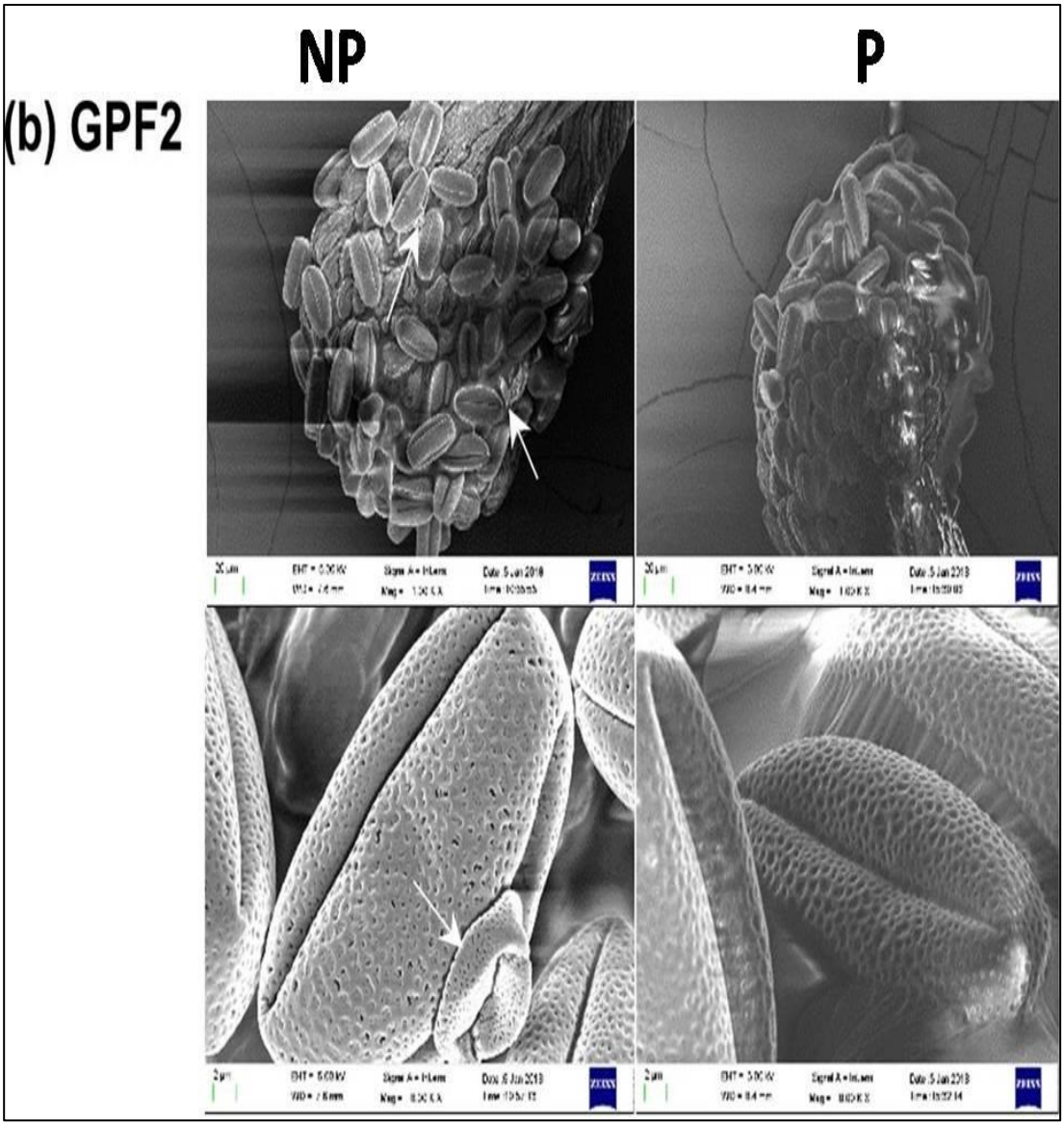


Figure 4.18 Field Emission Scanning Electron Micrographs of chickpea stigma showing formed and deformed (white arrows) pollens during cold stress in genotype GPF2.

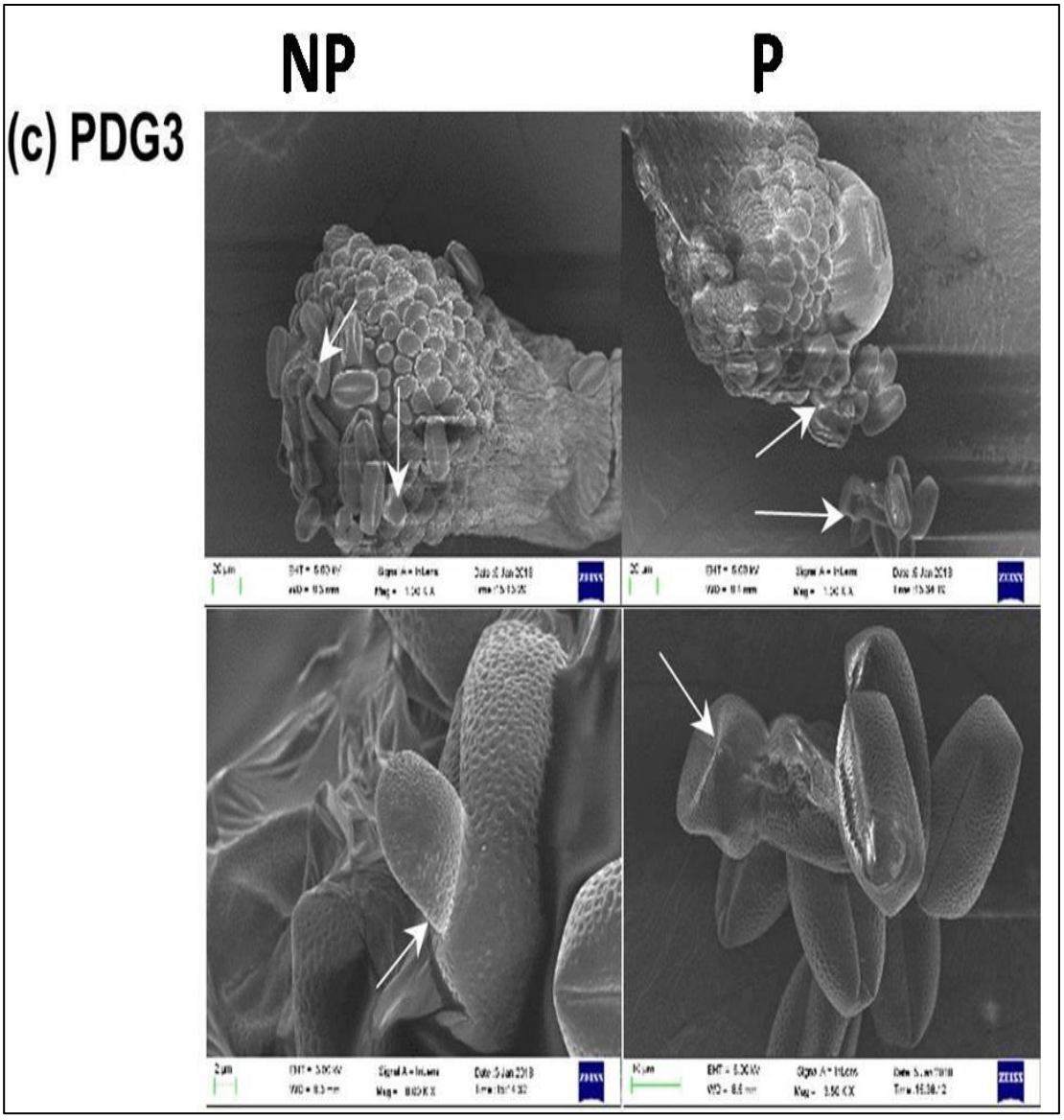


Figure 4.19 Field Emission Scanning Electron Micrographs of chickpea stigma showing formed and deformed (white arrows) pollens during cold stress in genotype PDG3.

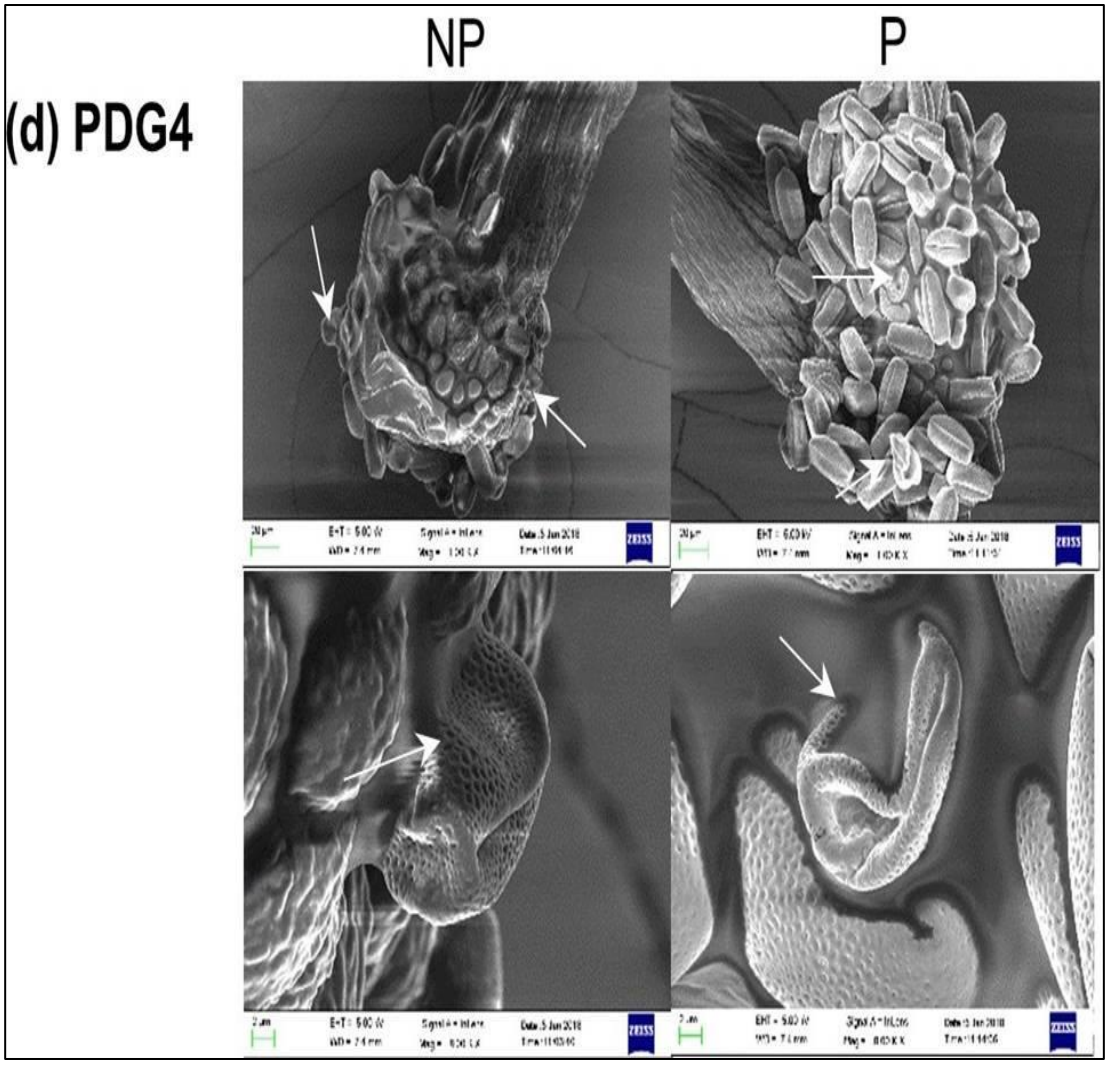


Figure 4.20 Field Emission Scanning Electron Micrographs of chickpea stigma showing formed and deformed (white arrows) pollens during cold stress in genotype PDG4.

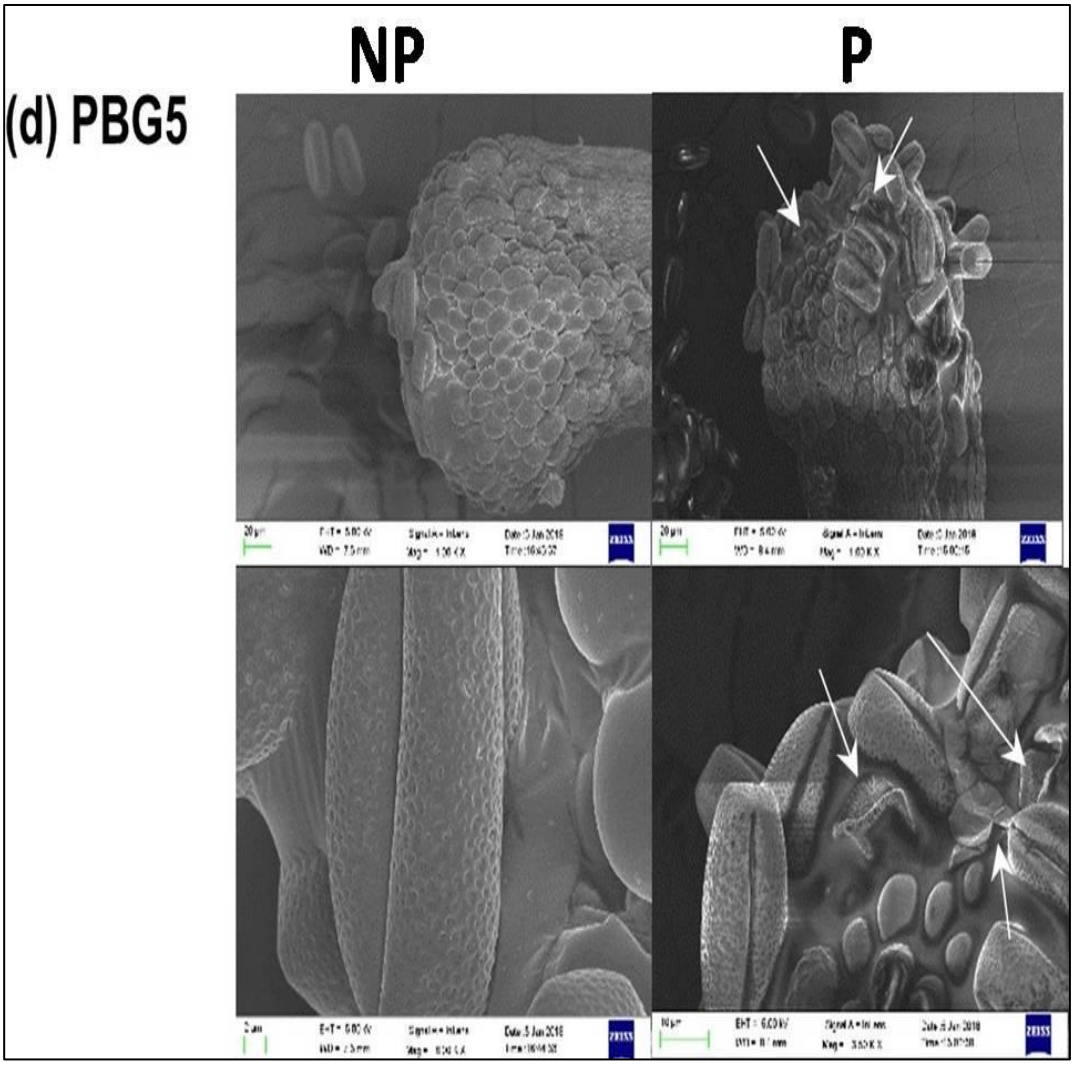


Figure 4.21 Field Emission Scanning Electron Micrographs of chickpea stigma showing formed and deformed (white arrows) pollens during cold stress in genotype PBG5

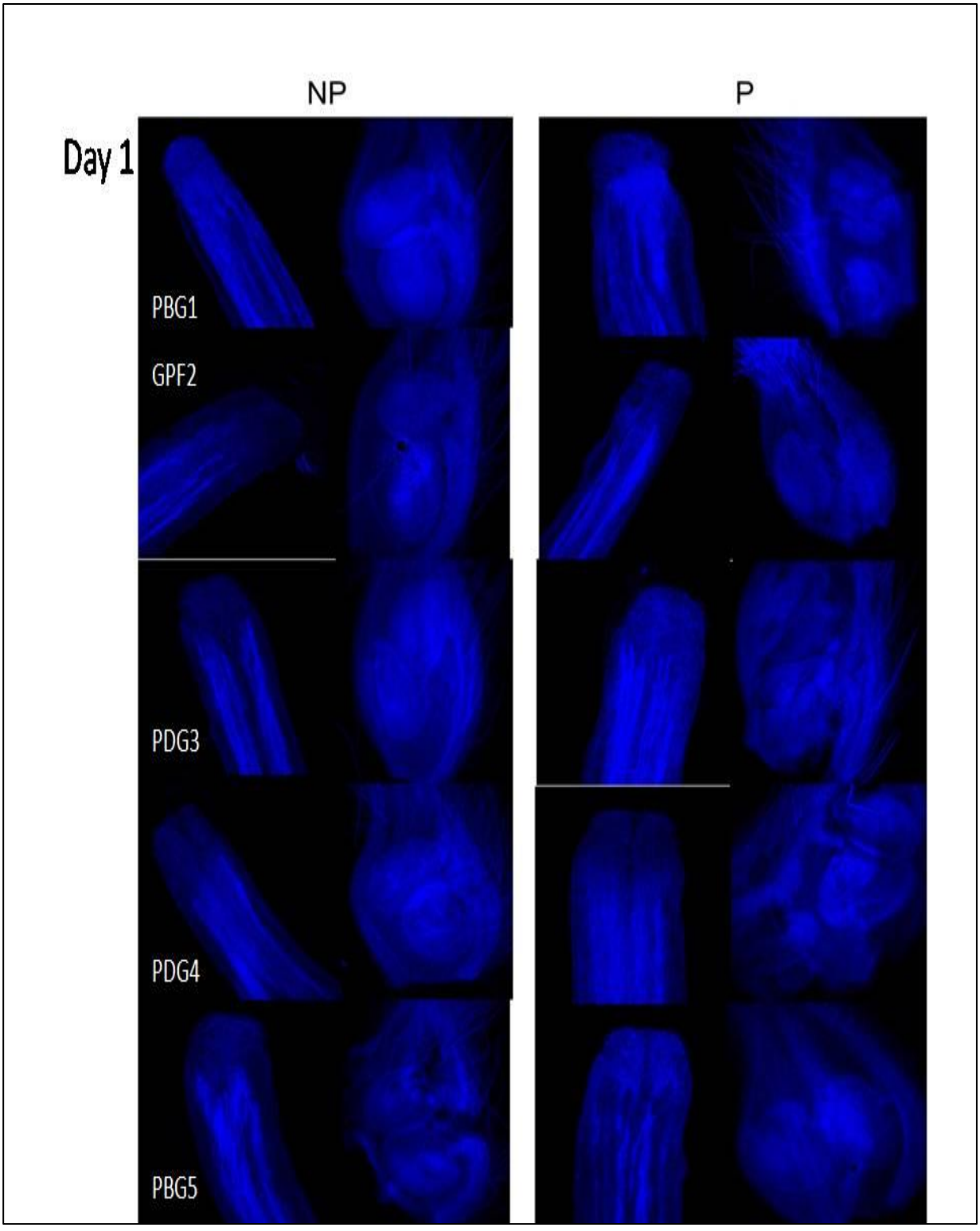


Figure 4.22 Confocal Mircographs showing *In vivo* pollen tube growth in 1st day open flower of five genotypes of chickpea during cold stress.

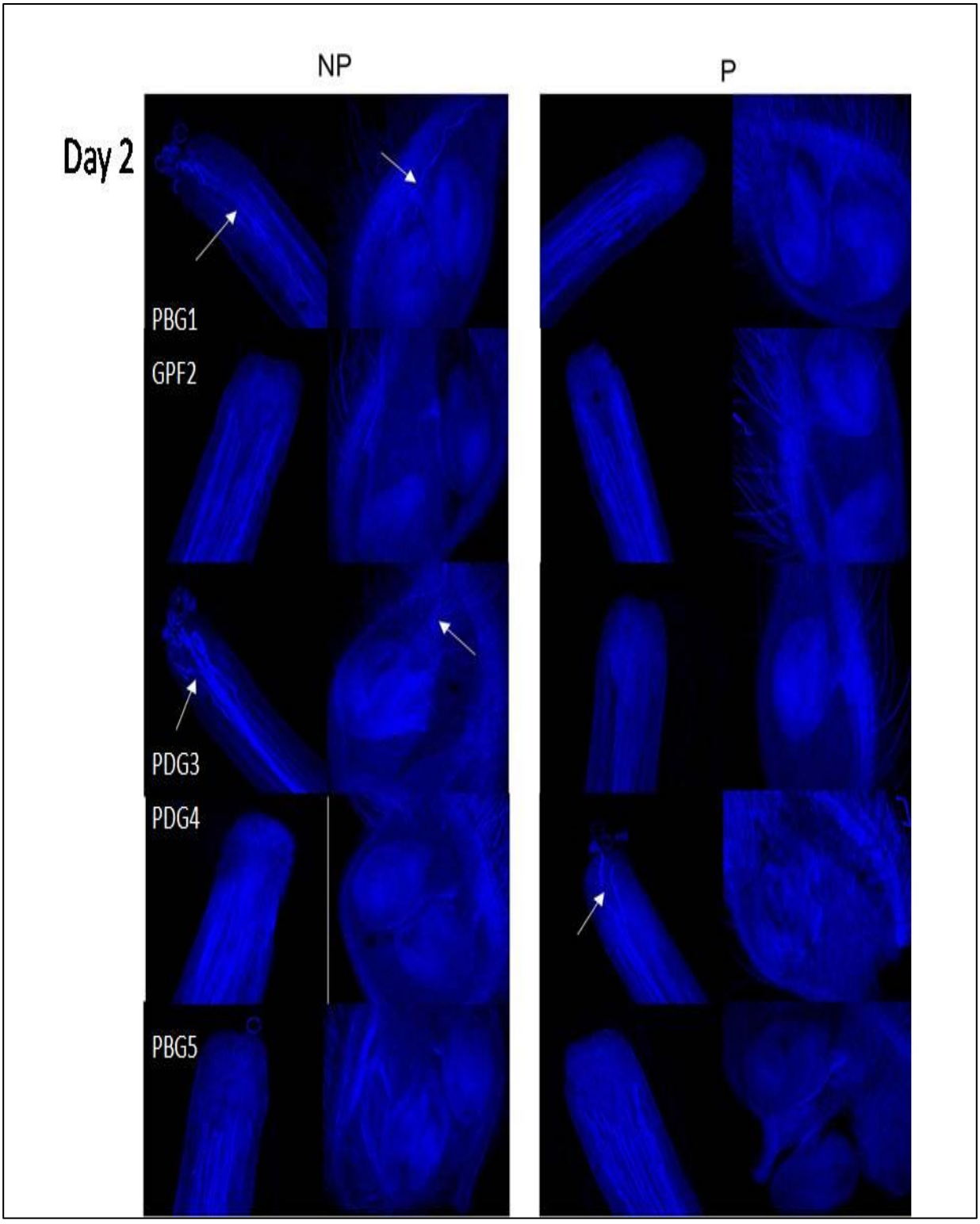


Figure 4.23 Confocal Mircographs showing *In vivo* pollen tube growth in 2nd day open flower of five genotypes of chickpea during cold stress.

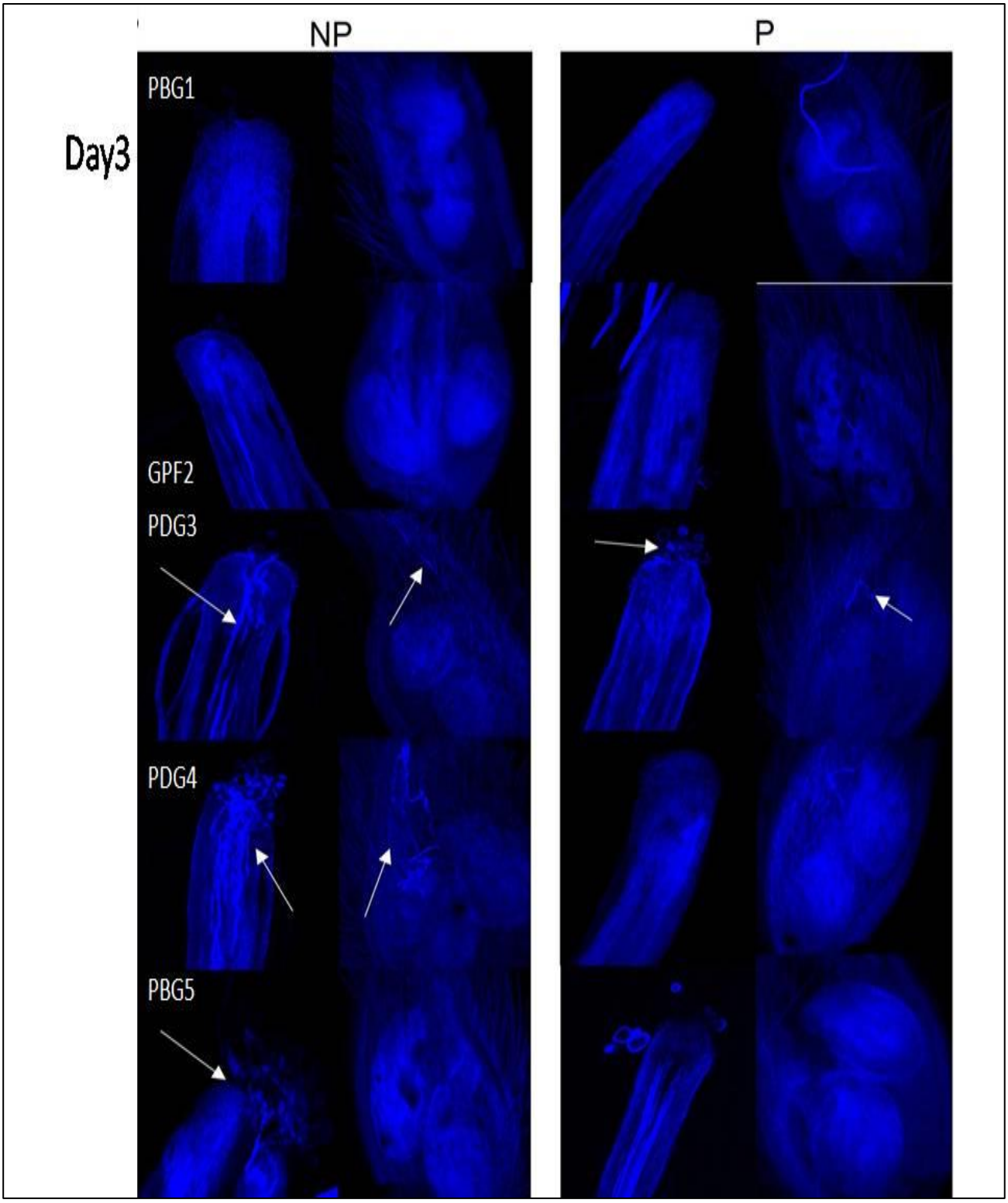


Figure 4.24 Confocal Mircographs showing *In vivo* pollen tube growth in 3rd day open flower of five genotypes of chickpea during cold stress.

Chapter – 5

Discussion

5. DISCUSSION

This study was conducted to understand the effect of lethal chilling stress in the month of December, January and February on the preconditioned genotypes of chickpea. Temperature below 10°C is detrimental for the reproductive growth of the chickpea. In present study, chilling stress during the January month remained <15°C. Preconditioned plants showed more number of flowers than non-precondition. Preconditioning has significantly increased the number of flowers in PBG1, GPF2 and PBG5. Abortion rate was highest in PBG1 and lowest in PDG3 while, retention rate showed the opposite results. PDG3 showed highest and PBG1 showed lowest retention rate. It means, preconditioned PDG3 positively responded to chilling stress while PBG1 is sensitive to low temperature. The difference in proportion of flower abortion can be related to fluctuating weather such as low temperature (Zaiter *et al*., 1995).

In case of pod formation, preconditioned PBG5 has shown significant increase. In PDG3 and PBG5 highest and lowest pod abortion rate was observed respectively while, maximum and minimum rate of pod retention rate was seen in PBG5 and PDG3 respectively. This signifies preconditioned genotype PBG5 is efficient in not only in pod formation but pod retention also. At this stage, PDG3 does respond to chilling stress. More number of pods can be related to more number of flowers. Variation in pod abortion/retention rate can be the consequence of differential sensitivity to chilling stress (Srinivasan *et al*., 1999). One reason can be defective source-sink relationships at the time of fruit formation during cold stress. If flowers don't undergo the pod formation in cool climate, it leads to wastage of sink capacity. But, this can be an adaptive mechanism for the formation of secondary branches for more flowers (Srinivasan *etal*., 1999).

Preconditioned PBG5 produced largest number of seeds, seed retention rate and seed yield. PBG1 showed highest abortion rate. Overall PBG5 has performed best among the five genotypes .During chilling stress, sucrose import at the time of seed development gets impaired at enzyme level (Nayyer *et al*., 2007). Higher rate

of seed abortion in preconditioning may be due to early flowering, podding and pod setting.

Pollen viability rate in preconditioned plants were higher than non-preconditioned chickpea plant during the chilling stress. PBG1 and PBG5 genotypes emerged with the significant increase in the rate of pollen viability. In case of *in vitro* pollen tube germination, PBG5 maintained its tolerance towards prolonged period of chilling stress while preconditioned PBG1 observed as sensitive. No significant difference was observed in both the treatments *in-vivo* pollen germination. Functioning of the pollen may be affected by the low temperature stress (Srinivasan *et al*., 2010). Abnormalities in the pollen morphology were observed in stressed plants. This may indicate their sensitivity towards the chilling stress (Kumar *et al*., 2010). Maximum pollen tube growth was seen in third day open flowers. There are chances that chilling stress delays the process of fertilization in the chickpea (Clarke *et al*., 2001).

Summary and Conclusion

SUMMARY AND CONCLUSION

Major loss in the crop yield is contributed by the various abiotic stresses. When plant is exposed to the temperature below 15°C, it experiences the chilling stress especially t reproductive stage.

To understand the effect of drought preconditioning on chickpea plant during lethal chilling stress this study was conducted. Five genotypes were selected such as PBG1, GPF2, PDG3, PDG4 and PBG5. Sowing of seeds were done in the month of October and after achieving the full vegetative growth, plants were exposed to four weeks of drought pretreatment (December). In the month of January, plants experienced chilling stress as the average temperature was below 15°C. Plants were harvested in March when terminal reproductive growth was attained. Number of flowers and retention rate was more in preconditioning as compared to non-preconditioned plant. Preconditioned PBG1 and PBG5 showed the highest number of flower/plant while no major significance was observed in abortion and retention rate. Pod/plant and seed yield was more in preconditioned PBG5.

Reproductive parameters in terms of pollen viability and *in vitro* pollen germination improved during the cold stress whereas, *in vivo* germination didn't show any significant difference. Stressed plants showed defects in their pollen morphology while 3rd day open flowers were observed with more pollen tube growth. This clearly indicated temperature is important factor for maintaining healthy pollen development and pollen-pistil interaction.

To conclude this study, it can be stated that preconditioning influences the growth of the plant as it increases the seed yield.

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Appendices

APPENDIX A

Preparations of staining solutions and accessories

I. **Iodine potassium iodide staining solution:** Mix1g of potassium iodide and .5g of iodide in 100 mL of distilled water.

II. **10% glycerine:** 1 mL of glycerol is dissolved in 9 mL of distilled water.

III. **Fixing agent:** Mix 112.50 mL of glacial acetic acid and 337.50 mL ethanol. Together making volume of 450 mL.

IV. **8 N NaOH:** Dissolve 144 g of NaOH pellets in 450 mL of distilled water.

V. **0.1% aniline blue:** First prepare .1M k2KPO4 by dissolving 0 .871 g of K2HPO4 in 50 mL of distilled water. Then, mix 50 mg of aniline blue in 50 mL of .1M K2HPO4.