PHYSIOLOGICAL STUDIES ON MOISTURE STRESS TOLERANCE IN CHICKPEA (*Cicer arietinum* L.) GENOTYPES

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
BOTANY
(Minor Subject: Biochemistry)

By

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LUDHIANA-141004

2016

CERTIFICATE I

This is to certify that the thesis entitled, **"Physiological studies on moisture stress tolerance in chickpea (*Cicer arietinum* L.) genotypes"** submitted for the degree of **Master of Science** in the subject of **Botany** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Vaishali Sharma (L-2014-BS-271-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled, **"Physiological studies on moisture stress tolerance in chickpea (*Cicer arietinum* L.) genotypes"** submitted by **Vaishali Sharma (L-2014-BS-271-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc** in the subject of **Botany** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee after an oral examination on the same.

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ACKNOWLEDGEMENT

All the unfathomable analogies humble thanks giving are preferred to Almightily Lord, the most gracious and compassionate, whose blessing and exaltation flourished my thoughts and thrived my ambitions to eventually shape up the cherished fruit of my modest endeavors to this manuscript.

I deem it an utmost pleasure to be able to express the heartiest gratitude and deep sense of indebtness to my esteemed Major Advisor ***Dr.(Mrs) Jagmeet Kaur****, Senior Botanist, Pulses section, Plant Breeding and Genetics PAU, Ludhiana for her unfailing patience, skillful, affectionate and generous guidance. Acknowledgement perhaps is too narrow in scope to express my reverence for her indelible help to elucidate my problems, unreserved enthusiasm and providing me the necessary research facilities for this study.*

My sincere thanks is reserved for ***Dr (Mrs) Seema Bedi****, Head, Department of Botany for providing me with the necessary facilities. I feel elated to acknowledge the cooperation extended by esteemed members of advisory committee,* ***Dr.(Mrs) Nirmaljit Kaur****, Botanist, Department of Botany,* ***Dr. Inderjit Singh****, Senior Plant breeder, Department of Plant breeding and genetics,* ***Dr.(Mrs) Satvir Kaur****, Assistant Biochemist, Department of Biochemistry,* ***Dr (Mrs) Seema Bedi,*** *Proffesor (Dean PG's Nominee) Department of Botany, for their invaluable insights and suggestion during the tenure of my work.*

I would like to thank all the special people who have taught me starting with my school teachers, my undergraduate teachers, my graduate teachers and examiners. It was my pleasure to work with my seniors Sukhpreet Kaur, Norah Johal, Priya, Mamta Gupta, Jaspreet Kaur and Manpreet Kaur they have been an invaluable support day in, day out during these years. Words are not enough to express my thanks to my abiding friends Sandeep Kaur, Silky Verma, Kawaljit Kaur and Manjeet Kaur for their valuable support.

A Father is someone who wants to catch you before you fall, but instead picks you up, brushes you off, and lets you try again. Special thanks to my Papa for ingraining in me the ways of logic which have become an intricate part of who I am and who I will be. It's a long journey, and he has always stood beside me giving all the support and encouragement. I owe a lot to my mother, who helped me at every stage of my personal and academic life, and longed to see this achievement come true. Special thanks to my both sisters Anshu and Kashish for their unflagging love and care throughout my life.

All may not have been mentioned but none is forgotten.

Vaishali Sharma

Title of the Thesis : Physiological studies on moisture stress tolerance in chickpea (*Cicer arietinum* L.) genotypes.
Name of the Student and Admission No. : Vaishali Sharma (L-2014-BS-271-M)
Major Subject : Botany
Minor Subject : Biochemistry
Name and Designation of Major Advisor : Dr. (Mrs.) Jagmeet Kaur, Senior Botanist
Degree to be Awarded : M.Sc.
Year of award of Degree : 2016
Total pages in thesis : 59+ Vita
Name of the University : Punjab Agricultural University, Ludhiana – 141 004, Punjab, India

ABSTRACT

The present investigation was carried out in the Department of Plant Breeding & Genetics, Punjab Agricultural University, Ludhiana to evaluate differential tolerance of chickpea genotypes under moisture stress condition. Two experiments were conducted, in first experiment twenty genotypes were raised in PVC pipes under three different conditions; control, 50% and 75% restricted irrigation whereas in second experiment same genotypes were sown in the field under irrigated and rainfed conditions. Growth behavior was studied during vegetative, reproductive and harvest stage. Significant changes in the shoot (plant height, biomass, shoot: root and stomatal frequency) and root traits (root volume, root mass, root area and nodule weight) under restricted irrigation indicated the effect of moisture stress on plant. During reproductive phase relative water content, photosynthetic rate and legahaemoglobin content declined under moisture stress. From shoot, root traits and physiological parameters, genotypes were categorized as tolerant and sensitive genotypes. Six selected genotypes (three sensitive GL 29095, GL 12003and GNG 2171and three tolerant BGD 1094, ILC 3279 and L 555) were further evaluated for biochemical studies in leaves and seeds. Under moisture stress conditions higher accumulation of osmotic solute proline and total soluble sugars were observed in tolerant genotypes as compared to sensitive genotypes. An increment in the activity of superoxide dismutase, peroxidase and catalase activity was also recorded in tolerant genotypes. Decline in starch content in seeds of chickpea was observed. Moisture imposed stress finally caused a decline in yield and yield attributes of sensitive genotypes as compared to tolerant genotypes. BGD 1094, ILC 3279 and L 555 chickpea genotypes were identified as moisture stress tolerant genotypes.

Keywords: Chickpea, moisture stress, growth, physiological traits, biochemical traits

Signature of Major Advisor

Signature of the Student

ਖੋਜ ਪ੍ਰਬੰਧ ਦਾ ਸਿਰਲੇਖ	:	ਛੋਲਿਆਂ ਦੀਆਂ ਕਿਸਮਾਂ ਉੱਤੇ ਨਮੀ ਤਣਾਅ ਸਹਿਣਸ਼ੀਲਤਾ ਅਤੇ ਸਰੀਰਕ ਪੜ੍ਹਾਈ ਦਾ ਮੁਲਾਂਕਣ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖ਼ਲਾ ਨੰਬਰ		*ਵੈਸ਼ਾਲੀ ਸ਼ਰਮਾ* (ਐੱਲ-2014-ਬੀ.ਐੱਸ.-271-ਐੱਮ)
ਮੁੱਖ ਵਿਸ਼ਾ	:	ਪੌਦਾ ਵਿਗਿਆਨ
ਨਿਮਨ ਵਿਸ਼ਾ	:	ਜੀਵ-ਰਸਾਇਣ
ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਮ ਅਤੇ ਅਹੁਦਾ	:	ਡਾ. ਜਗਮੀਤ ਕੌਰ ਸੀਨੀਅਰ ਬੋਟਨਿਸਟ
ਮਿਲਣ ਵਾਲੀ ਡਿਗਰੀ	:	ਐਮ.ਐਸ.ਸੀ.
ਡਿਗਰੀ ਮਿਲਣ ਦਾ ਸਾਲ	:	2016
ਖੋਜ ਪ੍ਰਬੰਧ ਦੇ ਕੁੱਲ ਪੰਨੇ	:	59 + ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ - 141004 ਪੰਜਾਬ, ਭਾਰਤ ।

ਸਾਰ

ਮੌਜੂਦਾ ਖੋਜ ਤਜ਼ਰਬਾ ਛੋਲਿਆਂ ਦੀ ਕਿਸਮਾਂ ਵਿੱਚ ਨਮੀ ਤਣਾਅ (ਹਾਲਤ ਅਧੀਨ) ਵਿੱਚ ਸਹਿਣਸ਼ੀਲਤਾ, ਵਿਭਿੰਨਤਾ ਨੂੰ ਜਾਨਣ ਲਈ ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ ਦੇ ਪਲਾਂਟ ਬਰੀਡਿੰਗ ਅਤੇ ਜੈਨੇਟਿਕਸ ਵਿਭਾਗ ਦੇ ਤਜ਼ਰਬਾ ਖੇਤਰ ਉੱਪਰ ਹਾੜ੍ਹੀ 2015-16 ਦੌਰਾਨ ਕੀਤਾ ਗਿਆ। ਪਹਿਲੇ ਪ੍ਰਯੋਗ ਵਿੱਚ ਵੀਹ ਕਿਸਮਾਂ ਨੂੰ ਤਿੰਨ ਵੱਖ-ਵੱਖ ਹਾਲਾਤਾਂ ਅਧੀਨ ਪੀਵੀਸੀ ਪਾਇਪ ਵਿੱਚ ਉਗਾਇਆ ਗਿਆ। ਜਦਕਿ ਦੂਜੇ ਤਜ਼ਰਬੇ ਵਿੱਚ ਵੀ ਉਸੇ ਕਿਸਮਾਂ ਨੂੰ ਬਿਰਾਨੀ ਅਤੇ ਸੇਂਜੂ ਹਾਲਾਤਾਂ ਵਿੱਚ ਤਜ਼ਰਬਾ ਖੇਤਰ ਵਿੱਚ ਲਗਾਇਆ ਗਿਆ। ਵਿਕਾਸ ਵਿਹਾਰ ਨੂੰ ਵੈਜ਼ੀਟੇਟਿਵ ਜਣਨ ਪੜਾਅ ਅਤੇ ਵਾਢੀ ਪੜਾਅ ਦੌਰਾਨ ਅਧਿਐਨ ਕੀਤਾ ਗਿਆ। ਤਣੇ (ਬੂਟਾ ਲੰਬਾਈ, ਬਾਇਓਮਾਸ ਤਣੇ ਤੇ ਜੜ੍ਹਾਂ ਦੇ ਨਿਸਬਤ', ਸਟੋਮੈਟਲ ਆਵਿਰਤੀ) ਅਤੇ ਜੜ੍ਹ ਦੇ ਗੁਣਾਂ (ਜੜ੍ਹ ਤੇ ਵੋਲੀਅਮ, ਪੁੰਜ, ਖੇਤਰ ਅਤੇ ਗੰਢ ਦੇ ਭਾਰ) ਵਿੱਚ ਸੀਮਤ ਸਿੰਚਾਈ ਦੇ ਅਧੀਨ ਮਹੱਤਵਪੂਰਨ ਬਦਲਾਵ ਪਾਏ ਗਏ ਅਤੇ ਇਸ ਗੱਲ ਦਾ ਸੰਕੇਤ ਮਿਲਿਆ ਕਿ ਨਮੀ, ਤਣਾਅ ਨੇ ਪੌਦਿਆਂ ਉੱਤੇ ਪ੍ਰਭਾਵ ਪਾਏ। ਜਣਨ ਪੜ੍ਹਾਅ ਦੇ ਦੌਰਾਨ ਪਾਣੀ ਦੀ ਮਾਤਰਾ, ਪ੍ਰਕਾਸ਼ ਸ਼ੰਸ਼ਲੇਸ਼ਣ ਦਰ ਅਤੇ ਲੈਗਹਿਮੋਗਲੋਬਿਨ ਸਮੱਗਰੀ ਨੂੰ ਨਮੀ ਤਣਾਅ ਦੇ ਅਧੀਨ ਘੱਟ ਦੇਖਿਆਂ ਗਿਆ। ਤਣੇ, ਜੜ੍ਹ ਦੇ ਗੁਣਾਂ ਅਤੇ ਫਿਜਿਓਲੋਜੀਕਲ ਮਾਪਦੰਡ ਦੇ ਅਨੁਸਾਰ ਸੰਵੇਦਨਸ਼ੀਲ ਅਤੇ ਨਾਜ਼ੁਕ ਕਿਸਮਾਂ ਨੂੰ ਸ਼੍ਰੇਣੀਬੱਧ ਕੀਤਾ ਗਿਆ । ਕਿਸਮਾਂ (ਤਿੰਨ ਨਾਜ਼ੁਕ GL 29025, GL 12003 ਅਤੇ GNG 2171) ਅਤੇ ਸਹਿਣਸ਼ੀਲ (BGD 1094, ILC 3279 ਅਤੇ L-555) ਨੂੰ ਅਗਾਂਹ ਜੀਵ ਰਸਾਇਣਿਕ ਮੁਲਾਂਕਣ ਲਈ ਚੁਣਿਆ ਗਿਆ। ਨਮੀ ਤਣਾਅ ਹਾਲਾਤਾਂ ਅਧੀਨ ਔਸਮੋਟਿਕ ਘੁਲਣਸ਼ੀਲ ਪ੍ਰੋਲੀਨ ਅਤੇ ਕੁੱਲ ਸ਼ੂਗਰ ਸਹਿਣਸ਼ੀਲ ਕਿਸਮਾਂ ਵਿੱਚ ਨਾਜ਼ੁਕ ਕਿਸਮਾਂ ਨਾਲੋਂ ਵੱਧ ਪਾਏ ਗਏ । ਛੋਲਿਆਂ ਦੇ ਬੀਜਾਂ ਵਿੱਚ ਸਟਾਰਚ ਦੀ ਮਾਤਰਾ ਵਿੱਚ ਗਿਰਾਵਟ ਦੇਖੀ ਗਈ। ਆਖੀਰ ਵਿੱਚ ਨਮੀ ਤਣਾਅ ਦੇ ਕਾਰਨ, ਝਾੜ ਅਤੇ ਉਸਦੇ ਗੁਣਾਂ ਵਿੱਚ ਵੀ ਫਰਕ ਪਾਇਆ ਗਿਆ । BGD-1094, ILC 3279 ਅਤੇ L-555 ਛੋਲਿਆਂ ਦੀਆਂ ਕਿਸਮਾਂ ਨੂੰ ਨਮੀ ਤਣਾਅ ਦੇ ਸਹਿਣਸ਼ੀਲ ਪਾਇਆ ਗਿਆ ।

ਮੁੱਖ ਸ਼ਬਦ: ਛੋਲੇ, ਨਮੀ ਤਣਾਅ, ਵਿਕਾਸ, ਫਿਜ਼ਿਆਲੋਜੀਕਲ, ਜੀਵ ਰਸਾਇਣਿਕ ਗੁਣ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ

ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

CONTENTS

CHAPTER I

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a legume of the family Fabaceae. It is one of the most important grain-legume crop, grown in more than 51 countries in the world on area over 13.5mha with an average global yield of about 967.6Kg/ha (FAOSTAT 2015). India is the largest producer and consumer of chickpea in the world, accounting for 67.02% of world's production (Ghosh *et al* 2015). It is generally sown in the month of October- November and harvested in February- April in north of India. The crop duration is 90-150 days, depending on the variety. Desi varieties are short duration while *Kabuli* varieties take relatively longer period to mature. The major sowing areas are in the arid and semi-arid regions. In these regions, chickpea is generally grown under rainfed conditions either on stored soil moisture in subtropical environments with summer-dominant rainfall or on current rainfall in winter dominant Mediterranean-type environments. In both environments, non-irrigated chickpea plantations suffer yield losses from terminal drought (Yadav *et al* 2006, Toker *et al* 2007).

Chemical composition and nutrient value of chickpea makes it an important food supplement for mankind. It contains 17-26% proteins, high fiber, vitamin B6, thiamin, magnesium, zinc, iron and phosphorus. Beside source of energy and protein in human nutrition, chickpea is used as animal feed also which helps to increase egg and milk production. Chickpea also plays an important role in maintaining soil fertility in the dry rainfed area (Gaur *et al* 2007). It increases the soil fertility by fixing atmospheric nitrogen through its symbiotic association with *Rhizobia spp.*

Drought, cold and salinity are the major abiotic stresses affecting chickpea in order of importance (Croser *et al* 2003). Among abiotic stresses drought is the most important stress in chickpea. The crop consistently suffers from moisture stress at one or the other stages of development depending on water accessibility in the soil. Chickpea faces two types of drought, terminal i.e. soil moisture content is continuously decreased towards the end of the growing season and intermittent drought i.e. soil moisture depends upon precipitation but rainfall is irregular and also insufficient. So during vegetative and reproductive growth phases plants are subjected to intermittent and terminal drought stresses respectively (Ganjeali *et al* 2005). The crop faces drought stress either when the water supply to roots is interrupted or when transpiration rate becomes very high. These two conditions often coincide under arid and semi arid climates. Besides arid and semi arid environment other factors may induce water stress during crop growth and development resulting in reduction of crop yield.

Plants respond to drought stress and become accustomed through various physiological and biochemical changes (Farooq *et al* 2009). These include changes of water use efficiency, pigment content, osmotic adjustment and photosynthetic activity (Dhanda *et al*

2004, Serraj *et al* 2004, Benjamin and Nielsen 2006, Kalefetoglu and Ekmekçi 2009, Praba *et al* 2009). These mechanisms play a key role in preventing membrane disintegration and provide tolerance against drought and cellular dehydration (Mahajan and Tuteja 2005). High relative water content (RWC) and low excised-leaf water loss are associated with drought resistance, and these parameters have also been proposed as more valuable indicators of plant water status in comparison to other water potential parameters under drought stress (Keles and Oncel 2004).

Moisture stress during seed filling stage has been reported to be highly detrimental to the yield in chickpea (Davis *et al* 1999). The reduction in the grain yield in chickpea under drought has been reported to be associated with significant decrease in the above ground dry matter or vegetative biomass. Plants adapt to drought environment either through escape, avoidance, or tolerance mechanisms. Increasing crop tolerance to water limitation would be the most economical approach to enhance productivity and reduce agricultural use of fresh water resources. Another plant response to drought stress is the change in photosynthetic pigment content. The contents of both chlorophyll (Chl) a and b can change under drought stress. Photosynthetic pigments play important role in harvesting light. The carotenoids play fundamental role and helps plants to resist drought stress. Drought stress inhibits chlorophyll synthesis and decreases the content of chlorophyll a/b-binding proteins, leading to reduction in the light-harvesting pigment protein complex associated with photosystem II.

The stress invariably leads to oxidative stress in plants due to higher leakage of electrons towards O2 during photosynthetic and respiratory processes causing enhanced generation of reactive oxygen species (ROS) (Munne-Bosch and Penvelas 2003). The ROS such as superoxide radical (O^{2-}), hydroxyl radical (OH^-) hydrogen peroxide (H_2O_2) and alkoxy radical (RO) are highly reactive and can alter normal cellular metabolism through oxidative damage to membranes, protein and nucleic acids. They cause lipid peroxidation, protein denaturation and DNA damage leading to cell death (Mittler 2002). When the crop experiences stress conditions, there is an activation and/or modulation of the activities of antioxidant enzymes which leads to enhanced cellular protection (Kaur *et al* 2012). Plant cells respond defensively to oxidative stress by removing the ROS and maintaining antioxidant defense compounds at levels that reflect ambient environmental condition (Scandalios 1997). Plants with higher levels of antioxidants have been reported to attain greater resistance to oxidative damage (Boo and Jung 1999). Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio *et al* 2002). Proline is one of the osmolyte which increase in plants under water deficit stress and help the plants to maintain cell turgor (Zhao *et al* 2008).

Root system is a primary sensor of drought stress (Davies and Zhang 1991). Root traits are likely to be one of the most important components of drought tolerance in chickpea

(Jayashree *et al* 2005). Rooting depth and density are among the main drought avoidance traits identified to confer seed yield in chickpea under terminal drought environment (Kumar *et al* 2007). In the rainfed environments, the depth of rooting is often cited as an important criterion because it has a major influence in determining the potential supply of water from the deep soil and thus improves yield (Kashiwagi *et al* 2005). Generally, legumes are highly sensitive to water deficit stress (Mahieu *et al* 2009). Drought conditions may limit production of legumes by affecting nodule functioning (Ashraf and Iram 2005, Clement *et al* 2008). Water deficit can also induce premature senescence of nodules. During this process, many changes occur in nodules, for example the external colour of the N-fixing tissues of the nodule changes from red (due to functional leghaemoglobin) to green (indicating alteration of this protein) (Swaraj and Bishnoi 1996), decrease in leghaemoglobin content (Garg and Manchanda 2008), decrease in nodule membrane integrity (Mhadhbi *et al* 2009), degradation of bacteroids (Herder *et al* 2008), increase of proteinase activities in nodules (Groten *et al* 2006) and loss of N-fixation activity regardless of physiological and biochemical mechanisms of nitrogen fixation inhibition by water deficit stress. There is evidence that legume species have significant genetic variation in their ability to fix nitrogen under stress conditions (Ashraf and Iram 2005, Charlson *et al* 2009).

So the crop loss due to moisture stress condition of the soil inflict the urgent development of the strategies to improve crop production and hence food availability. Keeping in view of the above facts the present investigation was undertaken with the following objectives:-

i. To compare root and shoot growth behavior under moisture stress condition.
ii. To evaluate physiological and biochemical parameters affecting yield contributing attributes under moisture stress condition.

CHAPTER II

REVIEW OF LITERATURE

Legumes are mostly grown under rainfed condition and experience water deficit at different developmental stages. Drought is a meteorological term and an environmental event, defined as a water stress due to lack or insufficient rainfall and/or inadequate water supply (Toker *et al* 2007). The intensity of drought depends on the environment and the crop species. Ulemale *et al* (2013) observed significant differences amongst the different chickpea genotypes for phenology, vegetative growth, generative growth and sink capacity, physiological parameters and drought characteristics under moisture stress and non-stress conditions. Significant reduction in the plant growth due to effect on various physiological and biochemical processes *viz.* photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Kawamitsu *et al* 2000). The development of deep roots is the adaptation and avoidance mechanisms of drought (Chandler and Bartels 2008). Roots are usually the site showing the highest resistance in the pathway for liquid-phase movement of water through the soil-plant-atmosphere continuum (Kramer and Boyer 1995). The efficiency of soil water uptake by the root system is a key factor in determining the rate of transpiration and the varying strategies of adaptation to drought. Water uptake by the root is a complex process which depends on root structure, root anatomy, and the pattern by which different parts of the root contribute to overall water transport (Cruz *et al* 1992). Root membrane cell contains lignin and suberin which makes them impermeable to water and ions. Root traits, such as rooting depth and root biomass, have been identified as the most promising plant traits in chickpea for terminal drought tolerance. Under rainfed conditions, there were significant differences in the rooting depth among the genotypes. The rooting depth remained higher under rainfed than irrigated environment. Serraj *et al* (2004) observed significant genetic variation amongst the recombinant inbred lines (RIL) population for root length density, root dry weight and shoot dry weight. The overall distribution of root length density and root dry weight among the RILs indicated that these traits are likely to be under polygenic control. The RILs exhibited a range of combinations of root size and seed yield, with a few RILs showing large root systems and high seed yield.

High relative water content (RWC) and low excised-leaf water loss (rate of water loss, RWL) are associated with drought resistance, and these parameters have also been proposed as more valuable indicators of plant water status in comparison to other water potential parameters under drought stress (Keles and Oncel 2004). As mechanisms of responses to drought stress varies with genotypes and growth stages of individual plants (Ashraf and Harris 2004), it would be much more valuable if biochemical indicators could be specified for individual crop species. Knowledge on interrelationships among various physiological responses to dehydration can offer insight for developing useful strategies to improve drought stress tolerance in chickpea. Mafakheri *et al* (2011) established that drought stress imposed during vegetative growth significantly decrease soluble protein content and increase water soluble carbohydrate concentration. Drought stress at flowering stage had significantly higher peroxidase (POX) activity compared to than at vegetative stage. These results suggest that catalase (CAT) and POX activities play an essential protective role against drought stress in chickpea. POX acts as the major antioxidant enzyme in chickpea leaves under oxidative stress condition. Deleterious response to drought can include reduction of growth, decrease in chlorophyll, increase in hydrogen peroxide, which causes lipid per oxidation and consequently membrane injury.

Relating the morpho-physiological traits associated with drought stress is very much important in selecting suitable selection criteria for moisture stress tolerance. The literature relevant to the root and shoot traits, physiological and biochemical parameters affecting yield contributing attributes under moisture stress has been reviewed under following headings:-

2.1 IMPACT OF MOISTURE STRESS ON PHYSIOLOGICAL TRAITS

The reduction in shoot and root dry biomass in stressed plants may be due to reduction in leaf expansion and the stomata number that limit water loss through transpiration. This limits CO_2 assimilation, disrupts the photosynthetic activity showing decline in shoot biomass, as well as the inhibition of photosynthates transport to nodules (Antolín *et al* 2010). The reduction of nodule biomass under water deficit could be explained primarily by the decrease in the number and diameter of root hairs, or the inhibition of the emergence and elongation of these bodies (Zahran and Sprent 1986); secondly, by the reduction of rhizobia growth and the initiation and development of nodules (Saadallah *et al* 2001, Antolín *et al* 2010).

The rate of electrolyte leakage is considered as good physiological index that reflects the degree of cell membranes stability in the plants under stressful conditions (Ghoulam *et al* 2002, Farissi *et al* 2013). An increase in electrolyte leakage indicates that the membrane integrity is affected. Under water deficit, osmotic stress suppresses enzymes of metabolic pathways involved in nitrogen-fixation. These effects decrease nodule functioning and accelerate their early senescence (Mhadhbi *et al* 2009). Positive relationship was found between the increase of antioxidant defense enzymes expressions and the enhancement of symbiosis tolerance to stress in nodules of alfalfa plants (Naya *et al* 2007).

Kannan amd Kulandaivelu (2011) reported that drought stress causes not only a substantial damage to photosynthetic pigments, but it also leads to deterioration of thylakoid membranes. The decrease in chlorophyll content is a commonly observed phenomenon under drought stress (Bijanzadeh and Emam 2010, Mafakheri *et al* 2010, Din *et al* 2011). Kulsherehtha *et al* (1987) found that there is no significant effect of drought stress on chlorophyll content in wheat. Ashraf and Karim (1991) reported an increase in some cultivars of black gram (*Vigna mungo*) and a decrease in others under water-deficit and suggested that it may be due to variation in chlorophyll synthesis among the cultivars mediated by the variation in the activities of specific enzymes involved in the biosynthesis of chlorophyll. However, studies on chlorophyllase and peroxidase revealed that the decrease may be attributed to accelerated breakdown of chlorophyll rather than its slow synthesis (Harpaz-Saad *et al* 2007, Kaewsuksaeng 2011). Jain *et al* (2010) reported that under drought stress the reduction of chlorophyll *b* is greater than that of chlorophyll *a*, thus, transforming the ratio in favor of chlorophyll *a*. These differences could be due to a shift in an occurrence of photosynthetic systems towards a lower ratio of photosystem (PS) II to PSI (Estill *et al* 1991).

A decrease in total chlorophyll with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Mafakheri *et al* 2010, Esfandiari *et al* 2008). Lawlor and Cornic (2002) suggested that actual plant water status in leaves depends on the osmotic conditions of cells and transport of water from the shoots. During the inhibition of water transport from the root, osmotic regulation may actively influence water potential in assimilating tissues and limits the detrimental effects of water deficiency on photosynthesis. Adaptation to low water potential was observed in sunflower under mild water stress in comparison to non-acclimated plants subjected directly to severe drought, in which full inhibition of photosynthesis occurred (Cornic and Fresnau 2002, Medrano *et al* 2002).

2.2 IMPACT OF MOISTURE STRESS ON ROOT AND SHOOT GROWTH

Drought stress is primarily perceived and responded by plant roots, particularly under field conditions. Therefore length and distribution of roots play an important role in influencing the ability of plants to absorb water and nutrients from soil. It has been postulated that deeper root system with greater root density is a great stress management tool as it not only facilitates better extraction of soil water but also helps the plant to sustain optimal growth and development under drought stress conditions (Lopes *et al* 2011). It has been reported that the number of lateral and fine roots increase under drought stress in several crop species which not only increases root surface area for water absorption but also increases root hydraulic conductivity (Miyahara *et al* 2011).

Root traits, such as rooting depth and root biomass, have been identified as the most promising plant traits in chickpea for terminal drought tolerance. Roots are much more exposed to drought stress than the upper plant parts. So the root system is more affected than the aerial part of the plant for drought stress (Franco *et al* 2011). Root development is strongly influenced by growing conditions such as drought stress. However, root growth is usually less affected by drought stress than shoot growth, since more severe water deficit conditions possibly developed in the transpiring shoots. Other root characteristics like root length, fresh weight, dry weight, diameter and surface area, deep rooting and cortex thickness are also strongly affected by drought. Franco *et al* (2011) also observed that deep rooting is a critical factor influencing the ability of the plant to absorb water from the deeper layers of the soil. Also, a greater percentage of fine roots, capable of penetrating smaller soil pores, presumably optimizes the exploratory capabilities of the root system as a whole, and may have an important role for survival of plants to drought stress.

2.3 IMPACT OF MOISTURE STRESS ON NODULATION

Legumes usually fix atmospheric nitrogen, owing to their ability to form nodules with host symbiotic bacteria. Generally it has been suggested that legumes and their symbiotic root nodule bacteria are sensitive to abiotic stresses. Drought strongly decreased total nodule biomass. The proportion of empty, dark nodules indicated the adverse effect of drought on individual nodule growth. Nodule blackening and emptying are indicators of degeneration (Gross *et al* 2002). The black color is due to the accumulation of a dark-staining material within the cortex cells (Ramos *et al* 2003). Figueiredo *et al* (2008) reported that under severe drought stress the dehydrated nodules are declined of leghaemoglobin content. The reduction of nodule leghaemoglobin content can be attributed to early nodules degeneration which leads to the production of O^{2-} radicals (Mhadhbi *et al* 2009).

According to Ashraf and Iram (2005) drought does not seem to influence the colonization of roots by *Rhizobia* but it suppresses the growth of nodules. The high sensitivity of chickpea nodule development as compared to other plant parts suggests that water deficit specifically affected nodule development. Inhibition of nodule development in stressed plants has been suggested to be due to restriction of carbohydrate transport from leaves to nodule (Singh and Singh 2006). However it has been reported that co-inoculation of polyglycerol polyricinoleate (PGPR) with nitrogen fixing bacteria augment nodule number of legumes grown in green house or field situations under normal or drought stress conditions (Figueiredo *et al* 2008).

2.4 IMPACT OF MOISTURE STRESS ON SOME BIOCHEMICAL PARAMETERS

It has been recognized that drought stress is a very important limiting factor at the initial phase of plant growth and organization. It affects both elongation and expansion growth (Kusaka *et al* 2005, Shao *et al* 2008). These physiological changes are due to the result of deleterious effect of water deficit on important metabolic processes as well as response of various defense mechanisms adapted by the plant under drought stress. Plants can partly protect themselves against water stress by accumulating compatible solutes which can stabilize proteins and cellular structures and are capable of maintaining cell turgor by osmotic adjustments (Mafakheri *et al* 2010). Proline is one of the most common compatible osmolyte in drought stressed plants, which maintains redox metabolism by removing excess levels of reactive oxygen species and re-establishing cellular redox balance (Bartels and Sunkar 2005). The proline content of the leaf, however, increased at water deficit environment in all varieties of chickpea. Chiang and Dandekar (1995) studied that proline content depends on plant age, leaf age, leaf position or leaf part. Accumulation of proline has been advocated as a parameter of selection for stress tolerance (Jaleel *et al* 2007). Matysik *et al* (2002) have attributed an antioxidant feature to proline, suggesting ROS scavenging activity and proline acting as a single oxygen quencher. In parallel to this, proline has also been reported to protect and stabilize ROS scavenging enzymes and activate alternative detoxification pathways in plants subjected to various abiotic stresses (Islam *et al* 2009, Khedr *et al* 2003). Proline, therefore, acts both as a direct antioxidant as well as an activator of mechanisms that act as antioxidants.

Proline and carbohydrates are the two most important organic solutes that are accumulated in higher plants under drought conditions (Changhai *et al* 2010, Sumera and Asghari 2010). It is well known that free proline level increases in response to drought (Chorfi and Taı ¨bi 2011, Moayedi *et al* 2011, Rampino *et al* 2006). Carbohydrates seem to play a key role in the integration of plant growth and appear to be part of a wider mechanism for balancing carbon acquisition and allocation within and between organs (Farrar *et al* 2000). It has been suggested that under water stress conditions, soluble sugars can function in two ways which are difficult to separate, namely osmotic agents and osmoprotectors (Yong *et al* 2006). As osmotic agent soluble sugars facilitate osmotic adjustment, as osmoprotectors they stabilize proteins and membranes, most likely substituting water in the formation of hydrogen bonds with polypeptide polar residues and phospholipid phosphate groups.

Higher amount of grain protein, carbohydrate and soluble sugars were recorded under moisture stress however starch content was decreased. Increase in sugar content in response to drought stress also reported by Hossein *et al* (2001). Higher accumulation of soluble sugars under moisture stress also exhibited higher depletion of starch. Minimum increase in soluble sugars content under moisture stress environment also reported minimum depletion of starch. This phenomenon of breaking down of starch into soluble sugars showed that under moisture stress or drought situations plants tend to convert starch into soluble sugars. These soluble sugars are glucose, sucrose, fructose etc. This mechanism in chickpea is analyzed to tolerate or escape the drought stress condition. Wang *et al* 2006 studied with a variety of plants to demonstrate that drought induced conversion of hexoses and other carbohydrates, such as sucrose and starch, into sugar alcohols (polyols) and proline.

Molecular markers have been used to study the extent of genetic variation. The protein profiling of germplasm have been widely and effectively used to determine the taxonomic and evolutionary aspects of chickpea (Nisar *et al* 2007, Hameed *et al* 2009). Drought tolerance trait is related to protein expression, proteins emerging while challenging with drought are called induced proteins as reported in chickpea (Kakaei and Mostafaie 2012). Decrease in protein content under water deficit conditions was observed in *Arachis hypogea* L. (Shradda and Naik 2011). Variation in protein yield was found in chickpea varieties under drought conditions (Hameed 2003, Akbar *et al* 2011, Shaban *et al* 2012). Kakaei and Mostafaie (2012) in an experiment analyzed the seed storage protein profiles of four chickpea cultivars (under drought stress and non stress conditions) which were studied on the basis of biochemical markers, by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). It was concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity.

2.5 IMPACT OF MOISTURE STRESS ON ANATOMICAL CHARACTERS

Along with morphological, physiological and molecular aspects, the anatomical changes occur during moisture stress. One of the most functional aspects related to root anatomy is water and nutrient transport capacity, because it is highly influenced by the number and size of the water conducting elements (Esau 1965, Steudle and Peterson 1998). In the soil-plant inter phase, root experiences the greatest resistance to the liquid water flow to normalize the absorption process with minimum energy (Rieger and Litvin 1999). Roots of Pearl millet were found with fewer xylem vessels arranged in a single layer below the endodermis. Gregory and Eastham (1996) reported that this fine root development and limitation in xylem vessel number in cereals like wheat is compensation of a large root length density of finer roots. Cereals are known to have a greater root length density than the legumes (Purushothaman *et al* 2013).

The presence of highly suberized exodermis, a definite cortex, a pericycle and the endodermis are clearly intended for better regulation and resistance that ensured very efficient but a conservative absorption of soil moisture which make the plants more appropriate to lighter soils with minimum water holding capacity as well as longer periods of water deficit. For maintaining the minimum water potential in the rhizosphere, thinner roots, wider xylem vessels and a thin cortex were positively related to the hydraulic conductivity; along with that xylem vessel elements also provide support and strength to the roots during stress condition (Hack and Sperry 2001). Chickpea had relatively thicker roots than other legumes like pearl millet, groundnut and pigeonpea. It also had a larger number of thinner vessels in a wider range of sizes than common bean, cowpea and soybean that had broader vessels.

Drought adapted plants generally had thick cuticles (Boom *et al* 2005). Under water stress conditions the plants with waxy cuticle in the leaves minimize the loss of water. The water deficit had little effect on number of trichomes, length and width of epidermal cells and length of stomata, and decreased the stomatal density especially on adaxial surface (Sam *et al* 2000). During stress, the anatomy of root tissues will change largely, because stress induces the development of apoplastic barriers for water and ion flow. Their formation represents a fundamental adaptive strategy of plants to survive in an adverse environment (Zimmermann *et al* 2000).

2.6 IMPACT OF MOISTURE STRESS ON ANTIOXIDANT SYSTEM

Antioxidant defense system plays an important role in plant's response to stress conditions. The accumulation of reactive oxygen species (ROS) was reported to be sensed as an 'alarm' signal that initiates pre-emptive defense responses (Shao *et al* 2005). Among the antioxidant enzymes, superoxide dismutase (SOD) constitutes the first line of defense, via detoxification of superoxide radicals (O^{2-}), (H_2O_2), and hydroxyl radical (OH^-) (Sairam and Saxena 2000), thereby maintaining membranes of plant tissue. Zaefyzadeh *et al* (2009) suggested that tolerant plant having higher activities of SOD might have better potential in decreasing the toxic concentrations of O^{2-} radicals as compared to the susceptible ones. The disproportionate reaction of O^{2-} leads to the formation of H_2O_2, which is then acted upon by catalase, another important enzyme of the defense system. Catalase is essential for the removal of H_2O_2; reducing the high levels of reactive oxygen species, under stress condition (Dat *et al* 2001). Hydrogen peroxide is a toxic compound and its high concentration is deleterious to plants, which results into lipid peroxidation and membrane injury.

According to Yang *et al* (2008) it is important to eliminate the reactive oxygen species by an efficient antioxidative defense system for avoiding the cellular damage under stress condition. Previous studies demonstrated that catalase activity in combination with SOD activity might have an essential protective role and thus essential for water deficit tolerance in the roots of chickpea seedlings. The combined action of CAT and SOD converts the toxic superoxide radical (O^{2-}) and hydrogen peroxide (H_2O_2) to water and molecular oxygen (O_2), thus averting the cellular damage under unfavorable conditions like drought stress. Peroxidase is also an important enzyme which regulates the intracellular level of H_2O_2. MDA has been reported to be a widely used marker of oxidative lipid injury and its concentration varies in response to abiotic stresses (Davey *et al* 2005, Moller *et al* 2007). Patel and Hemantaranjan (2012a) reported that lipid peroxidation is an indicator of the occurrence of free radical reactions occurring in tissues. The plant exhibiting better performance under water deficit conditions have been observed to have lower levels of MDA content in roots which correlated well with their H_2O_2 content and enhanced catalase activity, thus protecting the plants from lipid peroxidation of membrane system as compared to the plants which had higher levels of MDA content. Thus, the prevention of membrane damage is related to the induction of antioxidant responses which protect the plants from oxidative damage (Patel and Hemantaranjan 2012b).

Peroxidase activity increased under drought stress conditions in sunflower (Gunes *et al* 2008), poplar (Xiao *et al* 2008), liquorice (Pan *et al* 2006), brassica species (Das and

Uprety 2006) and wheat (Csiszar *et al* 2005). *Brassica napus* L. plants were grown under three irrigation regimes (FC; field capacity, 60% FC and 30% FC) in a greenhouse by Abedi and Pakniyat (2010). Drought stress preferentially enhanced the activities of superoxide dismutase (SOD) and guaiacol peroxidase (POD) whereas it decreased catalase (CAT) activity. SOD, CAT, GPOX activity increased as reported in sorghum (Saei 2004) and soybean (Shafei 2005) under water deficit conditions. Sharada and Naik (2011) in their research studied the effect of water deficit on early growth and biochemical constituents of two varieties of groundnut (*Arachis hypogaea* L.) plants and reported increased activities of antioxidant enzymes, SOD, CAT, POX, PPO and GR enzyme respectively under drought stress. Mafakheri *et al* (2011) reported in their studies that drought stress at flowering stage had significantly higher POX activity compared to than that at vegetative stage in various chickpea cultivars.

CHAPTER III

MATERIALS AND METHODS

The present investigation entitled "Physiological studies on moisture stress tolerance in chickpea (*Cicer arietinum* L.) genotypes" includes assessment of chickpea genotypes for root and shoot based traits. The work was carried out in the laboratory and field area of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Biochemical studies were performed on six (three sensitive, three tolerant) genotypes selected on the basis of field screening. Seeds of chickpea were made available from the Pulses section, Department of Plant Breeding & Genetics, PAU. A brief description of materials used, experimental procedures and techniques adopted in the study are presented in this chapter.

Seeds of following chickpea genotypes were used for field and laboratory studies for their growth behavior and yield attributes.

Table 3.1: List of chickpea genotypes used in the study

Sr. No.	Genotypes	Sr. No.	Genotypes
1	GL 12003	11	H-11-58
2	GL 12021	12	BGD 1094
3	GL 13037	13	ICC 4958
4	GL 13043	14	ILC 3279
5	GL 13050	15	CSJK 4
6	GL 29095	16	RVSSG 31
7	GL 96836	17	JG 41
8	GNG 2171	18	PDG 3
9	GNG 2249	19	L 555
10	GNG 2264	20	GPF 2

EXPERIMENT I: Assessment of chickpea genotypes for root and shoot based traits.

3.1 Shoot Traits (60 days after sowing)

3.1.1 Plant height

Five plants from each plot of three replications were selected randomly and their main stem length was measured and their average values are expressed in cm/plant.

3.1.2 Biomass accumulation

Three random plants were harvested at 60 days after sowing and dried in an oven at $80^{o}\pm1$C till a constant weight was attained and then weighed to determine dry weight. The mean of three plants was expressed in g plant^{-1}.

3.1.3 Shoot: Root ratio

Dry weight of the shoots and roots of same plants were recorded and their ratio was

Screening of chickpea genotypes for root traits under moisture stress.

calculated.

3.1.4 Stomatal frequency (mm^{-2})

Fresh leaves were taken and quick fix was applied on both the surfaces of leaves and peeled out with the help of forceps. The number of stomata was counted in ten microscopic fields (20X) and data were expressed as number of stomata mm^{-2}.

3.2 Root traits (60DAS)

3.2.1 Root volume

Three random plants were harvested and roots of the plant were dipped into the measuring cylinder filled with water up to a mark. Raise in water level due to root was noted and the difference between the initial water level and final water level indicated the root volume in cm^3.

3.2.2 Root mass

Three random plants were harvested and their roots were dried in an oven at 80^{o}±1C till a constant weight was attained and then weighed to determine dry weight. The mean of three roots was expressed in g $plant^{-1}$.

3.2.3 Root area

Root area of three randomly selected plants was recorded using Delta T root scanner. The mean of three roots were expressed in cm^2/plant.

3.2.4 Nodule weight

The nodules of roots were washed and then dried in an oven and weighed. The dry weight of nodules was expressed in g $plant^{-1}$.

3.2.5 Anatomical changes

To compare the structure of root of *C.arietinum,* the samples at pre-anthesis stage were collected and fixed in FAA (Formalin- acetic acid- ethyl alcohol) solution immediately. FAA was prepared by mixing 85 ml of 50% ethyl alcohol, 5 ml of glacial acetic acid and 10 ml of 40% formaldehyde. Transverse sections of roots were cut from the middle position with the help of blade. The sections were stained with an aqueous solution of 1.0% safferenin and 0.5% fast green. The stained sections were mounted in DPX under cover slip and observed under research microscope (20X).

EXPERIMENT 2: Biochemical changes and yield attributes of chickpea genotypes.

3.3 Phenophasic development

3.3.1 Days taken for flower and pod initiation

The number of days taken by the plants from the date of sowing to the period of first flower and pod initiation were recorded.

3.3.2 Days to 50% flowering

The number of days taken by the plants from the date of sowing to the period of 50%

flowering was recorded.

3.3.3 Days to maturity

The number of days taken by the plants from the date of sowing to the maturity was recorded.

3.4 Physiological parameters (During reproductive phase):-

3.4.1 Relative water content

Relative water content was recorded from the leaves of control and stressed plants. For this 100 mg fresh leaf tissue was submerged in 10 ml of deionised water in the petri dishes till saturation. After 24 hours, surface water of the leaves was blotted off without putting any pressure and then they were weighed to obtain saturated weight. Then leaves were dried in an oven at 70°C for 24 hr, and their dry weights were determined. From this data, RWC was calculated as follows:

$$\text{RWC} = \frac{\text{Fresh weight- Dry weight} \quad \text{x } 100}{\text{Turgid weight – Dry weight}}$$

3.4.2 Photosynthetic rate

Photosynthetic rate was recorded as µmole CO_2 m^{-2} s^{-1} by using "Portable Photosynthesis System (LI-6400XT, LICOR)"

3.4.3 Leghaemoglobin content in nodules (Wilson and Reisenauer 1963)

The leghaemoglobin content in nodules was determined by using drabkin's solution.

Procedure

Fresh nodules (0.5g) were collected and crushed in pestle and mortar with 9ml of Drabkin's solution. Solution was centrifuged at 12,000 rpm for 15 min. and then supernatant was collected. Optical density was recorded at 540nm using Drabkin solution as blank. The amount of lehaemoglobin was expressed as mg/g FW nodules.

3.5 Biochemical parameters

Table 3.2: List of selected chickpea genotypes used in the study

Sr. No.	Genotypes (Sensitive)	Sr. No.	Genotypes (Tolerant)
1.	GL 29095	4.	BGD 1094
2.	GL 12003	5.	ILC 3279
3.	GNG 2171	6.	L 555

3.5.1 Proline content (Bates *et al* 1973)

Extraction:

100 mg sample was weighed and extracted in 6 ml of Methanol: Chloroform: Water (12:5:1) by volume at room temperature. It was then centrifuged for 10 minutes and the supernatant was collected. Again the contents were centrifuged after adding 4 ml of Methanol: Chloroform: Water. Supernatant was pooled and final volume was made upto 10

ml with same solvent. To this, 6 ml of chloroform and 4ml of distilled water was added. After stirring it was allowed to stand for 15 minutes in separating funnel till the two layers get separated. Lower layer containing pigments was rejected and upper layer was collected. The final volume of upper layer was made to 10 ml by adding distilled water.

Reagents:

2 ml of acid Ninhydrin

Estimation:

5 ml of this solution was taken and 2.5 ml of acid ninhydrin (125 mg of Ninhydrin mixed in 3ml of acetic acid and 2ml of orthophosphoric acid, and then kept in an oven at 70^{o}C till a clear solution was formed) was added. The mixture was boiled for 45 minutes in boiling water bath till pink colour is formed. On cooling, 5 ml of benzene was added and shaken. Again allow it to stand in separating funnel for another 30 minutes till the two layers get separated. Lower layer was discarded and upper pink layer was collected. Optical density was recorded at 515 nm by using benzene as blank. Proline is used as standard to make standard curve. Proline content formed was expressed as µmole/g.

3.5.2 Total soluble sugars (Dubois *et al* 1956)

Sugars react with concentrated sulphuric acid to form a dehydration product i.e. furfural. This dehydration product then reacts with phenol which acts as a chromphore and gives orange yellow color.

Extraction:

100mg of dry leaves sample were collected and homogenized in 80% ethanol and centrifuged. The residue was re-extracted to ensure complete extraction; supernatants were pooled and used for estimation of total soluble sugars. Residue left after ethanol extraction was kept for analyzing starch content.

Reagents:

i. 80% ethanol
ii. 5% phenol
iii. Concentrated sulphuric acid

Estimation:

To 0.1 ml of sugar extract 1 ml of 5% phenol was added and kept for 10 minutes followed by addition of 5 ml of conc. H_2SO_4. The sulphuric acid was poured directly in middle of the test tube to ensure proper mixing of the solutions. After 10 minutes, the tubes were cooled to room temperature under running water. After another 20 minutes the absorbance was measured at 490 nm against ethanol. The concentration of total soluble sugars was calculated from the glucose standards (10-60 ug) run simultaneously.

3.5.3 Superoxide Dismutase (Marklund and Marklund 1974)

Superoxide dismutase catalyzes the disproportionation of superoxide anion to H_2O_2 and molecular oxygen.

$$O_2^{-} + O2^{-} + 2H^{+} \xrightarrow{\text{SOD}} H_2O_2 + O2$$

Extraction:

100mg of fresh leaves were extracted with 0.1M potassium phosphate buffer (pH 7.5) containing 1% PVP, 1 mM EDTA and 10mM β- mercaptoethanol. The extract was centrifuged at 10,000 rpm for 10 minutes. Supernatant was used for enzyme estimation.

Reagents:

i. 6 mM Pyrogallol (Fresh solution was prepared for assay).
ii. 6 mM EDTA
iii. 0.1M Tris-HCL buffer

Assay:

To a cuvette, 1.4 ml of 0.1M Tris-HCL buffer (pH8.2), 0.5 ml of 6 mM EDTA, 1ml of 6 mM pyrogallol solution and 0.1 ml of enzyme extract was added. Absorbance was recorded at 420 nm after an interval of 30 seconds up to 2 minutes. The reaction mixture without enzyme extract was taken as blank. A unit of enzyme activity has been defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank. Superoxide dismutase was expressed as unit enzyme g^{-1} fresh weight.

3.5.4 Peroxidase (Shannon *et al* 1966)

Peroxidases detoxify H_2O_2 in the cytosolic part of the cell. They are non specific in utilizing electron donor for oxidation of H_2O_2.

$$\text{Guaiacol} + H_2O_2 \xrightarrow{\text{POD}} H_2O + \text{Tetrahydroguaiacol}$$

Extraction:

The enzyme was extracted from the samples with 0.1M potassium phosphate buffer (pH 7.5) containing 1% PVP, 1 mM EDTA and 10mM β- mercaptoethanol. The extract was centrifuged at 10,000 rpm for 10 minute. Supernantant was used for enzyme estimation.

Reagents:

i. 0.05M guaiacol prepared in 0.1M potassium phosphate buffer (pH 6.5)
ii. 0.8M H_2O_2

Assay:

To a cuvette, 3ml of 0.05M guaiacol prepared in 0.1M potassium phosphate buffer (pH 6.5), 0.1ml of enzyme extract and 0.1ml of 0.8M H_2O_2 were added. The reaction mixture without enzyme extract was measured as a blank. The reaction was initiated by adding H_2O_2 and rate of change in absorbance was recorded at 470nm for 2 minutes at an interval of 30 seconds. Peroxidase activity has been defined as change in absorbance $min^{-1}g^{-1}$ of tissue.

3.5.5 Catalase (Chance and Maehley 1955)

Catalase is able to use one molecule of H_2O_2 as substrate or electron donor and another molecule of H_2O_2 as oxidant or electron acceptor.

$$2\ H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Extraction:

The enzyme was extracted from fresh tissue (0.1 g) with 50 mM sodium phosphate buffer (pH 7.5) containing 1% polyvinyl pyrrolidine (PVP) and centrifuged at 10,000 rpm for 20 minutes at 4°C. Supernatant was used for enzyme estimation.

Reagents

i. 50 mM sodium phosphate buffer (pH 7.5)
ii. H_2O_2 solution: 0.2ml of H_2O_2 was diluted to 50 ml with 50 mM sodium phosphate buffer (pH 7.5)

Assay:

In spectrophotometric cuvette, 1.8 ml of 50 mM sodium phosphate buffer (pH 7.5) and 0.2 ml of enzyme extract was added. The reaction was initiated by adding 1 ml of H_2O_2. Utilization of H_2O_2 was recorded at intervals of 30 seconds for 2 minutes by measuring the decrease in absorbance at 240 nm. Catalase activity was expressed as µmoles of H_2O_2 decomposed min^{-1} g^{-1} fresh weight.

3.5.6 Total soluble starch (Dubois *et al* 1956)

Starch is hydrolyzed with the help of HCl and free sugars are released which then form dehydration product with concentrated sulphuric acid. This dehydration product then reacts with phenol which acts as a chromphore and gives orange yellow colour.

Extraction:

100mg of dry seeds were homogenized in 80% ethanol and centrifuged. The residue was re-extracted to ensure complete extraction; supernatants were pooled and used for estimation of total soluble sugars. Residue left after ethanol extraction was kept for analyzing starch content. The pellet was then extracted for starch by boiling with 5ml HCl. The extraction was repeated twice and supernatants were pooled to be used for starch estimation.

Estimation:

The procedure for estimation of total soluble sugars was followed for starch as mentioned in 3.3.2. Starch content formed was expressed as mg g^{-1} dry weight.

3.5.7 Protein profiling using SDS PAGE (Laemmli 1970)

Sodium dodecyl sulphate- Polyacrylamide gel electrophoresis (SDS-PAGE)

Extraction of proteins:

1. 0.1 g (100 mg) powdered seeds extracted with 2 ml of 20 mM Tris HCl buffer

containing 0.5% NaCl (pH 7.5).

2. Centrifuged at 8000 rpm for 20 min.
3. Pellet was discarded.
4. Supernatant collected.

Proteins estimated by the method of Lowry *et al* (1951).

A part of supernatant was used for SDS-Polyacrylamide gel electrophoresis.

Preparation of stock reagents:

(a) Acrylamide stock (30%): Dissolved acrylamide and N,N,N,N- methylene bisacrylamide in the ratio of 30:0.8 in distilled water to prepare 1000 ml of solution. The mixture was filtered and stored at 4°C in dark brown bottle.

(b) 1.5 M Tris HCl buffer (pH 8.8): 18.17 g of Tris base was dissolved in 80 ml of distilled water. The pH adjusted to 8.8 with 1 N HCl and volume was made to 100 ml with distilled water.

(c) Stacking Gel buffer-0.5 M Tris-HCl buffer (pH 6.8): Dissolved 6 g of tris HCl in 80 ml distilled water. The pH was adjusted to 6.8 with 1N HCl and volume was made to 100 ml with distilled water.

(d) 10 % (W/V) Ammonium persulphate (APS): 10 mg of APS was dissolved in 100 µl distilled water. A fresh solution was prepared every time before use.

(e) 10% SDS: 1g of SDS was dissolved in distilled water with gentle stirring and volume was made to 10 ml.

(f) TEMED: N, N, N, N-Tetramethyl Ethylene Diamine.

(g) Running buffer (5X):

Tris buffer (7.55 g) and Glycine (36.0g) were dissolved in 450 ml of distilled water. The pH was adjusted to 8.2 with 1 N HCl and total volume made to 500 ml with distilled water. 1X buffer was used for running electrophoresis and to it 0.1% SDS was added just before use.

WORKING SOLUTION

(a) Resolving gel (12%)

1. 8.0 ml of 30% acrylamide
2. 5.0 ml of Tris HCl (pH 8.8)
3. 0.2 ml of 10% SDS
4. 6.6 ml of glass distilled water
5. 0.01 ml of TEMED

(b) Stacking gel (5%)

1. 1.02 ml of 30% acrylamide
2. 1.5 ml of Tris HCl (pH 6.8)

3. 0.06 ml of SDS
4. 3.33 ml of distilled water
5. 0.01 ml of TEMED

Above contents stirred and then 0.2 ml and 0.06 ml of freshly prepared 10% ammonium persulfate (APS) was added to the resolving and stacking gels respectively.

(c) Sample buffer

1. 1.2 ml of 0.5 M Tris HCl (pH 6.8)
2. 2.0 ml of 10% SDS
3. 1.0 ml of glycerol
4. 4.8 ml of glass distilled water
5. 0.5 ml of 0.5% Bromophenol blue
6. 0.5 ml of β-mercaptoethanol

β-mercaptoethanol was added just before use.

SAMPLE PREPARATION AND LOADING

1. Protein sample containing known quantity of protein as estimated by the method of Lowry *et al* (1951) were mixed with an equal volume of the sample buffer and kept in boiling water for 5 min.
2. Samples were loaded with molecular weight markers on to the wells in gel.
3. Electrophoresis was carried out at a constant voltage of 100 V until the samples travelled through the stacking gel. Voltage increased to 150 V when the bromophenol blue moved in to resolving gel and continued till dye reached at the bottom of the gel.
4. After completion of electrophoresis, the resolving proteins were prefixed by keeping the gel for 1 hour in 12.5% trichloroacetic acid, followed by immersing the gel in staining solution (0.1 g comassic blue, 100 ml of methanol, 20 ml of acetic acid and 80 ml of distilled water).

Then, destaining was done by immersing the gel in a mixture of methanol: acetic acid: distilled water (125: 35: 340).

3.6 Yield attributes

3.6.1 Number of pods per plant

The total number of pods obtained from five randomly selected plants were counted and expressed as number of pods plant^{-1}

3.6.2 100 seed weight

The hundred seeds were counted from the produce of each plot and then weighed in grams.

3.6.3 Yield per plant

Five plants were taken randomly from each plot and average seed yield per plant was recorded and expressed as in grams/plant.

3.6.4 Harvest index

HI defined as ratio of seed yield to the total biomass at maturity, was calculated as follows:

HI= (Seed Yield/ Total above ground biomass) ×100

3.7 Statistical analysis

The data on various parameters were subjected to statistical analysis. Critical difference values were calculates by doing analysis of variance (ANOVA).

CHAPTER IV

RESULTS AND DISCUSSION

The results of the investigation entitled 'Physiological studies on moisture stress tolerance in chickpea (*Cicer arietinum* L.) genotypes' are presented in this chapter. Two experiments were conducted, in the first experiment twenty genotypes were raised in PVC pipes in the field area of department of Plant Breeding and Genetics for shoot and root traits under varied moisture stress conditions. In the second experiment same genotypes were sown in the field to study physiological and yielding traits. The biochemical analysis was carried out on six (three sensitive and three tolerant) genotypes in the laboratory which were selected on the basis of first experiment. The results are presented in the form of tables and figures under these headings:-

4.1 Shoot traits
4.2 Root traits
4.3 Phenophasic development
4.4 Physiological evaluation
4.5 Biochemical estimations
4.6 Yield attributes

EXPERIMENT I: Assessment of chickpea genotypes for root and shoot based traits.

4.1 Shoot traits (60 days after sowing-DAS)

4.1.1 Plant height (cm)

Plant height is an important determinant related to yield potential. The plant height showed statistically importance by means of different water stress treatments. Under moisture stress, plants become stunted and variation in plant height (cm) of chickpea genotypes as influenced by restricted moisture conditions is presented in Table 1. There was a significant reduction in plant height when exposed to moisture stress; however maximum effect was observed at 75% restricted irrigation. The decrease in mean values is consistent with declining plant height viz. (50.2, 41.7 and 37.0 cm) respectively in control, 50% and 75% water reduction. Plant height ranged from 42.1 cm (GNG 2171) to 62.3 cm (ILC 3279) under control condition. GL 12003 exhibited maximum reduction (36.8%) with maximum restricted irrigation (75%), whereas GNG 2171 showed maximum reduction (32.54%) with 50% restricted irrigation. The similarities in results are in conformation to the results of Ulemale *et al* (2013) in chickpea where drought conditions resulted in decreased plant height. Ghiabi *et al* (2013) postulated that drought decreases the cell expansion properties which ultimately lead to lower plant height. Slower cell division and inhibition of growth occurs as a result of reduced cyclin dependent kinase activity under drought conditions as reported in wheat (Schuppler *et al* 1998).

Table 1: Variation in plant height (cm) and biomass accumulation (g/pl) of chickpea genotypes under restricted moisture conditions.

Genotype	Plant height (cm)			Biomass accumulation (g/pl)		
	Control	Restricted irrigation (50%)	Restricted irrigation (75%)	Control	Restricted irrigation (50%)	Restricted irrigation (75%)
GL 12003	43.5	33.7	27.5	1.25	0.82	0.56
GL 29095	44.2	33.2	28.3	1.12	0.75	0.54
GL 96836	44.5	35.1	30.4	1.31	0.89	0.64
GL 12021	47.7	41.4	35.4	1.93	1.45	1.13
GL 13037	52.7	45.7	37.3	2.39	1.82	1.21
GL 13043	50.6	43.5	37.5	2.15	1.67	1.24
GL 13050	48.7	39.4	35.4	2.06	1.43	1.13
GNG 2171	42.1	31.2	28.4	1.21	0.79	0.55
BGD 1094	61.4	52.1	49.1	3.06	2.51	1.95
GNG 2249	45.6	37.2	32.1	1.44	1.12	0.87
GNG 2264	51.3	43.2	37.2	2.14	1.64	1.24
ICC 4958	47.4	40.3	37.2	1.68	1.19	0.94
H-11-58	50.6	43.5	40.6	2.21	1.61	1.31
CSJK 4	52.6	44.3	43.2	2.51	1.87	1.52
ILC 3279	62.3	52.4	48.2	2.83	2.36	1.73
RVSSG 31	49.4	36.7	33.4	1.69	1.19	0.85
JG 41	49.2	42.6	38.4	1.54	1.16	0.83
L 555	59.2	52.1	46.2	2.94	2.42	1.83
GPF 2	52.4	44.2	38.4	2.47	1.86	1.35
PDG 3	48.1	41.5	36.4	1.96	1.47	1.04
Mean	50.2	41.7	37.0	1.99	1.50	1.12
CD (5%)	G= 0.843, T=0.326, GXT=1.459			G=0.2003, T=0.0776, GXT= 0.3471		

4.1.2 Biomass accumulation (g/pl)

Biological productivity of plant is judged from their actual ability to produce and accumulate dry matter. Biomass decreased with the increased moisture stress and the maximum effect was noted at 75% restricted irrigation (Table 1). The decrease in mean value is consistent with negating biomass accumulation (1.99, 1.49 and 1.12 g) at control, 50% and 75% restricted irrigation respectively. Under 75% restricted irrigation, diminishment of biomass accumulation over control varied between 36.3% (BGD 1094) to 55.2% (GL 12003) whereas under 50% restricted irrigation, maximum reduction was observed in GNG 2171 (34.7%) and minimum %reduction was exhibited in ILC 3279 (16.6%). Genotypic variations were prominent under stress conditions (Fig.1). The tolerant genotypes were observed with higher stem dry weight than the sensitive ones under water stress. Similar results were also observed by Thomas *et al* (2004), Ashraf and Iram (2005) and Slama *et al* (2006) who stated that shoot dry weight of two leguminous plants, *Phaseolus vulgaris* and *Sesbania aculeate* decreased significantly due to water deficit. Under water stress conditions, decrease in plant height alludes to decline in biomass (Labidi *et al* 2009) which is positively notified to studies of Ulemale *et al* (2013) where biomass accumulation declined with the water reduction. According to Schupper *et al* (1998) reduction in vegetative growth of chickpea plants under drought in particular biomass accumulation is due to slower cell division and inhibiton of growth.

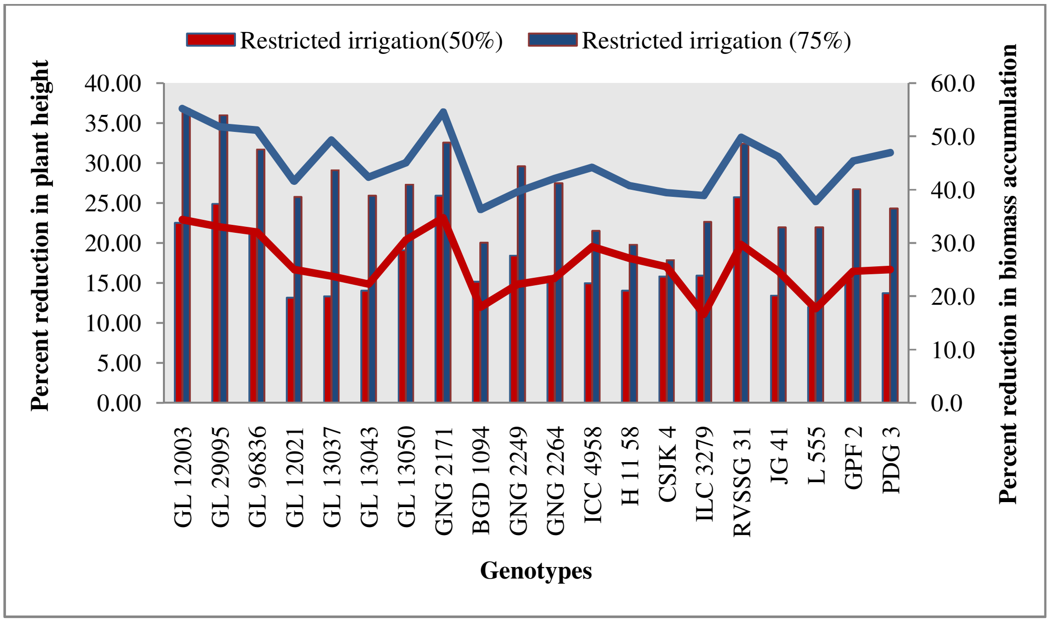


Fig 1: Percent reduction in plant height and biomass accumulation of chickpea genotypes as influenced by moisture stress.

4.1.3 Shoot: root ratio

The variation in extent of accumulation of dry weight in root and shoot of different genotypes of chickpea under drought conditions also affected the shoot to root ratio (Table 2). Under control condition, shoot: root ratio varied from 1.264 to 2.859 in GL 12021 and H-11-58, respectively. The mean values of the shoot to root ratio decreased with decline in water availability i.e. 1.964, 1.832 and 1.795 under control, 50% water reduction and 75% water reduction respectively. However, the differences between genotypes appeared for shoot: root ratio specifically in H-11-58 and CSJK 4 i.e. 7.48 and 11.90 percent increase under 75% restricted irrigation as compared to 50% restricted irrigation. Similarities in result were seen in work of Labidi *et al* (2009) in different chickpea genotypes. However, significant reduction in shoot to root ratio under restricted irrigation was seen in the *Medicago Sativa* by Mouradi *et al* (2015).

Table 2: Variation in shoot: root ratio of chickpea genotypes under restricted moisture conditions.

Genotype	Control	Restricted irrigation (50%)	Restricted irrigation (75%)
GL 12003	1.366	1.254	1.209
GL 29095	1.692	1.607	1.636
GL 96836	1.512	1.491	1.328
GL 12021	1.264	1.253	1.235
GL 13037	1.654	1.480	1.592
GL 13043	1.352	1.241	1.311
GL 13050	1.831	1.731	1.816
GNG 2171	1.465	1.403	1.291
BGD 1094	1.876	1.716	1.678
GNG 2249	1.955	1.914	1.812
GNG 2264	2.289	2.268	2.250
ICC 4958	2.361	2.121	1.918
H-11-58	2.859	2.568	2.775
CSJK 4	2.461	2.149	2.440
ILC 3279	1.747	1.655	1.539
RVSSG 31	2.091	1.941	1.954
JG 41	2.130	1.895	1.774
L 555	2.328	2.206	1.941
GPF 2	2.582	2.562	2.381
PDG 3	2.459	2.184	2.016
Mean	1.964	1.832	1.795
CD (5%)	G= 0.33461, T=0.1296, GXT= 0.157		

4.1.4 Stomatal frequency (number of stomata mm^{-2})

Under moisture stress conditions, stomatal control is a major physiological factor for optimizing the use of water. The mean frequency of stomata on adaxial surface under control, 50% and 75% restricted irrigation was 23.2, 26 and 27.6 mm^{-2} respectively (Plate I). Whereas,on abaxial surface it was 34.8, 39.2 and 41.8 mm^{-2} under control, 50% and 75% restricted irrigation respectively. Under 50% restricted irrigation, maximum % increase was observed in GL 29095 i.e. 13.3 and 14.1% on adaxial and abaxial surfaces respectively. However, under 75% restricted irrigation it was observed in GL 12003 i.e. 18.3 and 20.8% on adaxial and abaxial surfaces respectively (Table 3). Variability was observed in stomatal frequency on both leaf surfaces. Number of stomata on adaxial surface of leaves was lower as compared to abaxial surface. With increase in water stress the stomatal frequency also increased on both surfaces (Makbul *et al* 2011).

Table 3: Variation in stomatal frequency of chickpea genotypes under restricted moisture conditions.

Genotype	**Adaxial surface**			**Abaxial surface**		
	Control	**Restricted irrigation (50%)**	**Restricted irrigation (75%)**	**Control**	**Restricted irrigation (50%)**	**Restricted irrigation (75%)**
GL 12003	27.3	31.3	33.4	41.2	47.8	52.0
GL 29095	25.7	29.6	31.2	40.8	47.5	49.7
GL 96836	24.8	28.3	30.1	35.0	39.5	43.1
GL 12021	23.3	26.2	27.4	32.3	36.4	39.8
GL 13037	22.4	24.6	26.4	33.4	37.4	39.2
GL 13043	21.2	23.2	25.3	31.3	33.7	37.2
GL 13050	25.3	28.8	30.4	35.0	39.2	41.3
GNG 2171	26.7	30.7	32.4	41.0	46.8	51.6
BGD 1094	19.5	21.0	22.3	26.3	28.5	29.6
GNG 2249	22.5	24.7	26.4	40.5	45.2	47.6
GNG 2264	21.0	23.7	24.8	34.7	39.2	42.5
ICC 4958	22.3	25.1	25.7	32.3	36.2	37.2
H-11-58	25.1	27.8	29.7	38.4	43.1	45.3
CSJK 4	24.0	26.8	28.7	36.3	41.2	43.2
ILC 3279	20.2	22.2	23.4	23.6	25.4	26.4
RVSSG 31	24.3	27.6	29.2	36.4	41.3	44.2
JG 41	23.6	26.2	28.2	38.7	44.2	46.2
L 555	19.3	20.6	22.3	26.1	28.4	30.2
GPF 2	21.3	24.1	25.3	35.7	39.7	43.2
PDG 3	23.3	26.2	28.3	37.5	42.5	46.2
Mean	23.2	25.9	27.5	34.8	39.2	41.8
CD (5%)	G= 1.023, T=0.396, GXT=NS			G=1.091, T=0.422, GXT=1.889		

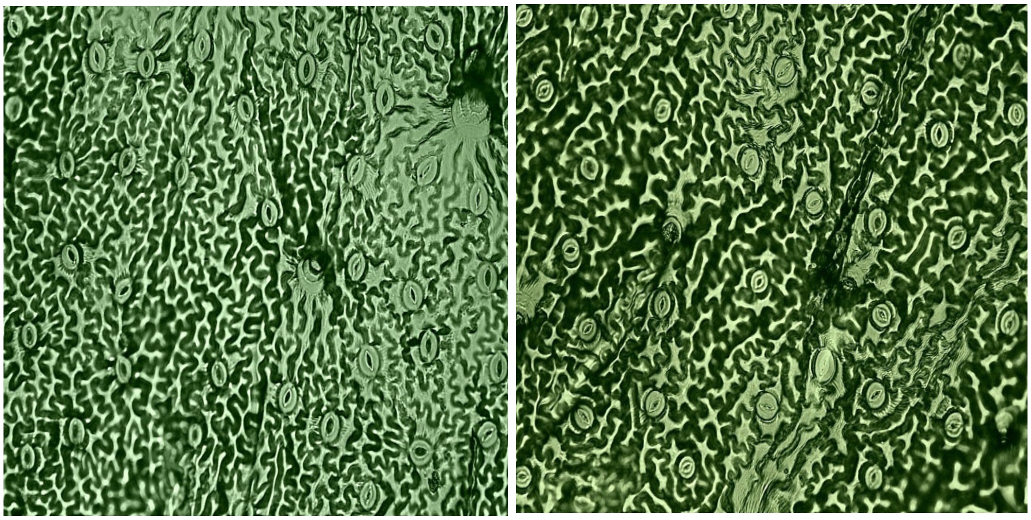
4.2 Root traits (60DAS)

4.2.1 Root volume (cm³)

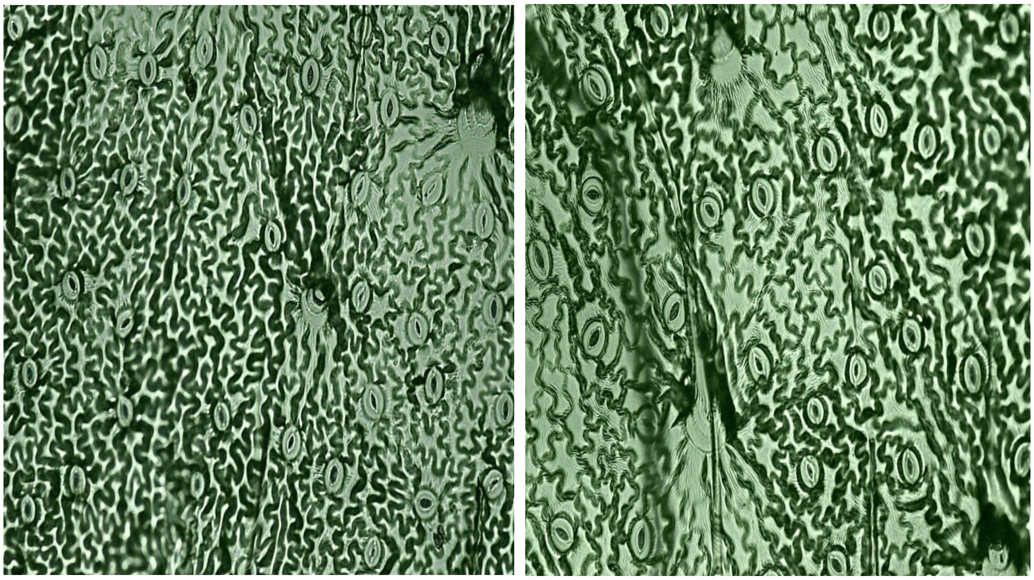
Like other root traits, root volume also declined under water stress conditions (Table 4). Root volume under control condition ranged between 5.5 to 12.1cm³ in GNG 2249 and BGD 1094, respectively. Reduction in root volume with restricted irrigation is shown in Figure 2 and the maximum decline was observed under 75% restricted irrigation. Under 50% restricted irrigation maximum and minimum % reduction was observed in GL 29095 (35.8%) and ILC 3279 (12.1%) respectively. Under 75% restricted irrigation maximum % reduction was observed in GL 29095 (56.6%) and minimum % reduction was observed in L 555(25.7%). The reduction in root traits as affected by drought have been reported in other crops such as in peanut (Songsri *et al* 2009) and Arabidopsis (Xiong *et al* 2006).

Table 4: Variation in root traits of chickpea genotypes under restricted moisture conditions

Genotype	**Control**			**Restricted irrigation (50%)**			**Restricted irrigation (75%)**		
	Root volume	**Root mass**	**Root area**	**Root volume**	**Root mass**	**Root area**	**Root volume**	**Root mass**	**Root area**
GL 12003	7.8	0.915	2346.2	5.5	0.654	1621.3	3.4	0.463	993.5
GL 29095	7.3	0.662	1834.2	4.7	0.467	1163.4	3.2	0.330	802.6
GL 96836	7.5	0.867	2614.8	5.4	0.597	1834.6	3.5	0.482	1145.2
GL 12021	10.4	1.530	3564.3	7.6	1.157	2413.6	5.9	0.915	1648.4
GL 13037	10.2	1.443	3254.2	8.5	1.230	2544.1	5.2	0.760	1527.5
GL 13043	11.1	1.590	3954.1	9.7	1.346	3154.6	7.2	1.124	2213.8
GL 13050	9.6	1.125	3217.6	6.9	0.826	2214.9	5.8	0.624	1834.7
GNG 2171	7.1	0.826	1934.5	4.8	0.563	1256.4	3.2	0.426	834.5
BGD 1094	12.1	1.631	4247.7	10.6	1.463	3561.2	8.5	1.162	3048.2
GNG 2249	5.5	0.737	2013.6	4.2	0.612	1536.4	3.5	0.486	1236.4
GNG 2264	8.8	0.935	2637.4	6.2	0.723	1834.1	5.2	0.551	1541.5
ICC 4958	5.4	0.713	2246.5	4.0	0.561	1631.8	3.7	0.490	1489.3
H-11-58	6.7	0.773	2868.0	5.3	0.627	2154.8	4.1	0.472	1749.5
CSJK 4	9.3	1.020	3614.4	7.5	0.870	2864.5	5.5	0.623	2058.1
ILC 3279	11.6	1.620	4593.6	10.2	1.426	3948.6	7.8	1.124	3157.8
RVSSG 31	7.1	0.810	2314.6	4.8	0.613	1531.8	3.7	0.435	1183.7
JG 41	6.3	0.723	2146.0	5.1	0.612	1647.5	3.9	0.468	1259.4
L 555	10.1	1.263	4135.9	8.3	1.097	3466.1	7.5	0.943	3142.7
GPF 2	9.1	0.957	3349.5	6.3	0.726	2247.8	5.3	0.567	1866.6
PDG 3	6.8	0.797	2946.1	5.3	0.673	2218.4	4.2	0.516	1737.8
Mean	8.5	1.047	2991.7	6.5	0.842	2242.3	5.0	0.648	1723.6
CD (5%)	G=0.482, T=0.187, GXT=0.835 (root volume), G=0.305, T=0.118, GXT=0.144 (root mass), G=90.663, T35.114, GXT=157.03 (root area)								



A (Abaxial surface) B (Adaxial surface)



C (Abaxial surface) D (Adaxial surface)

Plate I : Stomatal distribution on the leaf surfaces of GNG 2171 (A,B), a sensitive genotype and BGD 1094 (C,D), a tolerant genotype of *Cicer arietinum* (20X).

4.2.2 Root mass (g/pl)

Dry weight of roots of different genotypes of *C. arietinum* was observed under normal and stressed conditions (Table 4) and there was decrease in root dry weight in the latter conditions. Under control condition, dry weight varied between GL 29095 (0.662g) to BGD 1094 (1.631g). Among 50% restricted irrigation root dry weight decreased by 31.8 (GNG 2171) to 10.3 (BGD 1094) per cent and by 50.2 (GL 29095) to 25.3 (L 555) per cent among 75% restricted irrigation (Fig.2). In other crops like *Saccharum officinarum* (Jangpromma *et al* 2012) and *Medicago sativa* (Mouradi *et al* 2015) same decline was recorded in root biomass. Genotypic variation was also prominent in this study, tolerant genotypes showed less reduction in comparison to resistant chickpea genotypes. Decrease in root dry weight in peanut genotypes under moisture stress is due to reduction in number and weight of nodules and less overall partitioning of biomass towards root (Pimratch *et al* 2008).

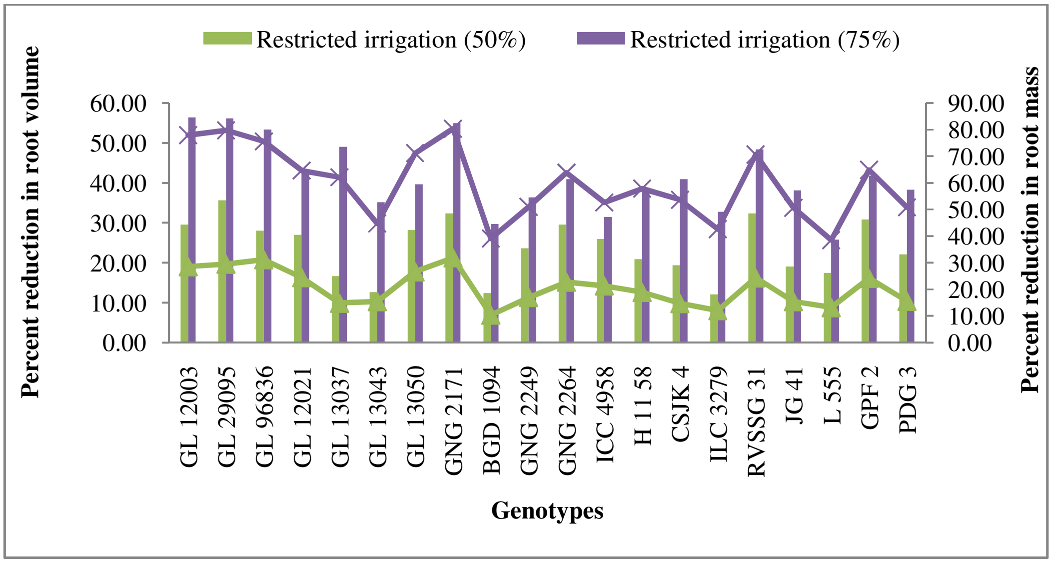


Fig 2: Percent reduction in root volume and root mass of chickpea genotypes as influenced by moisture stress.

4.2.3 Root area (mm²)

Variation in root area of chickpea genotypes under restricted moisture conditions is presented in Table 4. Root surface area decreased under stress over controlled environment. Decline under 75% restricted irrigation was comparatively higher than 50% restricted irrigated plants. Under control conditions, root surface area was found maximum in ILC 3279 (4593.6mm²) and minimum in GL 29095 (1834.2 mm²). Percentage reduction was least in ILC 3279 (14%) under 50% restricted irrigation and in L 555 (24%) under 75% restricted irrigation. Whereas, tremendous difference was observed in GL 29095 (36.6%) and GL 12003(57.7%) under 50 and 75% restricted irrigation, respectively (Fig.3). Benjamin and Nielsen (2006) demonstrated that in comparison to soybean, field pea and chickpea has a greater root surface area to weight ratio which indicated the more fine roots in the root system

L 555 (Control)

L 555 (Restricted irrigation 50%)

L 555 (Restricted irrigation 75%)

GL 29095 (Control)

GL 29095 (Restricted irrigation 50%)

GL 29095 (Restricted irrigation 75%)

Fig. 3: Variation in root area (mm²) of tolerant (L 555) and sensitive (GL 29095) genotypes under different moisture conditions.

for better soil exploration and water extraction. The results are in co-ordination to the reports in the literature.

4.2.4 Nodule weight (mg)

Low soil moisture during the early stages of the chickpea growth decreased nodule formation (Gan *et al* 2005). In comparison to the control condition, mean dry weight of root nodules under 50% restricted irrigation and 75% restricted irrigation decreased i.e. (67, 50 and 38mg). Under control condition maximum and minimum nodule dry weight was observed in L 555 (161mg) and GNG 2171 (30.0mg) respectively (Table 5). Diminishment of nodule dry weight in 75% restricted irrigation over control varied between BGD 1094 (34.09%) to GNG 2171(60%). Under 50% restricted irrigation, minimum % reduction was observed in L 555 (9.94%) and maximum % reduction was seen in GL 29095 (42.22%) (Fig.4).

The reduction in nodule dry weight were noted by Kumar *et al* (2010) in different chickpea genotypes where the similar decline was observed under drought conditions. Drought also affected the structure and weight of nodule in *Medicago sativa*. Under drought condition the proportion of empty nodules increases and it ceases to the senescence of nodules (Puppo *et al* 2005). Adverse effects of water stress in alfalfa on nodulation and nitrogen fixation was also reported by Athar and Johanson (1996). The reduction in nodule mass may be due to decreased number of nodules which is attributed to the effect of drought on the process of nodulation and the activity of nitrogenase enzyme as reported in chickpea (Repela and Bech 1990).

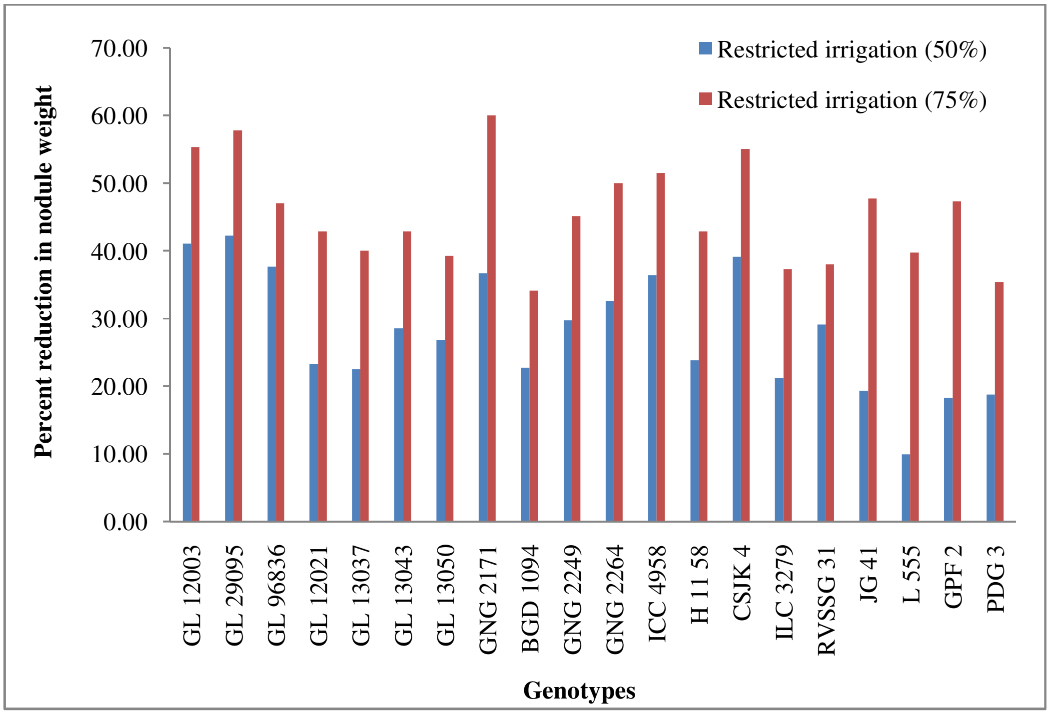


Fig 4: **Percent reduction in nodule weight (mg) of chickpea genotypes as influenced by moisture stress.**

Table 5: Variation in nodule dry weight (mg) of chickpea genotypes under restricted moisture conditions.

Genotype	Control	Restricted irrigation (50%)	Restricted irrigation (75%)
GL 12003	56	33	25
GL 29095	45	26	19
GL 96836	85	53	45
GL 12021	56	43	32
GL 13037	80	62	48
GL 13043	63	45	36
GL 13050	56	41	34
GNG 2171	30	19	12
BGD 1094	88	68	58
GNG 2249	58	41	32
GNG 2264	46	31	23
ICC 4958	33	21	16
H-11-58	42	32	24
CSJK 4	46	28	21
ILC 3279	85	67	53
RVSSG 31	79	56	49
JG 41	88	71	46
L 555	161	145	97
GPF 2	93	76	49
PDG 3	48	39	31
Mean	67	50	38
CD (5%)	G= 3.012, T=1.167, GXT= 5.217		

4.2.5 Anatomical changes

Water and nutrient transport capacity of plant roots is highly influenced by the number and size of the conducting elements (Steudle and Peterson 1998). Variability was observed among tolerant and sensitive genotypes for various root anatomical parameters *viz.* number of xylem vessels, average vessel diameter and diameter of largest vessel (Table 6). The number of xylem vessels per root cross section was significantly higher in tolerant genotypes as compared to sensitive ones (Plate II). Maximum number of xylem vessels was found in L 555 (70) which is closely followed by BGD 1094 (69), whereas minimum number was observed in GNG 2171 (58) under control condition. Under restricted irrigation i.e. 50% and 75% there was reduction in the number of xylem vessels and the maximum reduction was found in sensitive genotypes.

Variation in average vessel diameter in tolerant and sensitive genotypes under different moisture conditions was also noted (Table 6). It was observed maximum in L 555 (15.34mm) and minimum in GNG 2171 (12.78mm) under control condition. Largest vessel

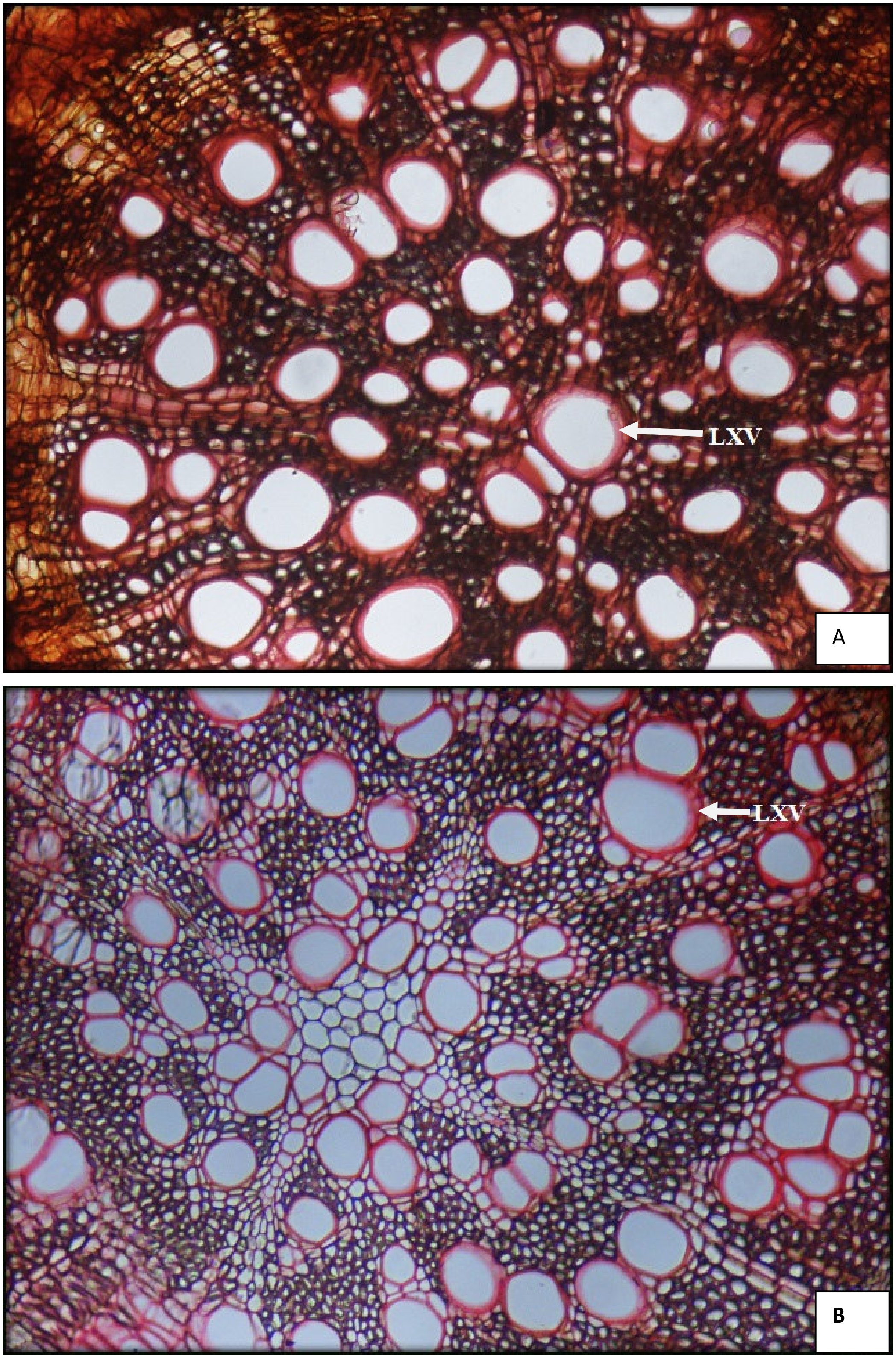


Plate II. Transverse section of *Cicer arietinum* root (20X).
A) Sensitive genotype GNG 2171 B) Tolerant genotype L 555
LXV-Large xylem vessel

Table 6: Variation in number of vessel elements and their features in root of different chickpea genotypes under varied moisture conditions.

Genotype	Number of xylem vessels			Average vessel diameter (mm)			Diameter of largest vessel (mm)		
	Control	Restricted irrigation (50%)	Restricted irrigation (75%)	Control	Restricted irrigation (50%)	Restricted irrigation (75%)	Control	Restricted irrigation (50%)	Restricted irrigation (75%)
GL 29095	61	52	43	13.15	11.16	10.63	16.78	14.15	13.75
GL 12003	63	51	44	13.11	11.23	10.06	16.23	14.23	13.64
GNG 2171	58	49	41	12.78	10.64	9.48	15.26	13.43	12.36
BGD 1094	69	63	55	15.12	13.56	12.25	18.13	17.26	16.84
ILC 3279	66	59	51	15.06	13.23	12.11	17.63	16.78	16.34
L 555	70	61	53	15.34	13.79	12.97	18.35	17.53	17.48

diameter among tolerant genotypes observed was 18.35mm (L 555) whereas; in sensitive genotypes it was 15.26mm (GNG 2171). Reduction in the vessel diameter was observed in all the genotypes under restricted irrigation. Maximum reduction was observed in sensitive genotype under 75% restricted irrigation i.e. 25.85% (GNG 2171) and minimum reduction was observed in tolerant genotype L 555 (15.45%). Reduction in vascular tissue area was reported under moisture stress condition by Ristic and Cass (1991) in *Zea mays*. The variation in width of cortex or width vascular bundle in stressed and unstressed plants was also reported.

EXPERIMENT 2: Biochemical changes and yield attributes of chickpea genotypes.

4.3 Phenophasic development

Due to onset of severe water deficit, early maturity is an important feature to avoid the moisture stress. There were differences in flower initiation among the different genotypes. Phenological parameters such as days to flower initiation, 50% flowering, pod initiation and

Table 7: Phenophasic development of various chickpea genotypes under irrigated and rainfed conditions.

Genotype	Days to flower initiation		Days to 50% flowering		Days to pod initiation		Days to maturity	
	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed
GL 12003	92	86	103	100	106	105	138	135
GL 29095	91	87	103	100	110	108	143	140
GL 96836	59	54	94	88	105	104	137	131
GL 12021	89	86	100	96	107	104	140	132
GL 13037	99	97	109	108	111	111	144	142
GL 13043	93	91	105	97	111	110	145	141
GL 13050	95	93	106	101	110	107	145	143
GNG 2171	89	88	100	99	109	107	146	139
BGD 1094	84	64	99	97	107	105	142	134
GNG 2249	70	63	97	84	109	104	142	133
GNG 2264	86	81	99	97	108	104	141	133
ICC 4958	68	56	98	87	109	105	142	135
H-11-58	96	93	105	102	111	108	145	140
CSJK 4	57	50	91	70	105	103	136	129
ILC 3279	95	79	109	99	111	110	143	135
RVSSG 31	54	50	77	65	102	96	133	128
JG 41	58	53	77	67	99	95	131	126
L 555	76	70	95	94	107	104	140	131
GPF 2	93	80	100	99	110	107	141	140
PDG 3	93	91	104	102	109	108	143	141
Mean	82	76	98	93	108	105	141	136

maturity were recorded and presented in Table 7. Moisture stress leads to early maturity and the twenty genotypes were categorized on the basis of early, moderately and late maturing genotypes. JG 41, RVSSG 31, CSJK- 4 and GL 96836 were noted to be early flowering and maturing genotypes, whereas BGD 1094, GNG 2249, L 555 and ILC 3279 were recorded to be moderately maturing and the genotypes GL 13037, H-11-58 and GL 13050 were observed to be late maturing types. Ulemale *et al* (2013) demonstrated that yield potential and early flowering are two major components of drought escape in lentil and chickpea.

4.4 Physiological evaluation

4.4.1 Relative water content (%)

Plant tolerance to drought stress may be associated with different systems involving the capability to maintain high relative water content (RWC) (Oukarroum *et al* 2007). Decreasing water availability under drought generally results in reduced total nutrient uptake and frequently reduces the concentrations of mineral nutrients in crops (Baliger *et al* 2001, Gunes *et al* 2006). Under irrigated condition, relative water content ranged from 78.54% to 69.03% in GPF 2 and GL 13037, respectively (Fig.5). It declined under rainfed condition, where least depletion was seen in GL 13043 (8.3%) and remarkable decline was seen in GL 12003(28.8%).Tolerance and sensitivity of chickpea to drought is related to its capability to maintain good leaf water status and utilization of leaf relative water content as an indicator of plant water status is usual (Lawlor and Cornic 2002).

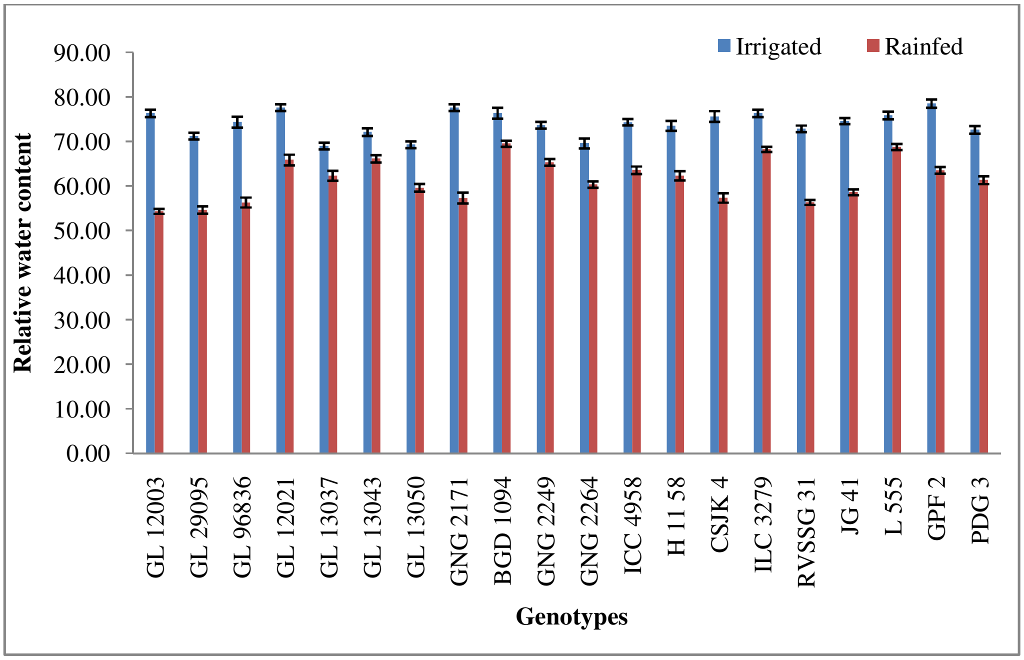


Fig 5: Variation in relative water content (%) of chickpea genotypes as influenced by moisture stress.

Siddique *et al* (1999) and Bahadur (2008) reported that there is significant reduction in relative leaf water content of plant subjected to water shortage. Significant differences in relative water content in tolerant and sensitive genotypes of maize (Pastori and Trippi 1992) and wheat (Sairam and Srivastava 2001) have also been reported. High relative water content may results from osmoregulation by osmoprotectants, as carotenoids or sugars are often accumulated in plants subjected to drought (Gunes *et al* 2008).

4.4.2 Photosynthetic rate (µmolCO_2 $m^{-2}s^{-1}$)

Low water potential due to reduced water availability negatively affected the photosynthetic activity. The significant decrease in net photosynthesis, stomatal conductance and transpiration rate under moisture stress which resulted into significant decrease in plant growth (Krouma 2009). Variation in photosynthetic rate in all chickpea genotypes as influenced by moisture stress condition is shown in Figure 6. Under irrigated conditions, photosynthetic rate varied between 7.68 to 12.78 µmolCO_2 $m^{-2}s^{-1}$, however under rainfed conditions remarkable reduction in photosynthetic rate over irrigated condition was observed. Reduction varied between lowest in GL 13043(7%) to highest in GL 29095 (59.2%) followed by GL 12003(54.7%).

Kumar *et al* (2010) also observed the reduction in photosynthetic activity in different chickpea genotypes under water deficit condition. Moisture stress induced decrease in the chlorophyll content in gerbera was reported by Qi-Xian *et al* (2007). Flexas and Medrano (2002) concluded that decreased photosynthetic rate is due to the stomatal closure which resulted into CO_2 scarcity in the chloroplasts. But Lawlor (2002) and Tang *et al* (2002) claimed that impaired ATP is a likely reason for decreased photosynthetic rate under water stress conditions. Later Basu *et al* (2004) also concluded that under moisture stress condition, limited photosynthesis is due to the stomatal closure.

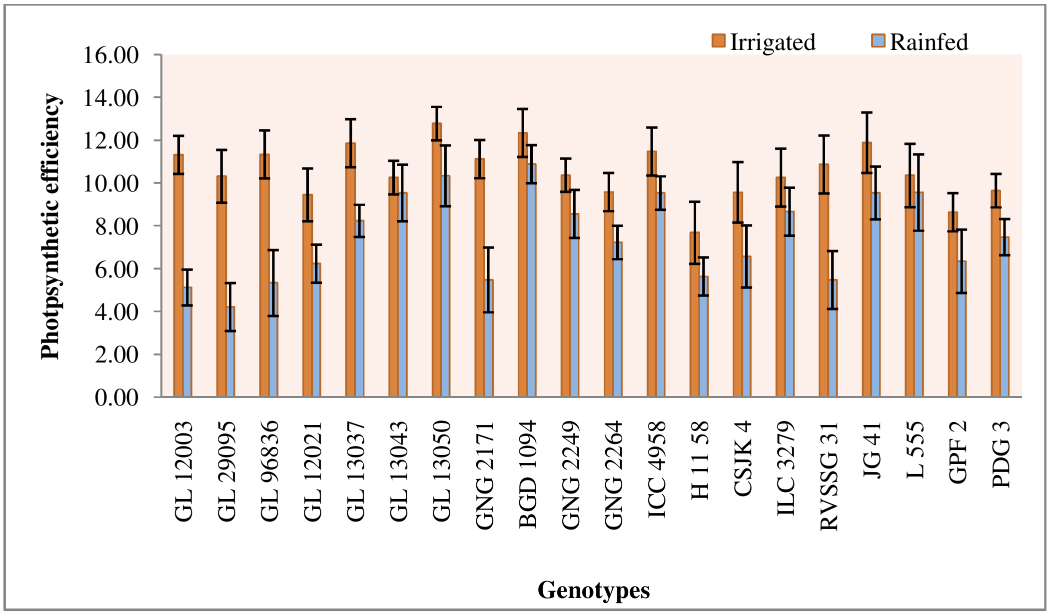


Fig 6: **Variation in photosynthetic rate (µmolCO_2 $m^{-2}s^{-1}$) of chickpea genotypes as influenced by moisture stress.**

4.4.3 Leghaemoglobin content (mg/g)

Variation in leghaemoglobin content in all chickpea genotypes is presented in Figure7. In tested genotypes, the leghaemoglobin content ranged from 4.65 to 8.28 mg/g FW in control. Under water deficit conditions leghaemoglobin content decreased in comparison to the control condition. Reduction was comparatively less in tolerant genotypes than in sensitive ones. Maximum reduction was recorded in GL 12003(50.15%) closely followed by GL 29095 (49.43%), whereas minimum reduction was observed in ILC 3279 (7.56%) and L 555 (10.76%).

Leghaemoglobin content in common bean was declined in the dehydrated nodules which were subjected to severe drought (Figueriedo *et al* 2008). Mhadhabi *et al* (2009) proposed that there could be the production of O_2^- radicals due to reduction of leghaemoglobin content in early degenerated nodules in *Medicago truncatula.* Singh and Singh (2006) observed that drought suppresses the growth of nodules, which may be due to restriction of carbohydrate transport from leaves to nodules as in *Dalbergia sissoo*.

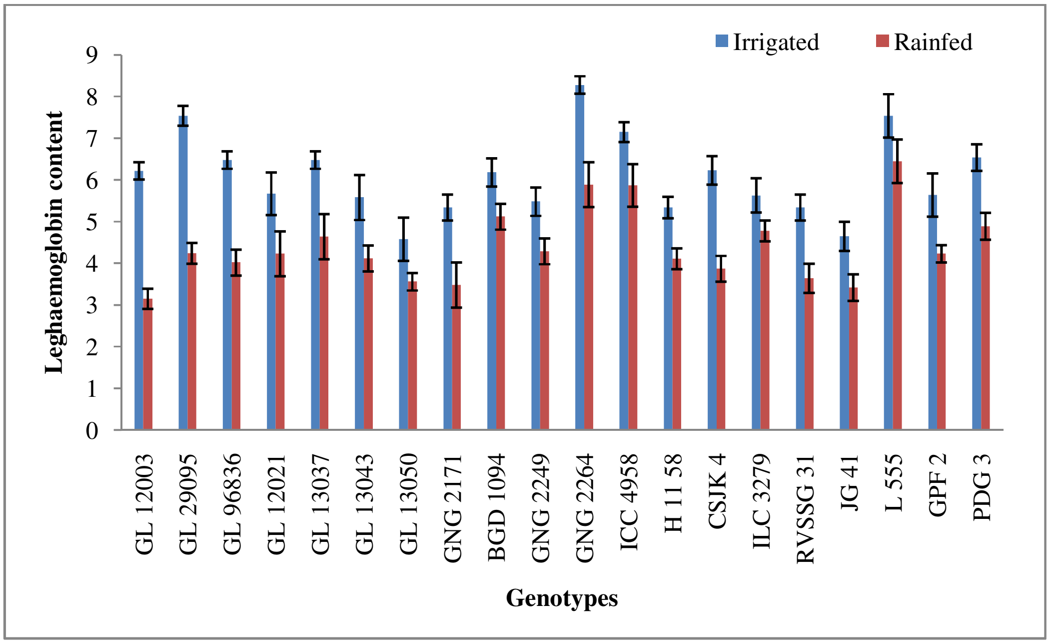


Fig 7: Variation in Leghaemoglobin content (mg/g FW) of chickpea genotypes as influenced by moisture stress.

4.5 Biochemical estimations

4.5.1 Leaves

4.5.1.1 Proline content

Proline accumulation is believed to play an adaptive role in plant stress tolerance (Verbruggen and Hermans 2008) and has been advocated as a parameter of selection for stress tolerance (Jaleel *et al* 2007). Increase in proline content under water stress condition as compared to the control condition is shown in Table 8. Highest value was observed in

ILC 3279 (1.53µmole/g) and lowest was observed in GL 12003 (0.95 µmole/g) under control condition. There was percent increase in proline content when subjected to water stress and maximum increase was in tolerant genotypes BGD 1094 (58.90%) and L 555 (57.54%) and minimum in sensitive genotypes GL 12003 (41.72%) and GNG 2171 (39.90%).

Several investigations indicated positive relationship between free proline content of leaves with drought tolerance in chickpea (Singh and Singh 1999), pigeonpea (Mukane *et al* 1996) and soybean (Heerden *et al* 2002). The singlet oxygen quencher and reactive oxygen species scavenging activity showed the antioxidant feature of proline (Szabados and Savoure 2010). Islam *et al* (2009) reported that, proline also protect and stabilize ROS scavenging enzymes and activate alternative detoxification pathways in plants subjected to various abiotic stresses. Therefore, proline acts as both as a direct antioxidant as well as an activator of mechanisms that act as antioxidants, as has also been observed in the present study.

Table 8: Proline content of chickpea genotypes under irrigated and rainfed conditions

Genotype	**Proline content (µmole/g)**	
	Irrigated	**Rainfed**
GL 29095*	1.02±0.20	1.86±0.46
GL 12003*	0.95±0.16	1.63±0.51
GNG 2171*	1.16±0.21	1.93±0.34
BGD 1094**	1.34±0.34	3.26±0.25
ILC 3279**	1.53±0.41	3.19±0.14
L 555**	1.38±0.42	3.25±0.51

Sensitive*   Tolerant**

4.5.1.2 Total soluble sugars (mg g^{-1} dry weight)

Higher level of various reserves especially total soluble sugars in tolerant genotypes might have resulted in maintenance of required turgor under conditions of drought stress. Total soluble sugar under control condition was found low in comparison to the water stress condition (Table 9). Maximum sugar content was observed in L 555 (38.12mg/g dry weight) and minimum was observed in GL 12003 (29.65mg/g dry weight) under control condition. Under water deficit maximum percent increase was noted in tolerant genotype ILC 3279 (42.57%) and minimum % increase was observed in GNG 2171 (19.04%). Jain and Jain (2015) observed the same trend of enhancement in sugar content under stress over control condition. Increase in sugar content in response to drought stress has also been reported by Hossein *et al* (2001). Total soluble sugars as osmolite materials increases flows of water into cells and maintain turgor of cells through adjusting water content (Rasaei *et al* 2013).

Table 9: Total soluble sugars of chickpea genotypes under irrigated and rainfed conditions

Genotype	Total soluble sugars (mg g^{-1} dry weight)	
	Irrigated	Rainfed
GL 29095*	32.31±1.35	46.31±1.52
GL 12003*	29.65±1.67	42.14±1.53
GNG 2171*	32.58±1.61	40.24±0.61
BGD 1094**	36.57±2.02	65.24±1.25
ILC 3279**	36.81±1.39	67.26±1.36
L 555**	38.12±1.46	68.31±2.15

Sensitive* **Tolerant****

4.5.1.3 Superoxide dismutase

Superoxide dismutase (SOD) constitute the first line of defense via detoxification of superoxide radicals (Sairam and Saxena 2000), thus maintain membranes of plant tissue. SOD converts the toxic O_2^- to H_2O_2 which then converted to O_2 and H_2O by other antioxidant enzymes (Peroxidase and catalase). The activity of SOD was found to be higher in leaves of tolerant genotypes as compared to sensitive genotypes (Fig.8). Highest activity was observed in tolerant genotype ILC 3279 (351.42 unit enzyme/g FW) whereas lowest activity was noticed in GNG 2171 (275.60 unit enzyme/g FW) under rainfed condition.

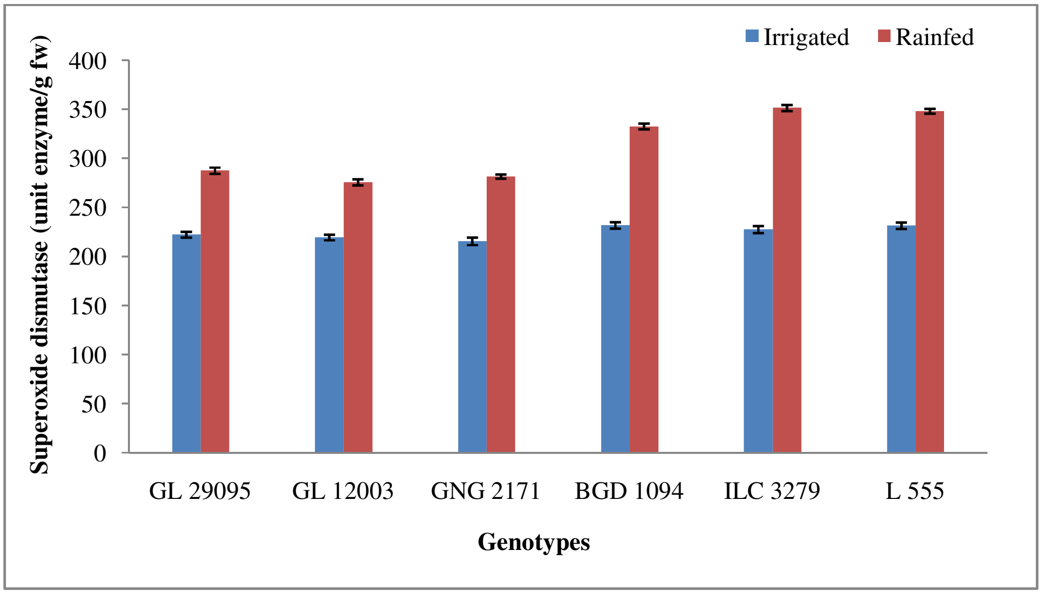


Fig 8: Superoxide dismutase activity (unit enzyme/g FW) of chickpea genotypes under irrigated and rainfed conditions

Patel and Hemantaranjan (2012a) also observed that the SOD activity increase in all chickpea genotypes under moisture stress condition. Higher superoxide dismutase activity during moisture stress might protect plants from oxidative injury (Arora *et al* 2002). Raheleh *et al* (2012) observed tolerant chickpea genotypes could decrease damaging effects of drought stress by an increase in antioxidant enzymes activity such as SOD enzymes, in the flowering and podding stages. Less increase in SOD activity in sensitive genotypes may be related to the low water potential of this cultivar to remove O^{2-} under water deficit.

4.5.1.4 Peroxidase activity

Peroxidase (POX) is among the major enzyme that scavenges H_2O_2 in chloroplasts which is produced through dismutation of O^{2-} catalyzed by superoxide dismutase as observed in *Phaseolus acutifolius* and *Phaseolus vulgaris* (Turkan *et al* 2005). Like superoxide dismutase, the activity of peroxidase enzyme was also found to be higher in leaves of tolerant genotypes as compared to sensitive genotypes (Fig.9). Under control condition, highest enzyme activity among tolerant genotypes was noticed in BGD 1094 (111.22 ΔA/min/g FW) while minimum activity among sensitive genotypes was exhibited in GL 12003 (98.87 ΔA/min/g FW). Under rainfed condition noticeable percent increase was observed in all the genotypes but maximum percent increase noticed was 38.07% in tolerant genotype (ILC 3279).

In contrast to catalase activity Dey *et al* (2007) found that POX plays more significant role in detoxifying the H_2O_2. It is well recognized that CAT is less efficient than POX in scavenging of H_2O_2 because of its low substrate affinity (Erdal and Dumlupinar 2010).Patel and Hemantaranjan (2012a) also found higher POX activity under drought stressed seedlings of chickpea which provide protection against the oxidative stress as has also been recorded in the present study.

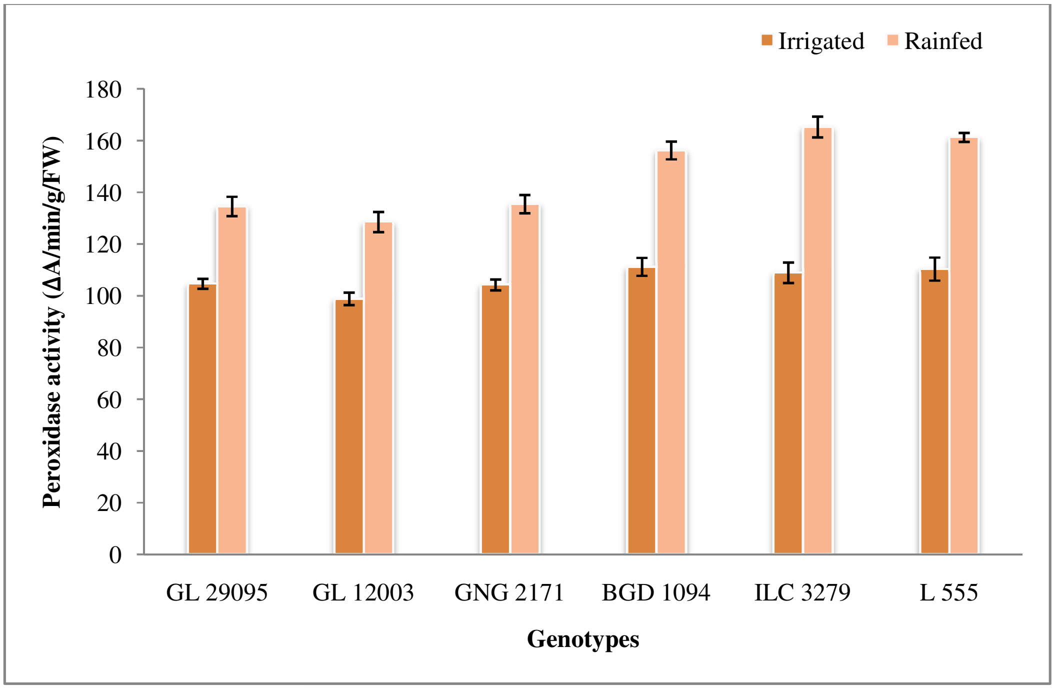


Fig 9: Peroxidase activity (ΔA min^{-1} g^{-1} fresh weight) of chickpea genotypes under irrigated and rainfed conditions

4.5.1.5 Catalase activity

Catalase eliminates H_2O_2 by breaking it down directly to water and oxygen. Catalase and peroxidases are the most important enzymes involved in regulation of intracellular level of H_2O_2. Catalase activity increased in tolerant chickpea cultivars in comparison to sensitive cultivars (Raheleh *et al* 2012) as also observed in present study (Fig.10). Under control condition highest activity of catalase was found in tolerant genotypes i.e. L 555 (943.13 ΔA min^{-1} g^{-1} fresh weight) and BGD 1094 (934.56 ΔA min^{-1} g^{-1} fresh weight) whereas, lowest activity was observed in sensitive genotype i.e. GNG 2171 (597.40 ΔA min^{-1} g^{-1} fresh weight). There was sharp increase in catalase activity when exposed to water stressed condition and it was noted in ILC 3279 (27.70%) and minimum percent increase was observed in GL 29095 (16.41%).

Catalase reduced the high level of oxygen species under stress condition. Helal and Samir (2008) also reported that the increased activity of catalase enzyme develops a potential for defence against damage in maize genotypes. The present results are in accordance with the findings in chickpea (Patel *et al* 2011, Patel and Hemantaranjan 2012a), *Catharanthus roseus* (Jaleel *et al* 2007), mungbean (Ahmed *et al* 2002), barley (Salekjalali *et al* 2012) and mulberry (Ramachandra Reddy *et al* 2000).

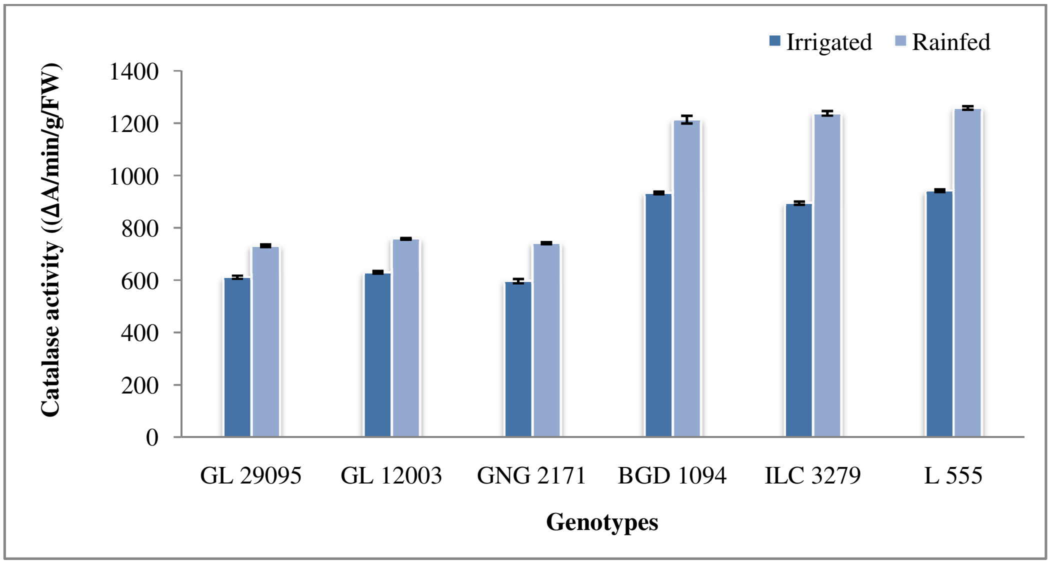


Fig. 10: Catalase activity of chickpea genotypes under irrigated and rainfed conditions

4.5.2 Seeds

4.5.2.1 Starch content

There is inter- relationship between starch and soluble sugars concentration. Nayer and Heidari (2008) reported that quantitative increase in the content value of soluble sugar concentration has a direct effect on the quantitative decrease of content value of starch concentration in chickpea. Under irrigated condition, highest starch content was observed in tolerant genotypes i.e. BGD 1094 (54.23 mg g^{-1} dry weight) closely followed by ILC 3279

(52.34 mg g^{-1} dry weight). Decline in starch content was seen when exposed to moisture stress condition (Fig.11). Sensitive genotypes showed maximum decline i.e. 32.36% (GL 12003) and 29.02% (GL 29095) while minimum decline was observed in tolerant genotypes i.e. 17.65% (L 555) and 19.11% (ILC 3279). Jain and Jain (2015) in their study on chickpea reported the decrease in starch content under water deficit condition and the present study results are also in accordance to the reports.

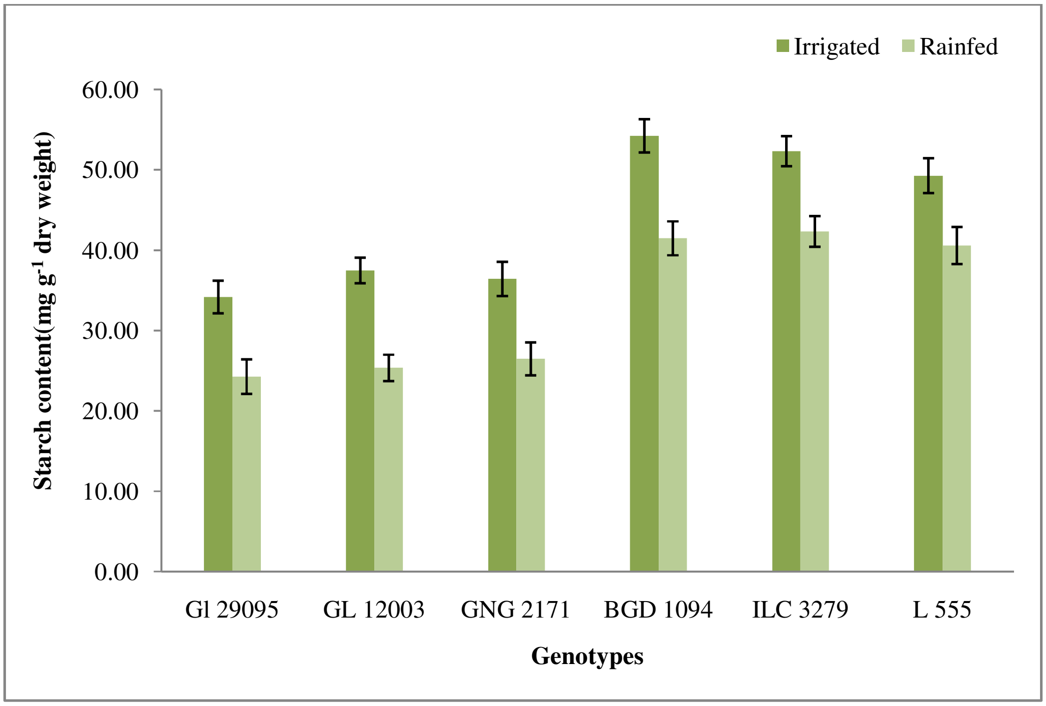


Fig 11: Starch content in seeds of chickpea genotypes under irrigated and rainfed conditions.

4.5.2.2 Proline content

Plants accumulate proline content under variety of stress conditions thereby, preventing stress-caused damages. It is an osmoprotectant accumulates in various plants when exposed to environmental stresses such as drought, salinity, extreme temperature, UV radiations and heavy metals (Eraslan *et al* 2007). Variation in proline content in dry seeds of selected chickpea genotypes under moisture stress condition is presented in Table 10. Proline content was found low in irrigated condition in comparison to the rainfed condition. Tolerant genotypes showed maximum increase in proline content as compared to sensitive genotypes under rainfed condition. Under irrigated condition, maximum and minimum proline accumulation was observed in L 555 (2.43 µmole/g) and GL 29095(1.52 µmole/g), respectively. Significant increase in proline content was observed when exposed to stress (rainfed) condition. Percent increase was highest in ILC 3279 (63.04%) closely followed by BGD 1094 (61.56%), whereas lesser increase in proline content was observed in GL 12003(46.73%).

Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure 2010). Proline influences protein recovery and preserves the quaternary structure of complex proteins, maintains membrane integrity under dehydration stress and reduces oxidation of lipid membranes or photoinhibition as noticed in rice (Demiral and Turkan 2004). Moreover, it also contributes to stabilizing subcellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions (Ashraf and Fooland 2007) as being corroborated in the present study.

Table 10: Proline content in seeds of chickpea genotypes under irrigated and rainfed conditions

Genotype	Proline content (µmole/g)	
	Irrigated	Rainfed
GL 29095*	1.52±0.21	3.12±1.31
GL 12003*	1.63±0.16	3.06±1.14
GNG 2171*	1.56±0.12	3.16±1.06
BGD 1094**	2.36±0.31	6.14±1.14
ILC 3279**	2.31±0.22	6.25±1.12
L 555**	2.43±0.32	6.10±0.76

Sensitive* Tolerant**

4.5.2.3 Protein profile

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protein profiling of seeds in chickpea genotypes under irrigated and rainfed conditions presented in Plate III. Electrophoretic analysis of total proteins in seeds from irrigated, revealed that bands near to 96 KDa molecular weights were more intense in BGD 1094 and L 555 and less intense in GNG 2171. In seeds derived from rainfed condition, differences were found more evident, bands of lesser intensity near 96 KDa molecular weights were visible in BGD 1094, ILC 3279 and L 555. However, 96 KDa molecular weight proteins were less intense in GNG 2171. Bands of 66 KDa molecular weights were more intense in control than stress treatments. 29 KDa bands were found more intense in GNG 2171 both in irrigated and rainfed conditions. Bands with molecular weight 20.1 KDa and 14.3 KDa were observed of high intensity under irrigated than rainfed conditions.

Molecular markers have been used to study the extent of genetic variation. The protein profiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops (Nisar *et al* 2007). The genotypic variations in banding pattern were higher in present research in analogy to water stress treatments. These results were in accordance with finding of Iqbal and Bano

(2009) in wheat. Severe drought stress had effect on protein banding patterns; however other water stress treatments showed no significant effect as observed in chickpea (Mansourifar *et al* 2011). Patel and Hemantaranjan (2013) revealed that protein accumulation and formation of new proteins is considered a way and an indicator of resistance towards drought.

4.6 Yield and yield attributes

Sadeghipour (2008) reported that environmental stresses such as water shortages, especially during grain filling, cause reductions in photosynthesis and remobilization of stored materials, rate and duration of grain filling and grain weight. Variation in yield attributes i.e. filled pods per plant, 100 seed weight, yield per plant and harvest index under moisture stress is presented in Table11. All the yield components significantly decreased under rainfed condition. Under control condition, maximum number of filled pods per plant was found in GL 12003 (40), while minimum was present in GL 13043(12). Under stressed condition there was significant reduction in filled pods per plants, least affected genotype was BGD 1094(14.29%), whereas immense percentage decline was seen in GNG 2171(65.71%).

Along with filled pods per plant, the seed per plant also declined under rainfed condition. Yield per plant among kabuli and desi genotypes varied from 11.3g to 4.5g and 10.5g to 1.1g respectively under irrigated condition. Under rainfed condition yield per plant among kabuli genotypes ranged between 9.3g to 3.3g and from 7.5g to 0.6g in desi genotypes. Pilbeam *et al* (1992) reported that decrease in yield of grain legumes grown under drought conditions is largely due to the reduction in number of pods per plant which also observed in present study.

Seed weight is an important attribute that significantly contributes to final seed yield in legumes. Drought stress imposed resulted in lower 100 seed weight as compared to control, as revealed in Table 11. The weight of 100 seeds of twenty genotypes ranged between 32.30g to 24.31g among the kabuli chickpea genotypes while of desi chickpea genotypes ranged between 26.50g to 13.30g under control conditions. Under rainfed condition, the 100 seed weight lies between 31.24g to 17.24g among kabuli genotypes whereas of desi genotypes the weight lies between 24.20g to 11.25g. The maximium 100 seed weight among kabuli was found in L 555 and BGD 1094, whereas in desi genotypes GL 13037 and GL 13050 showed the maximum weight under rainfed condition. Yadav *et al* (2005) revealed that the chickpea lines with higher plant water status and high leaf water potential produce higher number of seed per plant, seed/pod, harvest index and grain yield. Whereas, water stress generally accelerates leaf senescence and shorten grain filling duration (Chowdhury *et al* 2002). Oweis and Hachum (2009) demonstrated that the cultivars with deep root system can extract water at the greatest depths both under drought and irrigated conditions.

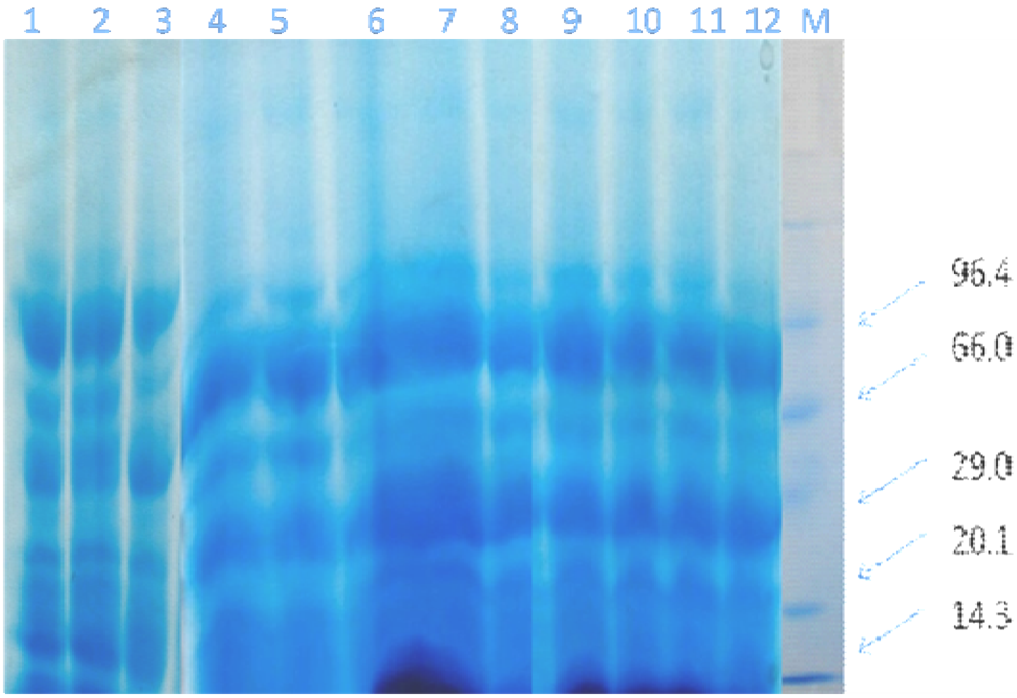


Plate III: Banding pattern in chickpea genotypes (GL 29095, GL 12003, GNG 2171, BGD 1094, ILC 3279& L 555) under irrigated and rainfed conditions by SDS-PAGE.

Lanes: M - Protein molecular weight marker (14.3-96.4 kDa)

Lane : 1- GL 29095 (irrigated), Lane : 2- GL 12003 (irrigated), Lane : 3- GNG 2171 (irrigated), Lane: 4- BGD 1094 (irrigated), Lane: 5- ILC 3279 (irrigated); Lane: 6- L 555 (irrigated); Lane 7- GL 29095 (rainfed), Lane : 8- GL 12003 (rainfed), Lane : 9- GNG 2171 (rainfed), Lane: 10- BGD 1094 (rainfed), Lane: 11- ILC 3279 (rainfed); Lane: 12- L 555 (rainfed).

Table 11: Yield and yield attributes of chickpea genotypes under irrigated and rainfed conditions

Genotype	Filled pod per plant		100 seed weight (g)		Seed yield per plant (g)		Harvest Index	
	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed
GL 12003	40	16	17.10	11.35	8.4	3.1	46.58	30.25
GL 29095	36	18	19.00	12.70	4.2	1.8	43.25	29.48
GL 96836	24	18	17.30	14.35	10.5	7.5	47.66	34.58
GL 12021	42	24	17.60	15.48	10.2	4.8	46.27	32.48
GL 13037	20	15	26.00	24.20	6.3	4.5	43.37	32.58
GL 13043	12	9	22.50	20.36	2.4	1.6	39.48	30.76
GL 13050	24	19	26.50	23.54	2.7	2.1	38.15	30.79
GNG 2171	35	12	16.00	11.25	6.4	1.6	44.47	28.47
BGD 1094	35	30	30.70	28.83	11.3	8.4	49.68	39.12
GNG 2249	15	10	13.30	11.50	1.9	1.0	34.56	27.13
GNG 2264	24	18	17.70	16.12	6.9	4.5	44.56	32.56
ICC 4958	14	9	18.30	13.24	1.1	0.6	35.47	26.52
H-11-58	36	24	17.60	14.90	10.8	7.2	47.25	36.48
CSJK 4	24	17	24.31	17.24	4.5	3.3	39.47	30.48
ILC 3279	21	15	28.20	25.36	8.1	6.3	45.17	36.48
RVSSG 31	26	15	28.10	19.80	6.3	3.6	45.86	33.78
JG 41	13	7	24.36	17.40	1.7	0.9	36.28	26.48
L 555	32	27	32.30	31.24	10.7	9.3	48.74	40.15
GPF 2	39	18	16.90	15.00	7.8	3.6	43.57	30.25
PDG 3	17	9	16.90	13.50	7.1	3.6	42.18	31.45
Mean	26	17	21.53	17.87	6.5	4.0	43.10	32.01
CD (5%)	G=2.421, T=0.766, GXT= 3.424		G=2.257, T=0.714, GXT= NS		G=0.447, T=0.141, GXT= 0.632		G=3.264, T=0.863, GXT=5.367	

CHAPTER V

SUMMARY

Chickpea (*Cicer arietinum* L.), is a legume of the family Fabaceae, subfamily Faboideae. It is generally sown in the month of October- November and harvested in February- April in north of India. The major sowing areas are in the arid and semi-arid regions. In these regions, chickpea is generally grown under rainfed conditions either on stored soil moisture in subtropical environments with summer-dominant rainfall or on current rainfall in winter dominant mediterranean-type environments. In both environments, non-irrigated chickpea plantations suffer yield losses from terminal drought.

The moisture stress invariably leads to oxidative stress in plants due to higher leakage of electrons towards O_2 during photosynthetic and respiratory processes causing enhanced generation of reactive oxygen species (ROS). They cause lipid peroxidation, protein denaturation and DNA damage leading to cell death. Plants respond to moisture stress and acclimatize through various physiological and biochemical changes. These include changes of water use efficiency, pigment content, osmotic adjustment and photosynthetic rate. These mechanisms play a key role in preventing membrane disintegration and provide tolerance against drought and cellular dehydration. High relative water content (RWC) and low excised leaf water loss are associated with drought resistance, and these parameters have also been proposed as more valuable indicators of plant water status in comparison to other water potential parameters under drought stress.

The present investigation entitled "Physiological studies on moisture stress tolerance in chickpea (*Cicer arietinum* L.) genotypes" was carried out during *rabi* 2015-16 with the objectives (1) To compare root and shoot growth behavior under moisture stress condition (2) To evaluate physiological and biochemical parameters affecting yield contributing attributes under moisture stress condition. The study comprised of two experiments and they were conducted in the laboratory and field area of pulses section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. In the first experiment twenty genotypes were raised in PVC pipes for shoot and root traits under restricted moisture stress. In the second experiment same genotypes were sown in the field to study physiological, biochemical and yielding traits. The observations pertaining to shoot, root traits, phenological development, physiological, biochemical and yield traits were recorded in relation to moisture stress condition. Salient results of the study are summarized below:

Shoot traits viz. plant height, biomass accumulation, stomatal frequency and shoot to root ratio were studied under control, 50% and 75% restricted irrigation. Biomass was recorded maximum in genotypes ILC 3279, BGD 1094 and L 555 under 50% and 75% restricted irrigation. Decline in plant height was observed under moisture stress, 50.2, 41.7

and 37 cm under control, 50% and 75% restricted irrigation respectively. There was an increase in stomatal frequency under moisture stress on both the leaf surfaces. Maximum stomatal frequency was observed in genotypes GL 29095, GL 12003 and GNG 2171on both the surfaces and these genotypes were categorized as sensitive genotypes. Shoot: root ratio also decreased with decrease in moisture stress and maximum decline was observed at 75% restricted irrigation.

Variation in root traits (root volume, root mass, root area and nodule dry weight) were also observed under restricted irrigation over the control. Percent reduction was calculated for all the root traits between control and restricted irrigations (50% & 75%). Genotypes which showed minimum percent reduction were ILC 3279, L 555 and BGD 1094 and these were categorized as tolerant genotypes, whereas maximum percent reduction was observed in genotypes GNG 2171, GL 12003 and GL 29095. All the genotypes under moisture stress showed early maturity in comparison to control. Physiological parameters (RWC, photosynthetic activity and leghaemoglobin content) were recorded during the reproductive phase. RWC indicated the plant water status and under moisture stress there was decline in RWC in all the genotypes. In control, RWC ranged from 78.54% (GPF 2) to 69.03% (GL 13037). Decline in RWC was recorded maximum in genotypes GL 12003, GL 29095, CSJK 4 and RVSSG 31 under moisture stress. Photosynthetic rate varied from 7.68 (H-11-58) to 12.78μmolCO_2 $m^{-2}s^{-1}$ (GL 13050) in control (irrigated), however remarkable reduction under moisture stress was observed. Leghaemoglobin content in nodules was estimated to be highest in GNG 2264 and L 555 in control and moisture stress respectively.

On the basis of all the above observations, six genotypes were selected; three tolerant (BGD 1094, ILC 3279 & L 555) and three sensitive (GL 12003, GL 29095 & GNG 2171). The biochemical estimations were carried out on the above mentioned tolerant and sensitive genotype leaves and seeds during reproductive phase. Biochemical estimations included proline content, total soluble sugars, superoxide dismutase-SOD, peroxidase-POX, catalase-CAT activity and starch content. Proline content in both leaves and seeds showed increase in under moisture stress and highest accumulation was recorded in tolerant genotypes. Total soluble sugars as osmolite material increased the slop flow of water in to cells and maintain turgor cells through adjusting water content. Maximum total soluble sugars were noted in tolerant genotype L 555 (68.31 mg/g dry weight) as compared to sensitive genotype GNG 2171 (40.24 mg/g dry weight). Under stress condition, antioxidant enzymes i.e. SOD, POX and catalase protect the cells from reactive oxygen species and they were found maximum in tolerant genotypes. Lower activity of SOD was found in sensitive genotype i.e. GNG 2171. Among all tolerant genotypes ILC 3279 showed maximum activity of SOD, POX and catalase which was followed by L 555 and BGD 1094. Starch content decline under moisture stress and maximum decline was noted in sensitive genotypes.

Yield and yield attributes were reduced under moisture stress condition. Maximum filled pods per plant were found in GL 12003 and minimum in GL 13043 in control. There was significant reduction in filled pods per plant and yield per plant under moisture stress and least affected genotypes were L 555, BGD 1094, ILC 3279,GL 13050 and GL 13037, whereas significant percent decline was observed in GNG 2171, GL 12003, GL 29095, GL 12021 and GPF 2. Seed weight varied from 11.3 g to 1.1 g in control condition and 9.3 g to 0.6 g under moisture stress. All these plant traits including physiological, biochemical and yield attributes contributes towards enhancing high productivity under normal and stress conditions. The information obtained from these traits in current study may be used to develop high yielding varieties which can produce economic yield and help the yield sustainability in the rainfed areas.

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