

**“PHYSIO-BIOCHEMICAL ANALYSIS FOR GROWTH AND YIELD
IN CHICKPEA UNDER MOISTURE STRESS”**

By

Vilas Ramrao Awari

M.Sc.(Agri.)

A thesis submitted to the

**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA**

**in partial fulfilment of the requirements for the degree
*of***

**DOCTOR OF PHILOSOPHY (AGRICULTURE)
in
AGRICULTURAL BOTANY
(PLANT PHYSIOLOGY)**

**DEPARTMENT OF AGRICULTURAL BOTANY
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
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MAHARASHTRA, INDIA**

2015

CANDIDATE'S DECLARATION

*I hereby declare that this thesis or a part
thereof has not been submitted
by me or any other person
to any other University
or Institute for
a Degree or
Diploma.*

Place : MPKV, Rahuri

Dated : / /2015

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This is to certify that the thesis entitled, " **PHYSIO-BIOCHEMICAL ANALYSIS FOR GROWTH AND YIELD IN CHICKPEA UNDER MOISTURE STRESS**", submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (M.S.) for the award of the degree of **DOCTOR OF PHILOSOPHY** (AGRICULTURE) in **AGRICULTURAL BOTANY (PLANT PHYSIOLOGY)**, embodies the results of a *bonafide* research carried out by **Mr. VILAS RAMRAO AWARI**, under my guidance and supervision and that no part of the thesis has been submitted for any other Degree or Diploma.

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

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LIST OF ABBREVIATIONS

%	:	Per cent
°C	:	Degree centigrade (Celsius)
ϵ	:	Molar extinction coefficient
APX	:	Ascorbate peroxidase
CAT	:	Catalase
C.D.	:	Critical difference
DAS	:	Days after sowing
EDS	:	Early Drought Stress
<i>et al.</i>	:	et alli (and others)
etc.	:	Etcetera
Fig.	:	Figure
FW	:	Fresh weight
g	:	Gram (s)
GB	:	Glycine betaine
GPX	:	Guaiacol peroxidase
h	:	Hour
ha	:	Hectare
kg	:	Kilogram
L	:	Litre
LDS	:	Late Drought Stress
M	:	Molar
μg	:	Microgram
μl	:	Microlitre
min	:	Minute
ml	:	Millilitre
mM	:	Millimolar
μmol	:	Micromole
MPa	:	Mega Pascal
nm	:	Nanometer
nmol	:	Nanomole
O.D.	:	Optical density
POX/POD	:	Peroxidase
ROS	:	Reactive oxygen species
S.E.	:	Standard error
Sec.	:	Second (s)
SCM	:	Soil Moisture Content
SDF	:	Stress Duration Frequency
SOD	:	Superoxide dismutase
SPAD	:	Soil and plant analyzer development

ABSTRACT

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VILAS RAMRAO AWARI

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MAHATMA PHULE KRISHI VIDYAPEETH

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Research Guide	:	Dr. S.N. Mate
Department	:	Agricultural Botany
Major Field	:	Plant Physiology

The present investigation entitled “Physio-biochemical analysis for growth and yield in chickpea under moisture stress” was undertaken at Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, during the *rabi* 2012 and 2013. Thirty genotypes were screened for osmotic stress induced by different osmotic potential level (0, -0.2, -0.4, -0.6 and -0.8 MPa) of PEG 6000. On the basis of germination, root length, shoot length, vigour index and proline content five susceptible and five tolerant genotypes were selected for pot and field evaluation.

The investigation was undertaken to evaluate morpho-physiological parameters, levels of osmolytes and activities of antioxidative enzymes, under PEG 6000 induced moderate and severe osmotic stress in pot and withholding irrigation at pre- and post-flowering growth stages in field.

Abstract contd.....**V. R. Awari**

The studies revealed that the water stress had a strong influence on the growth, yield and physiological parameters of almost all the chickpea genotypes. A significant decrease amongst all the genotypes for physiological parameters *viz.*, photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll index and RLWC under moderate and severe stress induced by PEG 6000 over unstressed control was observed. A similar trend of reduction was also observed under moisture stress at pre- and post-anthesis stages under field condition.

The tolerant genotypes recorded higher photosynthetic activity with lower rate of transpiration under stress condition as compared to susceptible genotypes. The tolerance of ICC 4958, Vijay and Phule G 0752 seems to be the result of equilibrium between many physiological parameters *viz.*, stomatal conductance, photosynthetic and transpiration rate and RLWC ensuring plant growth under stress.

The high proline accumulating genotypes, ICC 4958, Vijay and Digvijay also exhibited the higher accumulation of glycine betaine under PEG 6000 induced osmotic stress. A similar trend of osmotic accumulation observed even at pre- and post-flowering crop growth stages under field condition. The lowest decline in relative leaf water content was observed in tolerant types than susceptible types under stress condition.

The activities of antioxidative enzymes such as superoxide dismutase, catalase and ascorbate peroxidase were significantly higher in the leaves of tolerant than susceptible genotypes. The genotypes ICC 4958, Vijay, Digvijay and Phule G 0752 recorded higher antioxidative enzyme activities as compared to other genotypes under

study upon PEG –induced osmotic stress in pot and imposed water stress by withholding irrigation under field condition.

Among the tested genotypes, Vijay, ICC 4958, Digvijay and Phule G 0752 maintained higher growth, yield and yield traits showing their tolerance to water stress, while Phule G-0305-3, ICCV 11117, Phule G-405-44-2 were adversely affected both for growth traits and yield as well.

The moisture stress at pre-flowering is very critical to chickpea plants. Early drought stress (Pre-anthesis drought) was found to be more damaging than the late drought stress (post anthesis drought). The genotype Vijay and ICC 4958 were most tolerant to drought at pre flowering stage of crop growth.

In conclusion, the drought tolerant chickpea genotypes exhibited higher accumulation of osmolytes and higher levels of antioxidative enzymes under imposed osmotic stress and water stress created by withholding irrigation and could be used as biochemical markers for screening chickpea genotypes for drought tolerance. The genotypes, ICC 4958 and Vijay with prolific and deep root system and biochemical indices appeared to be the most promising ones for inducing a drought tolerant character in the existing high yielding chickpea genotypes through introgression.

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.), one of the important legume crops found in the semi-arid areas. Chickpea is the third most important grain legume crop in the world and first in the Mediterranean basin and South Asia that frequently experiences water stress during pod set and seed filling stage (terminal drought) in India and the Mediterranean basin, leading to substantial yield loss.

Asia contributes about 90 % of the global area and production. Globally chickpea is cultivated on about 13.20 million ha and produces 11.62 million tonnes with an average productivity of 880 Kg ha⁻¹. India stands first in terms of area (68 %) and production (70%). However, its productivity is slightly less than productivity of countries like Turkey (1046 Kg ha⁻¹), Mexico (1536 Kg ha⁻¹) and Canada (1340 Kg ha⁻¹). Besides India is also largest consumer as well as importer of chickpea in the world. In India, chickpea is grown on about 9.88 million ha producing 8.88 million tons of grain with productivity of 995 Kg ha⁻¹. Chickpea contributes 36 and 48% to the national pulse acreage and production, respectively. Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, Karnataka and Gujarat are the major chickpea producing states sharing over 80% of the area. Among chickpea growing states, Andhra Pradesh registered highest productivity (1448 Kg ha⁻¹) followed by Bihar (984 Kg ha⁻¹), Gujarat (977 Kg ha⁻¹), Madhya Pradesh (850 Kg ha⁻¹), Maharashtra (891 Kg ha⁻¹), Uttar Pradesh (745 Kg ha⁻¹), Karnataka (607 Kg ha⁻¹) and Rajasthan (466 kg ha⁻¹). The current growth rate (6.32 %) in production and productivity of Chickpea is increasing in recent years

but it is still low as compared to population growth due to various agro-ecological, biological, institutional and socio-economic constraints (Singh and Shiv Sewak, 2013).

Chickpea contains high protein (18-24 %) with balance amino acid profile. In developing countries like India where majority of population is vegetarian, it assumes special significance due to its varied uses. In addition to being an important in-gradient of human food, chickpea also plays an important role in sustaining soil fertility by fixing the atmospheric nitrogen. With the development of short duration varieties, chickpea has shown competitive advantage over other *rabi* crops and contributing to crop diversification through remunerative and intercrops.

Chickpea is an evolutionary crop evolved with some of the important morphological and physiological traits which enable it to adapt to diverse environmental conditions. These adaptive mechanisms include intermediate habit, high response to photo-thermo periods, deep root system, biological nitrogen fixing ability, phenotypic plasticity, osmotic adjustment and high degree of remobilization of efficiency of stored carbon and nitrogen from subtending leaves and stems towards growing sink. Photosynthetically, chickpea possesses C₃ mode of carbon fixation. Harvest index (HI) is still reasonably low mainly due to partitioning of quite appreciable amount of nitrogen to growing sink which is considered to be more energy requiring physiological processes than carbon fixation and its partitioning.

Chickpea is grown mainly on stored moisture which is progressively depleted with crop growth. The crop experiences drought stress from late vegetative stage until maturity. The intensity

of drought stress varies from year to year, depending on the amount and distribution of rainfall and on spring and early summer temperatures. Irrigation has been shown to significantly increase chickpea seed yield. Chickpea yield is reputed to be most responsive when irrigated at flowering and pod filling.

Drought lessens the yield and has the potential for leading into a total crop failure. However, chickpea is known for its better drought tolerance when compared to most of the other cool season legumes. Furthermore, drought stress is one of the fundamental reasons for reducing the amount of growth and yield of chickpea (Jaleel *et al.*, 2009). Plants show a lot of morpho-physiological and metabolic changes in response to drought stress. Consequently, these changes lead them to adapting to drought stress conditions. As Sanchez-Mora *et al.* (2008) mentioned response mechanisms to drought stress are very complicated because in addition to morphological and physiological and metabolic changes, interactions of these factors are also important in resistance to drought stress. Therefore, to investigate the mechanisms of resistance to drought stress and to identify drought tolerant genotypes, reviewing many physiological and biochemical studies and ultimately molecular research can be helpful (Chopra and Selote, 2007). Drought stress during seed filling has been reported to be highly detrimental to yield in chickpea (Patel and Hemantaranjan, 2012).

Water stress invariably decreases several vital processes of the plant and at the same time, modifies a number of morphological and physiological characters in a manner so that a plant can thrive well under drought. The effect of drought on grain yield depends on the aggregate response of morphological and physiological processes

such as photosynthesis, leaf area expansion, leaf senescence, partitioning of assimilates, and biomass production. It also depends on timing of the stress within the growth period and on climatic and soil condition. Reduction of photosynthesis is considered to be primary yield limiting factor under water stress. Damage in leaves as a consequence of drought stress causes leakage of electrolytes from cell membrane. Water stress causes loss within the plant and therefore a reduction in its relative water content. The metabolic activity in leaf tissues can be evaluated by measuring RWC that can be considered an integrated measure of plant water status, while loss of turgidity estimates the rate of leaf water loss during the stress. Leaf RWC and loss of turgidity are associated with water stress tolerance in chickpea cultivars in before flowering and pod formation periods. (Kayan and Tuthan, 2012). High relative water content 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 stress in comparison to other water potential parameters under drought stress. Drought tolerant cultivars have higher RWC, chlorophyll and carotenoid in comparison to drought-susceptible cultivars (Talebi *et al.*, 2013).

Drought affecting the uppermost soil layer is responsible for poor seedling establishment and affecting the uniformity of plant density with a decrease in plant growth and consequently in crop yield. Drought can be alleviated by using drought tolerant genotypes. Thus, identifying drought tolerant mechanism is essential for measuring stress resistance in large breeding population. For drought stress, polyethylene glycol (PEG) compound has been used to stimulate osmotic stress effect for plants maintain uniform water

potential throughout the experimental period. Using this methodology, selection from a large number of breeding lines can be shortly and economically. Water stress (0,-0.2,-0.4,-0.6 and -0.8 MPa) induced by PEG 6000 affected the germination and seedling development parameters in chickpea genotypes. Seedling growth decreased with decreasing the osmotic potential (Yucel *et al* , 2010).

Osmotic solution such as PEG has been used to impose water stress by exposing the root system of plants can resolve the problem. Addition of PEG to nutrient solution produces osmotic stress over a period of 3-4 weeks. PEG is used successfully to decrease the water potential of plants as it doesn't enter into the root and has been suggested as being superior to other solutes to induce water stress because of its inertness, non-toxic nature, not being readily absorbed by intact plants and impermeability to cell wall. This neutral polymer is being widely used to impose water stress in plant (Kumar, *et al.*, 2011b).

The accumulation of osmolytes may ensure the maintenance of the structural integrity of membranes. There are some evidences that plants are more tolerant to water deficit when water is withheld under condition that favour osmotic adjustment. Osmotic adjustment is part of drought avoidance mechanism. Proline is one of the osmolytes, which increases faster than other amino acids in plants under water deficit stress and help the plant to maintain cell turgour. The increase in proline is usually considered as a plant response to drought stress. Higher proline content in tolerant genotypes as compared to susceptible genotypes helps them to improve their cellular osmotic adjustment and also the stabilization of enzymes proteins under drought stress (Kumar *et al.*, 2006). Proline

imparts partial heat tolerance to chickpea's growth by reducing the cellular injury and protection of some vital enzymes related to carbon and oxidative metabolism and exogenous application of proline appears to have a countering effect against elevated high temperature on chickpea. (Kaushal *et al.*, 2011). Accumulation of proline under water deficit condition is considered to be an important character responsible for drought tolerance (Blum and Ebercon, 1976). It involves two major key regulatory enzymes such as pyrroline-5-carboxylate synthetase (P₅CS) and proline oxidase. The former is involved in proline biosynthesis, and the latter in its degradation. The proline accumulation may be due to increased synthesis from glutamate. The glutamate may be produced by the degradation of proteins or by the increased conversion of α -keto glutarate into glutamate. An increase in proline synthesis can be assessed by monitoring the activities of P₅CS and pyrroline-5-carboxylate reductase (P₅CR) during stress.

The glycine betaine is synthesized from phosphatidylcholine which accumulates in plants in a large quantity. Genetic transformations have allowed the introduction of new pathways for biosynthesis of various compatible solutes in plants, resulting in the production of transgenic plants with improved tolerance to stress. Transgenic plants accumulate GB at various levels and exhibit enhanced tolerance to several stresses (Sakamoto and Murata, 2002). The concentration of GB in such transgenic plants is generally low compared to the levels observed in stressed plants of species that normally accumulate glycine betaine under stress. Two major factors have been identified that the limit accumulation of glycine betaine in transgenic plants: the availability

of endogenous choline *itself* (McNeil *et al.*, 1999), and the transport of choline across the chloroplast envelope.

Shora and Ali, (2013) reported that the GB showed the highest absolute accumulation in chickpea seedlings in response to salinity. Wu (2014) observed that the glycine betaine in young soybean seedlings was increased by 59.4 and 62.9 per cent under 300 mM NaCl treatment compared to control, respectively.

Drought is the most severe abiotic stress factor limiting plant growth and crop production. When plants are subjected to various abiotic stresses, some reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$) and singlet oxygen ($^1O_2$) are produced.

However, under various abiotic stresses the extent of ROS production exceeds the antioxidant defense capability of the cell, resulting in cellular damages (Almeselmani *et al.*, 2006). One of the main reasons that why environmental stress inhibits growth and photosynthetic abilities of plants is the breakdown of the balance between the production of reactive oxygen species (ROS) and the antioxidant defense. This activated oxygen's injure the cellular components of proteins, membrane lipids and nucleic acids. To mitigate and repair damage initiated by ROS, plants have developed a complex antioxidant system. Water deficit is also known to alter a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation (Hu and Schmidhalter, 1998).

SOD is a major scavenger of O_2^- and its enzyme action results in the formation of H_2O_2 and O_2 . Catalase is localized in the mitochondria, peroxisomes and cytoplasm of higher plants (Bray *et*

al., 2000). It is instrumental in the decomposition of H_2O_2 , which is produced outside the chloroplasts by the H_2O_2 generating oxidases present in the peroxisomes. Catalase is a tetrameric heme protein, occurring in almost all aerobic organisms, and one of the few enzymes showing dual activity: it has hyperoxidase activity (catalytic activity) when it catalyzes the breakdown of hydrogen peroxide into water and oxygen. It also shows peroxidase activity (Luhova *et al.*, 2003). POX is a major enzyme scavenging H_2O_2 in chloroplasts produced through dismutation of O_2^- catalyzed by superoxide dismutase. Several physiological processes are dramatically affected by peroxidase over-production, and severe wilting was found in transgenic plants (Arora *et al.* 2002). Rasool *et al.* (2013) and Kaur *et al.*, (2013) reported that SOD activity showed significant variation in all the chickpea genotypes at all stress levels.

An alternative defensive system called antioxidative is also activated to protect cells against oxidative stress and support plants against oxidative hurt. The endogenous supportive mechanisms consist of some enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) as well as, peroxidase (POX) that can effectively scavenge the toxic oxygen species. If the plants are confronted to environmental stress situations, such as drought conditions, the capacity of leaves for active oxygen generation will increase. This situation mostly results in oxidative damages (Chopra and Selote, 2007).

Furthermore, Bian and Jiang (2008) reported that there is a close relationship between increased antioxidant enzyme activity and enhanced resistance to environmental stresses. Simova *et al.* (2008) reported that the effects of drought stress on wheat species

depends on variety, intensity and duration of the stress as well as, on the developmental stages. Masoumi *et al.* (2010) reported that drought stress caused a significant increase in antioxidant enzyme activities such as CAT, APX and glutathione reductase, but it had no significant effects on SOD and POX activity. Keles and Unyayar (2004) reported that drought stress had different effects on antioxidant enzymes. They further believed that the relative tolerance of a genotype to water stress as reflected by its comparatively lower lipid peroxidation, higher proline and chlorophyll contents was closely associated with its antioxidant enzyme system. Cevik *et al.* (2014) revealed that the SOD and APX activity significantly increased in *C. arietinum* under water deficit, compared with the control.

Nevertheless, according to Muhammad Zia-Ul-Haq (2008) antioxidant enzymes activity and production of active oxygen species under drought stress and their relationship with photosynthetic rate and cell membrane destruction is not yet clear, and therefore, it has been the subject of intensive study for a long time. The responses to drought stress depend on the species and genotype, the length and severity of water deficit and the age and stage of development. This study is designed to investigate the effects of drought stress on the production of glycine betaine and proline. In addition, the study aims at determining the role of enzymes involved in antioxidative defense mechanism, including CAT, APX and SOD in drought stressed chickpea plants. Plant responses to drought stress can be different in each growth stage as compared to other stage. So, in this study we tried to investigate differences of chickpea genotypes in responses to drought stress at pre and post anthesis stage.

This research explored further the possibility of evaluating different physiological and biochemical traits at different growth stages as an aid in screening drought tolerance of chickpea genotypes.

Considering all these aspects, the present study on **“Physio-biochemical analysis for growth and yield in chickpea under moisture stress”** was undertaken with the following objectives:

1. To screen the chickpea genotypes for water stress tolerance under PEG 6000 induced osmotic stress,
2. To study the variation in morpho-physiological, biochemical parameter among various chickpea genotypes and their relationship at moderate and severe stress, and
3. To find out the correlation between physio-biochemical parameters and yield and yield contributing character in chickpea under moisture stress at pre and post anthesis stages.

2. RIVIEW OF LITERATURE

Chickpea (*Cicer arietinum* L.) is the fourth largest grain legume crop in the world. About 90% of the chickpea in the world is grown under rainfed conditions where drought is one the major constraints, limiting its production. Drought is a world-wide problem seriously influencing global crop production and it will become progressively important due to the global climate change. Drought tolerance is a typical quantitative trait and breeding programmes to increase yield under reduced water supply conditions are rather challenging task. A physiological approach can complement empirical breeding to enhance the rate of yield improvement. Now-a-days, effort is focused on improving crop genotypes for drought-prone areas. Understanding of the mechanisms behind drought tolerance, which can lead to the restitution of physiological function and to hardening of plants under drought stress, is essential for the achievement of such goal. Drought stress cause to many changes on plants in terms of biologically, physiologically and biochemically.

Breeding for resistance or tolerance to drought and high temperature stress in chickpea is limited by the lack of adequate selection criteria for stress tolerance. Most breeding programmes are based on visual scoring in the controlled or field conditions. Understanding the drought tolerance process in plants and considering biochemical and physiological traits in breeding programmes would probably provide high returns.

Review of literature provides a general orientation about the topics of investigation by creating an insight and sense of integration about the subject as a whole and attempt was made to

review the literature on the problem in respect **“Physio-biochemical analysis for growth and yield in chickpea under moisture stress”**

Limited research has been reported on chickpea particularly with reference to physiological and biochemical aspects. The present review is an attempt to collect the available literature, particularly with reference to physiological and biochemical aspect in relation to moisture stress i.e. drought tolerance in chickpea. The research work carried out in the past on the aspects related to the present investigation is summarized in this chapter under appropriate headings.

2.1 Screening of chickpea genotypes under PEG 6000 induced osmotic stress

Kaur *et al.* (1998) reported that, on increasing the concentration of PEG in the MS medium from 5 to 15 per cent, the percent germination, lengths and weights of roots and shoots decreased, in comparison to control. With 15 per cent PEG in the medium, only 33 per cent germination of chickpea seeds was observed. A decrease of about 40% in germination and 60-70 per cent in lengths and weights of roots and shoots as compared to control was observed with 10 per cent PEG.

Okcu *et al.* (2005) observed that the water stress depressed the shoot growths of the cultivar rather than their root growths in pea.

Sanchez-Mora *et al.* (2008) observed a significant correlation between growth and osmotic adjustment and turgor maintenance, but only at the highest concentrations of PEG assayed. The coefficient of correlation was at 30 mM PEG, $r = 0.70$ ($P < 0.01$)

and $r = 0.79$ ($P < 0.01$), and at 46 mM PEG, $r = 0.64$ ($P < 0.05$) and $r = 0.89$ ($P < 0.01$) for osmotic adjustment and turgor maintenance, respectively. Free proline in the epicotyls increased between 5- and 50-fold. A significant correlation was found for turgor maintenance ($r = 0.78$; $P < 0.01$). The results obtained indicate that measurements made at early stages of development could be used to identify drought-tolerant genotypes. Gurbuz *et al.* (2009) also observed that PEG adversely affects the germination percentage.

Macar *et al.* (2009) investigated the effects of water deficit induced by different osmotic potential levels (0, -0.4, -0.6 and -0.8 MPa) of PEG 6000 and NaCl treatment on chickpea cultivars and lines at germination and early growth stages. All these treatments affected germination percentage of all the genotypes but PEG was more effective in inhibition than NaCl at the MPa levels tested. Increasing PEG potentials to -0.8 MPa caused approx. 40 per cent reduction in germination percentage in FLIP 87-59C. The length of epicotyls of all cultivars decreased significantly with increasing PEG potentials. There was no considerable epicotyls elongation in all genotypes at -0.8 MPa. Results showed that drought stress brought by PEG 6000 inhibited epicotyls elongation more than the root growth and decreased the shoot/root ratio.

Farsiani and Ghobadi (2009) reported that the decrease of osmotic potential induced a decreasing of root growth. As a result of decreasing of root lengths, root fresh and dry weight decreased in drought stress.

Xu and Huang (2010) concluded that, among 56 stress-responsive protein spots, 46 were identified using mass spectrometry. Some proteins involved in primary nitrogen and carbon metabolism

were down-regulated by PEG-induced water stress in two cultivars. The abundance of antioxidant enzyme proteins (ascorbate peroxidase, catalase and glutathione-S-transferase) increased under water stress, particularly ascorbate peroxidase in 'Penn-A4'. The results suggest that proteins involved in membrane synthesis, cell wall loosening, cell turgor maintenance, and antioxidant defense may play roles in perennial grass adaptation to PEG-induced water stress.

Yucel *et al.* (2010) found that, the germination and seedling growth in all chickpea genotypes stopped completely in -0.8 MPa. Shoot length decreased drastically with decreasing osmotic potential and more adversely affected than the root length.

Homayoun *et al.* (2011) evaluated five wheat genotypes in five levels of drought treatment (distilled water, -2, -4 and -8 bars). Results indicated that, significant decrease was observed in percentage of germination, germination rate, length of radical and plumule and radical and plumule dry matter. Also reported that the genotypes didn't show any germinated seed in -8 bars.

Kumar *et al.* (2011b) demonstrated that the sensitive cultivars showed no germination at -0.89 MPa osmotic potential, while germination was inhibited ~92% in moderately tolerant cultivars but tolerant cultivars showed about 20% germination. The germination was not seen beyond -0.89 MPa. The increase in drought stress reduced the germination rate, seedling fresh and dry biomass, seedling vigour index and germination index in all cultivars.

Sleimi *et al.* (2013) studied the influence of temperature and seven water stresses induced by different solution of PEG 4000 (0, 1, 2, 3, 4, 5, and 10 g/L) on germination of four varieties of chickpea.

At the high PEG concentration (10g/L), the inhibition of seed germination was highly significant for all the varieties studied.

2.2 Phenological and Morphological traits

Mishra (1972) observed that the plant height exhibited highly significant negative correlation with grain yield probably because the plants with greater height could not produce more number of branches, leaves, flowers and pods and could not accumulate more photosynthates for grain development resulting in poor yield.

Birari, (1976) reported that the height of chickpea plant increases progressively with the advancing age of the crop up to 75 DAS. Saxena (1987) reported the technique for analyzing and selecting genotypes for drought resistance. In a group of chickpea genotypes with a wide range of days to flowering (30-77days), a significant correlation between days to flowering and stress was observed at Patancheru.

Wanjari (1988) observed in black gram that flowering had negative effect and also negative association with yield hence early flowering and maturity are desirable traits to get higher yield.

Singh and Singh (1991) studied the influence of water deficit on phenology in chickpea cv. JG-74. The crop was subjected to increasing intensities of water deficits during vegetative and reproductive phase by applying gradient irrigations. Duration of emergence to flowering, flowering to beginning of pod filling and beginning of pod filling to physiological maturity were inversely correlated with normalized evapotranspiration deficit experienced by crop during a growth period.

Under severe drought condition Silim and Saxena (1993) noticed that, early flowering was associated with high harvest index, large number of pods and high seed mass. Johansen *et al.* (1994) observed that, increasing drought stress accelerates the flowering process and maturity in chickpea.

Gupta *et al.* (1993) studied the response of chickpea to water deficits and drought stress and reported that, under non-irrigated condition there was an increase in numbers of leaflets per leaf and decreased leaf area compared to irrigated conditions. There were correlation between prolonged water stress and decreased height, leaf dry weight and increased number of leaflets and branches per plant.

Schmidt *et al.* (1996) in the field experiment conducted at Mosonmagyaravar examined the changes in the plant height of 5 chickpea genotypes due to water stress conditions as compared to irrigated conditions.

Pandey *et al.* (2000) reported in maize that increasing moisture stress resulted in progressively less leaf area, crop growth rate, plant height, shoot dry matter and harvest index.

Malhotra and Saxena (2002), early flowering remains the main component of the chickpea water stress avoidance. This mechanism was largely used, especially through the selection of genotypes for an early flowering.

Kumar *et al.* (2004) reported that under moisture stress conditions seed yield in chickpea was governed by

agronomic traits *viz* number of branches, pods per plant, biomass and harvest index.

Lakshmamma *et al.* (2004) reported that the drought tolerant line maintained growth in terms of plant height, branches production and stem girth, less than 30% yield reduction and low drought susceptibility index as that of control plants even under stress.

Chauhan *et al.* (2007) observed that plant height, primary branches, secondary branches per plant, 1000-seed weight and seed yield were reduced under rain-fed conditions.

Fatokun *et al.* (2009) evaluated cowpea germplasm for drought tolerance and observed that drought reduced number of days to flower by 12 days on an average and the mean grain yield per plant was also reduced by 67.28 percent. Hundred seed weight and number of seeds per pod were not significantly affected by drought. A few of the cowpea lines stayed green for up to six weeks after irrigation was stopped but some of them did not produce pods.

Lakshmamma and Laxmi (2010) observed plant height, leaf number and tertiary branch production was reduced significantly with stress.

Yaqoob *et al.* (2012) observed that the days to flower initiation were highly significantly affected due to moisture levels and the plant under moisture stress had produced early flowering whereas well-watering treatment had delayed flowering. As a matter

of fact, moisture stress always shortens the life cycle of crop plant which results in early flowering as well as early maturity.

Hinnkossa *et al.* (2013) reported that the effect watering regime was significant for the number of pods per plant, seeds per pod, seed yield and harvest index. In both crosses, drought stress imposed at flowering stage resulted in to considerable reduction in all the yield related traits. On over all basis, drought stress reduced the number of pods per plant, seed per plant, seed yield and harvest index by 18, 8, 28 and 8 per cent, respectively relative to non-stress treatments. He further concluded that drought stress imposed at flowering stage reduced all growth physiological and yield related traits of the basic generations of both crosses in common bean.

Randhawa *et al.* (2014) reported that the significant reduction occurred in plant height and number of branches under varies stress treatment. Under water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from xylem to the surrounding elongating cells leading to reduced number of branches.

2.3 Physiological parameters in relation to drought

2.3.1 Photosynthetic rate

Chaves (1991) described that the stomatal closure is among the first responses to water stress and is assumed to be the main cause of impaired photosynthesis induced by drought, as stomatal closure limits CO₂ availability to the mesophyll. Soussi *et al.* (1998) emphasized that the photosynthetic carbon assimilation process in chickpea cultivar ILC1919 was found to be greatly depressed by high levels of NaCl .

Leport *et al.* (1999) revealed that the mean photosynthetic rate of the irrigated plants, except at 151 and 167 DAS, ranged from 21 to 27 mmol m⁻²s⁻¹ min all chickpea genotypes. The decrease of the rate of net photosynthesis in irrigated plants at 151 and 167 DAS. In the rainfed plants, the rate of net photosynthesis decreased rapidly to values of 2 to 2.5 mmol m⁻²s⁻¹ after 124 DAS.

Ohashi *et al.* (2000) postulated that the low water potential due to reduced water availability negatively affects plant growth and photosynthesis.

Kawamitsu *et al.*, 2000) found that drought stress decreases the rate of photosynthesis. Ashraf and Bashir, (2003) determined that the per cent reduction in net photosynthesis (*P_n*) under saline conditions was more drastic in *P. vulgaris* than in *S. aculeate*.

Cornic, (2000) and Flexas *et al.* (2004) quoted that, under mild or moderate drought stress stomatal closure (causing reduced leaf internal CO₂ concentration) is the major reason for reduced rates of leaf photosynthetic.

Decreased CO₂ diffusion from the atmosphere to the site of carboxylation is generally considered the main cause of decreased photosynthesis under wild to moderate water limitations (Chaves *et al.* 2003, 2009 and Grassi and Magnani, 2005)

Zanella *et al.* (2004) reported that there was a direct relationship between reduced intercellular CO₂ concentrations due to stomatal closure and decreases in CO₂ assimilation. Although drought impaired photosynthesis, it did not damage the photosynthetic apparatus. The reduction in photosynthesis in

Myracrodruon urundeuva under drought conditions occurred primarily because of stomatal closure, rather than damage to PSII.

Basu *et al.* (2007) reported that the water stress resulted a significant decline in the light-saturated rates of photosynthesis in Tyson and M 129. Decreased CO₂ diffusion from the atmosphere to the site of carboxylation is generally consider the main cause of decreased photosynthesis under mild to moderate water limitation

Konwar *et al.* (2007) reported that the moisture stress significantly influence the various physiological processes. They observed a significant reduction in rate of photosynthesis, respiration, intercellular CO₂ and stomatal conductance. These attributes reflected adversely the yield components viz., *number* grains per panicle, thousand grain weight and sink capacity.

Chaves *et al.* (2009) observed the decrease in photosynthetic rate under stressful condition (salinity, drought and temperature) was normally attributed to a suppression in the mesophyll conductance and the stomata closure at moderate and severe stress.

Channappagoudar *et al.* (2010) observed that the drought resistant promising entries exhibited higher values of grain yield, panicle dry weight, 1000 grain weight, partitioning efficiency, photosynthetic rate at flowering, total dry matter, lower transpiration rate higher and values of RWC. Khan *et al.* (2010) observed that the linseed plants exposed to NaCl stress exhibited reduction in net photosynthesis (P_N).

Mafakheri *et al.* (2011) concluded that photosynthesis decreased as a result of the drought stress in all three varieties of chickpea.

Kumar *et al.* (2010) described that the photosynthetic rates were significantly reduced by drought in all genotypes of chickpea.

Krouma (2010) reported that all the cultivars under drought stress exhibited a significant decrease in photosynthetic activity, as compared to the controls. All chickpea genotypes cultivated under drought stress exhibited a significant decrease in photosynthetic activity, as compared to the controls. Water deficit significantly decreased net photosynthesis, stomatal conductance, and transpiration. Amdoun genotype of chickpea was the least affected by drought, followed by Kesseb and Chetoui. Net photosynthesis decreased by 33, 48 and 51 per cent, respectively, in Amdoun, Kesseb, and Chetoui .

Under severe drought stress, dehydration of mesophyll cells take place causing a marked inhibition of basic metabolism process of photosynthesis as well as reduction of plant WUE (Damayanti *et al.* 2010 and Anjum *et al.* 2011)

As salinity stress, drought stress causes not only a substantial damage to photosynthetic pigments, but it also leads to deterioration of thalokoid membrane (Anjum *et al.* 2011 and Kannan and Kulandaivelu, 2011)

Kanwal *et al.* (2011) reported that photosynthetic rate is adversely affected by salinity stress with maximum reduction in net carbon dioxide assimilation at higher level of salinity.

Kataria and Singh, (2013) found that under water stress, photosynthetic rate significantly decreased in chickpea genotypes. Potassium application significantly increased the rate of photosynthesis under stressed conditions.

Iqbal *et al.* (2014) observed that net photosynthetic rate (PN) decreased substantially in mustard cultivars at 100 mM NaCl stress. Maximum decrease was noted in RH30 whereas Pusa Jai Kisan maximally protected its net photosynthetic rate. Net photosynthesis decreased minimally by 13.3% in Pusa Jai Kisan, whereas, maximum decrease was observed in RH30 by 47% under salt stress in comparison to control.

2.3.2 Transpiration

Yao *et al.* (2001) reported that the transpiration rate was significantly decreased by drought, Crop plant with limited water availability seems to use water more economically. Water stress conditions leads to change in leaf hydraulics, which leads to closure of stomata due to fall of water potentials. Stomatal closer inhibits transpiration process.

Ashraf and Bashir, (2003) showed that in chickpea, some genotypes have limited transpiration rate under vapor pressure deficit greater than 2 kPa. Percent reduction in transpiration rate (*E*) was significantly higher in *P. vulgaris* than in *S. aculeate*.

Sinclair *et al.* (2005) reported that the limitation on maximum transpiration rate may be caused by restricted hydraulic conductance in the plant or by limited supply of water to the plant from uptake by the roots.

Jyothi *et al.* (2010) observed that during and after stress period the castor entries recorded high transpiration rate, leaf water status, low SLA, dry matter accumulation, capsules per plant and test weight.

Krouma (2010) reported that the transpiration rate of Amdoun genotype of chickpea was the least affected by drought,

followed by Kesseb and Chetoui. The transpiration decreased by 27, 54 and 61%, respectively, in Amdoun, Kesseb, and Chetoui. He also reported that water deficit significantly decreased transpiration.

Sikuku *et al.* (2010) emphasized that the stomata closure is known to have more inhibitory effect on transpiration of water than that CO₂ diffusion into the leaf tissue

Sinclair *et al.* (2005) concluded that a limited transpiration rate has been shown in simulation studies to be a desirable trait for sorghum and soybean.

2.3.3 Stomatal conductance

Amede and Schubert, (2003) studied that under water stress conditions, the decrease in transpiration rate was much more substantial in common bean than in chickpea. This may be attributed to the decreased stomatal conductance in haricot bean, which sharply dropped within the first three days of stress. Recovery in transpiration rate of chickpea plants was presumably due to a recovery in stomatal conductance. This was not the case in common bean plants. Stomatal conductance of well-watered plants at later growing stages increased more in beans than in chickpea plants. Ashraf and Bashir (2003) observed that the percent reduction in stomatal conductance (*gs*) was significantly higher in *P. vulgaris* than in *S. aculeate*.

The regulation of leaf stomatal conductance (*gs*) is a key phenomenon in plants as it is vital for both a prevention of desiccation and CO₂ acquisition (Dodd, 2003 and Medici *et al.*, 2007)

Khetarpal *et al.* (2009) found that stomatal conductance was decreased in the cultivars under elevated temperature significantly at all the growth stages.

Krouma (2010) revealed that the stomatal conductance of Amdoun genotype of chickpea was the least affected by drought, followed by Kesseb and Chetoui. The stomatal conductance decreased by 28%, 64%, and 70%, respectively, in Amdoun, Kesseb, and Chetoui.

Khan *et al.* (2010) observed that the linseed plants exposed to NaCl stress exhibited reduction in stomatal conductance (g_s). Galle *et al.* (2009) reported that the stomatal closure has been shown to act as the initial and most prominent limitation to CO₂ assimilation, as diffusion of CO₂ from the atmosphere to the sites of carboxylation in the chloroplast is impaired.

Hirich *et al.* (2014) revealed that the chickpea genotypes exposed to increasing irrigation water salinity, exhibited a significant decrease in stomatal conductance (g_s) both during growth period and during the day. The salinity stressed treatment T4 (10 dS m⁻¹) showed the lowest stomatal conductance compared to the control treatment.

Iqbal *et al.* (2014) reported that the stomatal conductance (g_s) decreased substantially in mustard cultivars at 100 mM NaCl stress. Maximum decrease was noted in RH30 whereas; Pusa Jai Kisan maximally maintained its stomatal conductance. Stomatal conductance decreased minimally by 27.5% in Pusa Jai Kisan, whereas, maximum decrease was observed in RH30 by 55.7% under salt stress in comparison to control.

2.3.4 SPAD chlorophyll meter reading

Ommen *et al.* (1999) in their investigation on effect of drought stress on wheat verified that increasing stress led to significant increase in chlorophyll content.

Ahmadi and Baker (2000) indicated that the moderate water stress (15 per cent of FC) significantly reduced wheat leaf chlorophyll content. The ability to maintain high chlorophyll density under water deficit conditions has been suggested as a drought tolerance in barley (This *et al.* 2000).

Naderikharaji *et al.* (2008) reported that with increase in drought stress, SPAD meter readings (the relative chlorophyll concentration of leaves) were decreased. Similarly, the result showed that, drought stress had significant effect on net photosynthesis (A), Stomatal conductance (gs), intra cellular CO₂ concentration (Ci) and leaf area (LA) of rapeseed at both vegetative and flowering stages. In general, Hayola 401, had highest seed yield in both control and drought treatment followed by Hayola 308, whereas RGS had lowest seed yield among the cultivars. Hayola 401 and Hayola 308 had the highest gs in control and lowest gs in drought.

Songsri *et al.* (2009) reported that the drought reduced WUE, root dry weight and HI across both seasons, Tifton-8 and ICGV 98300 had high WUE and also had large root systems under drought conditions. ICGV 98324 and Tifton-8 had low SLA and high SCMR under stressed and non stressed condition.

Lakshamma and Lakxmi(2010) observed that SPAD Chlorophyll meter reading (SCMR) was significantly more in stress at 55 DAS. Specific leaf area (SLA) did not differ significantly in control and stress. Strong negative correlation of SCMR with SLA was seen ($> - 0.90$.) both in control and stress treatments. Genotype RG297 showed high SCMR, low SLA, and high total dry matter (TDM) both in control and stress treatments. This genotype also

showed higher seed yield and harvest index (HI) in stress with less percent reduction in seed yield compared to control with low drought susceptibility index (DSI) values.

Rajababu *et al.* (2010) observed that the groundnut cultivars recorded significantly higher relative water content (RWC), pod yield, SPAD values, higher root-shoot ratio, lesser shoots and higher functional leaves under moisture stress conditions. These characters are contributed the drought tolerance mechanism in groundnut.

Shinde *et al.* (2010) reported concomitant reductions were observed in RWC, MSI, HI and DTI in groundnut varieties with increasing water stress levels. Among the four varieties TG-26 and WEST-20 were found to be more sensitive to drought stress, as compared to var.TAG-24 and var.WEST-44.

2.3.5 Relative leaf water content

Ritchi *et al.* (1990) observed that high RLWC under moisture stress denotes ability of plants to tolerate moisture stress. Under moisture stress conditions tolerant types shows less reduction in RLWC as compared with susceptible ones (Sairam, 1997).

Deshmukh *et al.* (2000) examined gradual decrease in RWC with increase in stress and greater reduction afterwards under severe stress has been reported in chickpea.

In several legume cultivars of *Phaseolus vulgaris* (Costa-Franca *et al.* 2000), *Vigna glabrescens* (Scotti *et al.* 1999), and *Lupinus albus* (Pinheiro *et al.* 2001) the close relationship between RWC and predawn water potential observed during progressive water deficit supports its utilization as an indicator of plant water status.

Gonzalez and Gonzalez (2001) reported that relative water content (RWC) represents a useful indicator of the state of water balance of a plant, essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation.

Lawlor and Cornic (2002) found that the drought decreased the relative water content, water potential, and osmotic adjustment. The utilization of leaf RWC as an indicator of plant water status is usual.

Sairam *et al.* (2002) observed that RWC decreased with increase in salinity levels. Under control and S1 (5.4 dS m⁻¹) KRL 19 maintained highest RWC at all stages while at S2 (10.6dS m⁻¹) wheat genotype Kharchia 65 showed highest RWC.

Moinuddin and Khanna-Chopra (2004) described that the relative water content (RWC), often considered as an excellent indicator of the water state of plant and directly related to the cells volume and can reflect the balance between leaves water provision and transpiration rate. Under drought conditions, RWC was strongly affected by the increase in the water deficit and the plant age.

Upreti *et al.* (2007) showed that the high temperature induced higher transpiration, situation similar to water stress is created and RWC becomes important under heat stress. Various studies have reported that maintenance of favorable water status is essential for plant tolerance to heat stress as it involves water deficit and cell turgor loss.

Turner *et al.* (2007) described that RWC has been considered as a better indicator of water status as it reflects the balance between water supplied to leaves and transpiration rate

through its relation to cell volume. Heat tolerance may be related to the maintenance of plant water relations by reducing water loss and or increasing water uptake capacity. Decrease in relative water content was a main factor resulting in reduced growth in response to osmotic stress in chickpea. Under drought stress, sensitive chickpea genotypes were more affected by the decline in RWC than tolerant ones.

According to result of Bayoumi *et al.* (2008) RWC may be attributed to differences in the ability of the variation to absorb more water from the soil and / or the ability to control water loss through stomata and RWC parameter can be used to select high yielding genotypes that maintain cell turgor under water stress environment to give relative high yield.

Gorai *et al.* (2010) reported that the RWC decreased significantly as water stress intensified. A similar behavior was observed in *Phaseolus vulgaris* and *Medicago truncatula* (Nunes *et al.* 2008). BGD 72 registered high RWC than ICCV2 under drought stress (Jain and Chattopadhyay, 2010).

Singh *et al.*, (2013) studied that the chickpea genotypes, BGD 72 and Pusa 1103 possessed significantly higher values of RWC, so these genotypes performed better under limited water environments. RWC was a measure of stress adaptation and accounts for osmotic adjustment, which is considered to be one of the important mechanism for adaptation to water limited environments in plants.

Ahmad (2010) observed that presence of NaCl in the growth medium induced significant decrease in RLWC, whereas maximum RLWC was recorded in unstressed linseed plants (Khan *et*

al. 2010). RLWC declined significantly in *B. juncea* plants with salinity stress in S1 and S2 as compared to control.

Sheokand *et al.* (2010) examined that a sodium chloride treatment adversely affected the relative water content (RWC). The RWC decreased with increasing duration of stress.

Kumar *et al.* (2011b) observed that a decrease of 25% RWC was noted in moderate stress condition as compare to control while 56% decrease in RWC was observed in severe stress in Pigeon pea. They also reported that PEG induced significant water stress in plant and not having toxic effects. A progressive moderate stress (-0.5 MPa) cause a significant decrease in RWC whereas decrease in RWC seen at progressive severe stress (-1.22 MPa).

Sen *et al.* (2012) observed that there was a significant reduction in relative leaf water content (RWC) and membrane stability index (MSI) of chickpea genotypes under moisture stress as compared to fully irrigated condition.

Patel and Hemantrajan (2012) observed that the chickpea genotypes JG 315 and DCP 92-3 showed lower RLWC compared with Tyson and ICC 4958 at pre anthesis drought which maintained higher RWC at both the stages. RWC was in the range of 71.6-74.4 (unstressed) and 67.9-71.6 (stressed).

Kataria and Singh (2013) emphasized that exposing the chickpea plants to water stress by withholding irrigations resulted in a significant decrease in RWC for the three growth stages.

Ulemale *et al.* (2013) reported that the relative water content (RLWC) was relatively low under moisture stress as compared to non-stress condition.

Khodadadi (2013) reported that the leaf relative content was decreased over the non-stress condition.

Meena *et al.* (2014) studied that the RWC in leaves of chickpea genotypes showed a significant decrease under high temperature stress as compared to control and showed a continuous decline with the duration of heat stress. In this study the RSG 888 maintained higher RWC, under both normal and high temperature at different stages of treatment followed by Pusa 256 and JG 11.

Rizvi *et al.* (2014) reported that the significant reduction was observed in RWC under moisture stress in all the cultivars. Under moisture stress condition the highest RWC at flowering stage was observed in Pusa-362 (86.33%) and at pod formation stage in Pusa-1103 (81.07%), while the lowest RWC was observed in SBD-377 (63.81%) and Flip 90-166 (52.64) at flowering and pod formation stages, respectively.

2.4 Biochemical parameters in relation to drought

2.4.1 Accumulation of osmolytes under drought

Osmoprotectants include proline, sucrose, polyols, trehalose and quaternary ammonium compounds such as glycine betaine, alanine betaine, proline betaine, choline O-sulphate, hydroxyproline betaine and pipercolate betaine (Rhodes and Hanson, 1993). The accumulation of compatible solutes such as betaine, proline and sugar alcohol is a widespread response that may protect plants against environmental stresses. It is not yet fully understood how these compounds are involved in the stress tolerance of whole plants. Some plants have been genetically engineered to express enzymes that catalyze the synthesis of various compatible solutes.

Some interventions have improved the tolerance of some crop plants to abiotic stresses (Chen and Murata, 2002).

2.4.1.1 Proline

Bates *et al.* (1973) revealed that in a number of crop plants, proline increases proportionately faster than any other amino acid in plants under water stress and is an evaluating parameter for selecting drought tolerance.

Savoure *et al.* (1995) reported that the accumulation of proline was proposed to play an important role in the osmo-adaptation of the plant cell.

Yadav and Khare (1995) observed that in chickpea, proline content in leaves and seeds were higher under rainfed than under irrigated condition. They also reported that high leaf proline content at the vegetative stage was associated with high yield under rainfed condition. The degree of moisture stress tolerance has been positively correlated with the level of proline.

In response to osmotic stress stimulated by drought, salt, abscisic acid (ABA) and cold, proline accumulates in many plant species. Proline performs an important function of facilitating a correction of altered redox potential by replenishing the NADP⁺ supply (Hare *et al.* 1999).

Kocheva and Georgiev (2003) observed proline accumulation under severe PEG stress (25 %) more than moderate stress (6.25 %) in barley cultivars. The maximal imposed stress of 25 % PEG showed 31-fold increase of proline in the leaves of Houstera cultivar, whereas 70 times increase in cultivar Odeskill in comparison to control.

Jafar *et al.* (2004) reported that the higher accumulation of proline has been reported in seedlings of forage sorghum at various levels of osmotic stress induced by polyethylene glycol (PEG).

Kavi Kishor *et al.* (2005) studied in higher plants there was a dramatic accumulation of proline due to increased synthesis and decreased degradation under a variety of stress conditions such as salt or drought .

Ahire *et al.* (2005) reported a much higher magnitude of proline accumulation in the leaves of stressed plants of drought tolerant chickpea cultivars.

Djibril *et al.* (2005) reported that the proline content was increased upon PEG-induced water stress to date palm seedlings. PEG was used for screening of drought tolerance. The proline content was increased progressively and significantly in drought tolerant cultivars when amount of PEG increased in the growth medium. Proline content was 279 to 651 η moles g^{-1} of fresh matter respectively at 6.9 to 27.5 g/L of PEG. In contrast, in non tolerant cultivar proline increased quickly and significantly 200 to 1000 η moles g^{-1} of fresh matter, respectively for control and stressed plants.

Kavi Kishor *et al.* (2005) studied decrease in the level of accumulated proline in rehydrated plants is due to both down regulation of proline biosynthetic pathway enzymes and up regulation of proline degrading enzymes. But the role of proline during plant development and the molecular basis of the effect of proline accumulation during stress and upon relief of stress are still largely obscure.

Zgalli *et al.* (2005) reported tenfold increase in proline accumulation under PEG induced water stress condition in tomato

plants that shows that a high proline accumulation observed under PEG stress may produce adaptive responses for pigeon pea under water stress.

Kumar *et al.* (2006) reported that the proline content in leaves was significantly higher under rainfed condition in all the chickpea genotypes as compared to irrigated control at both the stages of crop growth. The genotype ICC-4958 possessed highest proline content followed by BG-362 under irrigated condition. Whereas, the genotype K-850 possessed the highest proline content followed by ICC-4958 and C-214 under rainfed condition.

Dalvi *et al.* (2007) were evaluated drought tolerant and susceptible cultivars of both sorghum and chickpea for proline accumulation, P₅CS activity and soluble proteins in relation to imposed water stress. The proline content increased from 14.58 to 70.75 $\mu\text{mol g}^{-1}$ fresh weight in drought tolerant sorghum cultivar and from 11.74 to 131.30 $\mu\text{mol g}^{-1}$ fresh weight in chickpea with an increase in P₅CS activity and soluble proteins.

Bhatnagar-Mathur *et al.* (2009) reported that, the accumulation of proline in the selected events was more pronounced that increased significantly in the leaves when exposed to water stress. However, the over expression of P5CSF129A gene resulted only in a modest increase in TE, thereby indicating that the enhanced proline had little bearing on the components of yield architecture that are significant in overcoming the negative effects of drought stress in chickpea.

Garg and Noor (2009) reported that proline accumulation was higher in the salt tolerant genotypes of pigeonpea PUSA2002-2, HPM-1, RC01-69N, PUSA2001-7 and PUSA2002-1 than in the salt

sensitive RG01-73N, AK2000-3N3, AL1455 and PUSA2004-1 under salt stress conditions.

Najaphy *et al.* (2010) suggested that, by increasing irrigation intervals, leaf proline content increased. Water deficit stress increased concentration of soluble proteins in the leaves up to 43% in comparison with normal watering treatment .

Kumar *et al.* (2011b) reported that under progressive mild stress increased free proline content increased upto 12.17 $\mu\text{Mgm}^{-1}\text{FW}$ and 54.47 $\mu\text{Mgm}^{-1}\text{FW}$ accumulation of proline was observed in old leaves under severe stress condition. Severe progressive stress in pigeon pea leads to about 25 fold more accumulation of proline as compare to control, while 6 fold increase was observed in progressive mild stress.

Rasool *et al.* (2012) observed that the proline content was maximum in salt-tolerant (SKUA-06, SKUA-07) and minimum in salt-sensitive chickpea genotypes (SKUA-04, and SKUA-05). The percent increase in proline content in SKUA-06 and SKUA-07 from T1-T2 was 20.3–79.1% and 49.5–201.0%, respectively at 5 DAT (days after treatment).

Ulemale *et al.* (2013) reported that the genotypes Phule G 09103, Phule G 2008-74, Digvijay exhibited higher values for drought tolerance efficiency, proline content.

Manaa *et al.* (2014) observed that the accumulation of osmoregulatory compound, proline in shoots and roots of the medicinal plant *Lepidium sativum* L. (commonly called garden cress) was greatly increased by NaCl stress.

Rizvi *et al.* (2014) observed increase in proline content under moisture stress over irrigated control at flowering stage was

highest in Pusa-1108 (3.23 times), while at pod formation stage the highest increase was recorded in Pusa-362 (2.5 times).

Hirich *et al.* (2014) found that the concentration of proline in leaves and roots of chickpea genotypes was significantly affected by salinity. Proline concentration in both leaves and roots has been increased as irrigation water salinity increased. Treatment T4 (10 dS m⁻¹) led to high proline accumulation in both leaves and roots compared to the less saline treatments.

2.4.1.2 Glycine betaine

Glycine betaine (GB) and proline are the two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity and extreme temperatures, UV radiations and heavy metals. Betaines are quaternary ammonium compounds in which the nitrogen atom is fully methylated. The most common betaines in plants include glycine betaine.

Sakamoto and Murata (2001) observed that the glycine betaine was accumulated in transgenic plants at low levels only to the extent of protection against a given stress and was correlated with the level of accumulated glycine betaine.

In addition to its role as an osmoprotectant, GB has been reported to protect the oxygen-evolving PSII complex by stabilizing the structure of extrinsic proteins of PSII complex, and ATP synthesis as well as protection of cell membranes and activation of enzymes in onion and rice (Mansour, 1998; Rahman *et al.*, 2002).

Yokoi *et al.* (2002) postulated that the glycine betaine (GB) accumulation enhanced plant salt tolerance as it preserved thylakoid

and plasma membrane integrity effects of salt, freezing or high temperature stresses. It was mainly localized in chloroplasts and plays a vital role in chloroplast adjustment and protection of thylakoid membranes, thereby maintained photosynthetic efficiency and plasma membrane integrity.

Li *et al.* (2003) revealed that the betaine aldehyde decarboxylase from the halophyte *Suaeda liaotungensis* was introduced into tobacco plants and the *in vitro* plantlets were significantly resistant to salt conditions.

Mickelbart *et al.* (2003) studied a recombinant-inbred (RI) population, developed from a cross between GB deficient such as IS 2319 and GB-accumulating such as P932296 sorghum genotype. The proportions of GB-accumulating lines in both the F₅ and F_{7:8} generations of RI population were higher than expected, possibly due to some beneficial effect of GB accumulation on plant growth.

Yang *et al.* (2003) analyzed 240 sorghum genotypes, sampled at the post-flowering stage, the total quaternary ammonium compound (QAC) levels in the betaine fraction of the flag leaves were found to range from as low as 0.1 $\mu\text{mol g}^{-1}$ FW to as much as 33 $\mu\text{mol g}^{-1}$ FW. GB levels were highest in the youngest leaves of GB-accumulating sorghum genotypes.

Similar results were obtained by transforming rice with the choline dehydrogenase gene (*cod A*) from *Arthrobacter globiformis*; the gene product of *cod A* catalyzing the oxidation of choline to GB *via* betaine aldehyde as an intermediate. Tomato plants transformed with a bacterial *codA* gene targeted to the chloroplast were highly tolerant to chilling and oxidative stress, showing an increase in the

photosynthetic rate, plant survival, flower retention and fruit set (Park *et al.*, 2004).

The effects of salinity at the level of 300 to 600 mmol L⁻¹ on some growth and physiological parameters in *Prosopis alba* seedlings were investigated by Meloni *et al.* (2004). Leaf relative water content, nitrate content and nitrate reductase activity in leaves and roots was decreased, whereas the GB content was significantly increased in both the leaves and roots. Total soluble carbohydrates increased only in roots. These results suggested that GB enhancement may be important for osmotic adjustment in *Prosopis alba* under salinity stress.

Zhu *et al.* (2005) observed that the glycine betaine level was markedly enhanced in drought-tolerant wheat cultivar Dingxi 24 at the two later growth stages under drought stress compared with the unstressed control.

Ashraf and Foolad (2007) in their review article reported that genetically-engineered plant containing transgenes for production of GB or proline have thus far faced with the limitation of being unable to produce sufficient amount of these compounds to ameliorate stress effect. In many plants but not all plant species 'shot-gun' application of GB and proline led to significant increase in growth and final crop yield under environmental stress.

Sankar *et al.* (2007) reported an increase in GB in all *bhendi* varieties under drought stress condition at 50 and 70 DAS.

Jaleel *et al.* (2008) emphasized that the drought stress caused an increase in the GB content, paclobutrazol and ABA acts as stress ameliorating agents by further enhancing this parameter in water stressed *Cajanas cajan* plants.

Garg and Noor (2009) reported that the salt tolerant genotypes accumulated more GB than salt sensitive pigeon pea genotypes. Leaves and roots of the salt tolerant genotypes of pigeonpea PUSA 2002-2 showed maximum synthesis of glycine betaine than the leaves and roots of the salt sensitive RG01-73N but roots of all genotypes accumulate more glycine betaine than the leaves .

Khan *et al.* (2010) reported that the GB content showed a significant increase in linseed plant under NaCl stress compared to the control. The level of GB content was lower in unstressed plants supplied with CaCl₂ or GA₃ alone when compared with salt-stressed plants.

Shora and Ali (2013) reported that the GB showed the highest absolute accumulation in chickpea seedlings in response to salinity. Wu (2014) observed that the glycine betaine in young soybean seedlings of ZH13 and BB52 was increased by 59.4% and 62.9% under 300mM NaCl treatment compared to control, respectively.

2.4.2 Antioxidative Enzymes

2.4.2.1 Superoxide dismutase

Barlets (2001) quoted that enhanced superoxide dismutase (SOD) activity has been associated with induced resistance of plants to drought stress.

Shalata *et al.* (2001) proposed that the salt stress tolerance is related to a higher activity of SOD enzyme and that lower activity is found in sensitive species. Sairam *et al.* (2001) have reported that water stress tolerance is associated with the increase in SOD activity.

Sairam *et al.* (2002) found that the SOD activity was increased significantly under salinity stress at all three stages in both the genotypes and more activity was observed at S2 salinity level. SOD activity was highest in Kharchia 65 wheat genotype at 50% anthesis in both control and salinity treatments as compared with other two stages.

Sairam *et al.* (2006) reported significant increase in SOD activity under temperature stress in wheat genotypes, and a greater increase in tolerant genotype C 306, while the susceptible genotypes showed lower activity. This shows that the tolerant genotypes combated the ROS by maintaining efficient antioxidant mechanism.

Eyidogan and Oz (2007) studied that in both tissues (leaf and root) of chickpea, Cu/ZnSOD activity comprised 50–53% of total SOD activity followed by MnSOD (44–47%) and FeSOD (6% in leaves). Cu/ZnSOD activity was increased significantly in both tissues at all doses of stress after 2 and 4 days of salt treatment. Similarly, MnSOD activity was significantly increased under all salt levels in all tissues with the exception of 0.1 M NaCl treatment for 2 days in root tissues.

Dutta and Bera (2007) found an increase in superoxide dismutase (SOD) activity in both leaf and stem parts of the seedlings of mungbean cultivars under water stress was noticed in the genotypes studied upto 4th day.

Kumar *et al.* (2008) reported that the SOD activity was higher in control and increased under high temperature treatment in both the genotypes of chickpea. Genotype Pusa 1103 showed 28 per cent more SOD activity compared to Pusa 261 under high temperature.

Esfandiari *et al.* (2009) in their study found a negative relationship between SOD activity and lipid peroxidation or MDA content. When SOD activity was high, ROS, especially superoxide radical, scavenging was done properly and thus, damage to membranes and oxidative stress decreased, leading to the increase of tolerance to oxidative stress.

Dolatabadian and Saleh-Jouneghani (2009) identified that superoxide dismutase activity was increased in salt stressed plants of *Phaseolus vulgaris* L. in response to salt stress.

Garg and Noor (2009) reported that the salt stress induced an increase of SOD activity, which could be directly correlated with salt stress tolerance of all the genotypes of pigeonpea. Leaf tissue had higher SOD activity as compared to roots in all the genotypes.

Gill and Tuteja (2010) quoted that the SOD provides the first line of defense against the toxic effects of elevated levels of ROS.

The SOD activity was increased in linseed plants by NaCl, CaCl₂ and GA₃ treatments compared to the control. Being equally effective, CaCl₂ or GA₃ separately enhanced SOD activity compared to the plants grown with NaCl only (Khan *et al.*, 2010).

Kukreja *et al.* (2010) found that the specific activity of SOD was increased in chickpea cultivar from 10% to 133% under salinity. Du *et al.* (2010) emphasized that the SOD activity in cucumber leaves increased by 354.34 unit mg⁻¹ protein three days after starting NaCl treatment compared with the control. Hernandez *et al.* (2010) found that total SOD activity was highly induced in zone I root of broccoli at 7 d and 14 d in both salt treatments.

Sheokand *et al.* (2010) revealed an increase in superoxide dismutase (SOD, EC 1.15.1.1) activity was observed with NaCl stress.

Increasing the duration of stress further increased the SOD activity indicating an induction of the defense system. Increased activity of SOD observed in the present studies points towards its induction to quench higher levels of superoxide radical generated due to NaCl stress.

Ahmadizadeh *et al.* (2011) studied activities of SOD, CAT, yield and index of damage (ID) of stress were studied after exposure to drought stress phases after 30 days and in control condition. The results analysis showed significant differences between genotypes and stress condition in terms of SOD, and CAT enzyme activity and ID parameters. The mean comparison showed that the activity of SOD and CAT decreased in susceptible landraces, where as in resistant landraces SOD and CAT remained unchanged and in some cases they showed an increase under stress condition. In these genotypes (resistant) increasing SOD and CAT accompanied with ID decrease in the membrane. Cluster analysis on the basis of ID (20% PEG), ID (30% PEG) and on the basis of SOD, CAT and yield in the stress conditions classified resistant and other landraces into different groups.

Mohammadi *et al.* (2011) analyzed that, treatments significantly affected the activity of antioxidant enzymes like SOD, CAT and GPX increases under stress condition.

A significant increase in the specific activity of SOD by 2 fold was reported in roots of chickpea cultivars at 10.0 dS m⁻¹ (Kukreja *et al.*, 2005). The increase in SOD activity was higher in plants treated with SA @1.5mM [255.07 units mg⁻¹(protein) min⁻¹] than plants treated with 1.0 mM SA [247.35 units mg⁻¹(protein) min⁻¹] in genotype ICC 4958.

Rahelech *et al.* (2012) observed that the SOD activity was increased in MCC392 and MCC877 genotypes and decreased in MCC448 genotype under drought stress as opposed to the control. SOD activity in MCC877 was significantly higher than other investigated genotypes under drought stress.

Rasool *et al.* (2013) reported that the SOD activity showed significant variation in all the chickpea genotypes at all stress levels. The percent increase in SOD activity in SKUA-06 and SKUA-07 was 57.8 and 49.1 %, respectively, at 100 mM NaCl stress at 5 DAT. Other genotypes showed non-significant increase in SOD activity after 50 mM NaCl treatment.

Kaur *et al.* (2013) studied that the chickpea genotype, GL-28184 exhibited better performance in terms of superoxide dismutase activity under both control and stress conditions.

Meena *et al.* (2014) observed significant increase in SOD activity under high temperature compared to normal temperature. Among the chickpea genotypes, RSG 888 showed significantly higher SOD activity under both normal as well as high temperature conditions followed by JG 11 and Pusa 256.

Cevik *et al.* (2014) revealed that the SOD activity significantly increased in *C. arietinum* under water deficit, compared with the control. SOD activity increased rapidly in *C. reticulatum* on day 1 of water deficit, by approximately 5.5-fold.

2.4.2.2 Catalase

Catalases (CAT, EC 1.11.1.6) are tetrameric homoproteins that exist as multiple isozymes encoded by nuclear genes. They are

located mostly in peroxisomes and glyoxisomes (Scandalios *et al.*, 1997).

Shalata *et al.* (2001) proposed that salt stress tolerance was related to a higher activity of CAT enzyme and that lower activity was found in sensitive species.

Sairam *et al.* (2002) reported that the wheat genotype, Kharchia 65 showed higher CAT activity than KRL 19 at all the stages and salinity treatments. Kharchia 65 showed continuous increase in CAT activity with increasing salinity levels, while KRL 19 showed marginal increase at lower salinity level.

Kukreja *et al.* (2005) found a significant increase in the specific activity of CAT by 5 fold was reported in roots of chickpea cultivars at 10.0 dS m⁻¹.

Dutta and Bera (2007) revealed that the tolerant genotype of mungbean K 851 showed higher activity of catalase than susceptible PDM 84-139 in response to water stress.

Dolatabadian and Saleh-Jouneghani (2009) found that the total CAT activity was mostly increased in salt stressed plants, but it was higher at 400 mM NaCl than at 100 mM NaCl stress treatment to *Phaseolus vulgaris* L.

Garg and Noor (2009) examined that each saline treatment brought about significant increase in the catalase activity in the salt tolerant genotypes of pigeonpea when compared with untreated control.

Khan *et al.* (2010) reported that activity of CAT was increased in linseed plants by NaCl, CaCl₂ and GA₃ compared with the control.

Kukreja *et al.* (2010) observed that the specific activity of CAT was increased in chickpea cultivar from 9% to 109% under salinity. CAT activity in leaves cucumber increased by 29.1 U/mg protein three days after starting NaCl treatment compared with the control (Du *et al.*, 2010).

Mafakheri *et al.* (2011) reported that an increase was observed in POX and CAT activity of three cultivars under stress condition throughout the experiment. CAT activity increased in drought resistance cultivar under drought stress. Also, observed that there was significantly higher CAT activity in all three varieties of chickpea upon exposure to drought stress during the vegetative stage as compared to control.

Habibpour *et al.* (2012) reported that the CAT activity with cooperation of other antioxidant enzymes like GPX and PPO could play a significant role in defense against H_2O_2 , especially after short term cold accimilation in accession 4322 under cold stress. The accession 4322, as an appropriate cold tolerant candidate, showed an early higher tolerance to cold-induced oxidative stress compared to other accessions, as inferred from antioxidative activities of enzymes and higher yield (YLD) in field conditions.

Rahelech *et al.* (2012) revealed that a remarkable increase in CAT activity was observed in MCC68 and MCC448 genotypes at both seedling and podding stages under drought stress condition.

Kaur *et al.* (2013) found that the chickpea genotypes, *viz.*, PDG-3, PDG-4 and GL 27104 exhibited higher catalase activity as compared to other genotypes, under control as well as under stress conditions. Some of the genotypes, *viz.*, PBG-1, BGM-569, PBG-5 and

GPF-2 also expressed increased catalase activity under stress conditions.

Ceylan *et al.* (2013) emphasized that, under all stress conditions, H_2O_2 , MDA, and Pro levels increased sharply, but pretreatment with COR significantly reduced them. Moreover, COR increased the activities of H_2O_2 scavenger enzymes such as catalase (CAT) under heat stress, ascorbate peroxidase (POX) under PEG stress, and CAT and POX under combined stresses. CAT activity was increased (2.62-fold) under heat stress compared to the control group in chickpea seedlings.

Rasool *et al.* (2013) found that among the varieties of chickpea, 'ILC482' had the highest CAT activity whereas 'Bivaniej' showed the lowest CAT activity. Catalase activity of chickpea genotypes SKUA-06 and SKUA-07 was increased significantly up to 100 mM of NaCl treatment. The genotypes SKUA-06 showed an increase of 73.8 per cent catalase activity and SKUA-07 showed 75.5 per cent at 5 DAT. Other genotypes showed significant increase up to 25 and 50 mM NaCl stress. After 75 mM NaCl stress no significant change in CAT activity was observed.

Kadkhodaie *et al.* (2014) observed that the activity of CAT was increased in most of the sesame genotypes under water deficit treatments as compared to the control level of irrigation. However, the activity decreased in Kal at I_2 and in Ultan, Shahreza, Markazi, and Kal at I_3 irrigation levels compared to the control.

Battana and Ghanta (2014) emphasized that the catalase activity of the roots and shoots of Pigeon pea cultivars treated with PEG, Cd and Cr registered higher values when compared to their respective controls except in Ws + Cd and Ws + Cr combinations.

2.4.2.3 Ascorbate peroxidase

APX, together with monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and GR, removes the H_2O_2 via the Foyer–Halliwell–Asada pathway (Foyer and Halliwell, 1976 and Halliwell, 1987). CAT ($K_m = 20\text{--}124\text{ mM}$.) can also reduce H_2O_2 to water but it has lower affinity for H_2O_2 than APX ($K_m = 20\text{--}74\text{ }\mu\text{M}$. (Mittler and Zilinskas, 1991 and Ishikawa *et al.* 1998). Ascorbate peroxidase (APX, EC 1.11.1.11) is the most important peroxidase in H_2O_2 detoxification, catalysing the reduction of H_2O_2 to water using the reducing power of ascorbate (Noctor and Foyer, 1998).

Shalata *et al.* (2001) proposed that salt stress tolerance is related to a higher activity of APX enzyme and that lower activity is found in sensitive species.

Vardhini and Rao (2003) reported that in sorghum ascorbate peroxidase activity was increased upto 20 per cent PEG-induced osmotic stress in drought tolerant as well as susceptible cultivars than the unstressed control.

Remarkably higher APX activity was observed in leaf tissues of chickpea at 0.2 and 0.5 M NaCl stress (Eyidogan and Oz, 2007). Kukreja *et al.* (2005) reported that the APX activity was increased up to 141 % in roots of chickpea cultivars at 10.0 dS m^{-1} .

Turkan *et al.* (2005) reported that -0.4 MPa PEG -induced osmotic stress increased APX activity in seedlings of drought tolerant *Phaseolus acutifolius* than the susceptible *Phaseolus vulgaris* up to day 14.

Dolatabadian and Saleh-Jouneghani (2009) reported that ascorbate peroxidase activity strongly increased under salt stress

specifically at 400 mM NaCl in *Phaseolus vulgaris* L. as compared to control condition.

Garg and Noor (2009) indicated that each saline treatment brought about significant increase in the ascorbate peroxidase (APX) activity in the leaves of salt tolerant genotypes and was at a maximum at 8 dSm⁻¹, whereas in salt-sensitive genotypes, APX activity in leaves declined with 6 and 8 dSm⁻¹.

APX is thought to play the most essential role in scavenging H₂O₂ in water–water and ASH–GSH cycles and protecting cells. Several researchers have suggested an increase in APX activity in cotton (Pace *et al.*, 1999), pea (Zlatev *et al.*, 2006 and Terzi *et al.*, 2010), and wheat (Al-Ghamdi, 2009) under drought stress.

Wang *et al.* (2009) reported an increased APX activity in shoots and roots of 7-day-old drought tolerant Xinmu No. 1 alfalfa seedlings on imposition of PEG stress than the sensitive cultivar Northstar. Thus, it indicates that plants have developed an antioxidant defense system to cope with oxidative damage under extremely adverse conditions that include H₂O₂ sensitive antioxidative enzyme APX.

Sheokand *et al.* (2010) reported that 11% increase in ascorbate peroxidase (APX) activity was recorded 2 DAT which further increased to 21% 4 DAT and to 31% 6 DAT with NaCl stress in chickpea.

Rahelech *et al.* (2012) found that in the seedling stage, the leaf APX activity increased significantly in MCC392 and MCC877 genotypes under drought stress, as compared to the control.

Rasool *et al.* (2013) reported that APX activity showed large variation in all the chickpea genotypes at all the levels of NaCl

treatment. The APX activity was maximum in SKUA-06 and SKUA-07 and minimum in SKUA-03, SKUA-04, and SKUA-05. The percent increase in APX activity in SKUA-06 and SKUA-07 was 162.3 and 112.3 %, respectively, at 100 mMNaCl stress at 5 DAT. No significant change in APX activity was observed in other genotypes after 75 mM NaCl stress.

Ceylan *et al.* (2013) found that application of coronatine under normal conditions increased APX activity by 67.9 %, while PEG stress with coronatine increased APX (10 %) activity compared to PEG stress without coronatine pre-treatment in chickpea seedlings.

Cevik *et al.* (2014) emphasized that the activity of APX was significantly increased in both species of chickpea under water deficit, compared with the control plants. APX level was always higher in *C. reticulatum* than in *C. arietinum* under water deficit.

2.5 Yield and yield contributing characters

Ali *et al.* (1998) observed the superiority of CM-2 to other genotypes observed under drought conditions is due to the production of more number of pods per plant, yield per plant and leaf area per plant. Water stress imposed from flowering to maturity resulted in low 100 grain weight and grain yield per plant as compared to non stress chickpea plants.

Rosales *et al.* (2004) reported similar results in common bean subjected to drought and observed that overall yield reductions due to drought treatments were greater in the drought susceptible cultivars than in the tolerant cultivars.

Mathur *et al.* (2005) observed that under terminal moisture stress and normal irrigation control genotype H99-270

recorded significantly highest yield although it was at par with H 00 53 under normal irrigated condition. This might be attributed to the better yield attributes of these genotypes such as branches per plant and yield per plant.

Bakhsh *et al.* (2007) reported that the yield and most of yield components were improved with the application of irrigation. On average basis 48% increase in number of pods per plant, 36% in total dry weight and 17% in grain yield was recorded due to irrigation. The genotype that responds positively to irrigation with respect to seed size coupled with increase in number of pods per plant will be the most suitable for irrigation areas. The number of pods per plant was the most important character that responded positively to irrigation and hence contributed significantly to yield increase.

Islam *et al.* (2008) reported that high yielding genotypes of chickpea, in general, produced higher number of secondary branches per plant. It means that yield is positively correlated with secondary branches. Ali *et al.* (1999) indicated the importance of secondary branches and pods per plant in determining the yield of chickpea.

Thomas A. (2010) suggested that chickpea lines should be given moisture stress at pre flowering stage while screening against drought stress conditions. Among the irrigation levels two irrigations at pre-flowering and pod filling stages of chickpea recorded significantly higher seed yield as compared to all other treatment.

Shamsi *et al.* (2010) reported that compared to treatment without irrigation, the treatments with only one-time irrigation at 50%flowering and pod filling stages had 149.9 and 117.9 % increases in the number of pod per plant, respectively. Grain yield was

positively correlated with number of pods per plant ($r=0.654$), number of grain per plant ($r=0.902$) and 100 grain weight ($r=0.707$)

Patel *et al.* (2011) investigated that, reduction in relative injury was observed in plants treated with SA @1.5 mM as compared to control seedlings. Results indicate wide variation in tolerance to drought stress amongst chickpea cultivars at both the critical stages i.e. pre- and post-anthesis. On the basis of growth and antioxidant activity better genotypes Tyson and ICC-4958 appear to be adapted to drought stress tolerance. Early drought stress (pre-anthesis drought) was found to be more damaging than the late drought stress (post-anthesis drought).

Yaqoob *et al.* (2011) reported that among the various drought stresses at different crop growth stages, the stress at pre-flowering stage has been established to be the more fatal. It is also suggested that the chickpea lines should be given moisture stress at pre-flowering stage while screening against drought stress conditions.

Patel and Hemantrajan (2012) reported that pre-anthesis stage was most sensitive whereas genotype ICC 4958 was most tolerant to drought.

Pouresmaeil (2012) showed that the mean comparisons of some traits showed that genotype 216066 produced the highest yield under severe drought. This accession also possessed higher values in plant canopy width, harvest index, biological yield, pod number and seed weight per plant. On the other hand, reduction of these traits due to moisture stress was more obvious in genotype 215979.

Hinkossa *et al.* (2013) reported that drought stress imposed at flowering stage reduced all growth , physiological and yield related traits of the basic generations.

Khodadadi M. (2013) showed that the chickpea grain yield, number of second branch and biomass yield decreased significantly with the drought stress. Mean comparison of drought stress showed that it causes 34, 22,7,51 and 38 per cent reduction in seed, biological yield, 100 seed and relative water content respectively across all treatments.

Nagar *et al.* (2013) reported that number of pods per plant at maturity indicated the positive effect of irrigation in all the genotypes in both the years. The availability of moisture played a significant role in contributing towards higher pod weight in general in all the genotypes grown under irrigated conditions. Better source sink relationship was observed in the tolerant types. The mean plant height in response moisture stress declined by 28 per cent and 26 per cent respectively in season 2009-10 and 2010-11, respectively at maturity at IARI, New Delhi farm. The irrigation caused significant increase in plant height at maturity. The moisture stress has reduced the days to 50 per cent flowering by 7.19 to 8.8 per cent, maturity with 4.5 and 4.9 per cent reduction and number of pods 8.8 and 5.4 per cent, in season I and II, respectively.

Shinde *et al.* (2013) investigated the grain yield of chickpea was significantly declined due to moisture deficit, which appeared as result of reduction in photosynthetic rate, RLWC, plant height, number of pods and harvest index. The plant height and number of pods significantly declined under moisture deficit while

maximum plant height and more number of pods were recorded under irrigated condition.

Talebi *et al.* (2013) reported that drought stress reduced the seed yield of all genotypes. Yield reduction of different genotypes varied from 34.3 to 78.1%.

Yaqoob *et al.* (2013) suggested that various chickpea lines had a quite divergent response to drought stress conditions. Similarly, plant recovery response revealed that more than 50% lines could not properly convalesce after termination of drought stress. The sensitivity of 30 lines suggest that moisture stress at pre flowering is quite dangerous to chickpea plants.

Kumar *et al.* (2014) reported that seed yield per plant showed highly significant positive correlation with number of pods per plant, number of seeds per plant and number of secondary branches per plant. Results showed that number of secondary branches per plant, number of pods per plant and number of seeds per plant can be considered as a selection criteria for the improvement of seed yield.

3. MATERIAL AND METHODS

The investigation entitled “Physio-biochemical analysis for growth and yield in chickpea under moisture stress” was carried out during *rabi* 2012-13 and 2013-14 under rain out shelter at Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (MS). The details of the methods adopted to conduct the experiment are presented in this chapter.

3.1 Experiment 1 : “Physiological screening of chickpea genotypes under polyethylene glycol (PEG-6000) induced water stress.”

The assessment for drought tolerance at early stage of seedling is most frequently carried out by inducing chemical drought induced molecules like polyethylene glycol (PEG 6000) in the medium. This technique would appear to be suitable for initial screening of large population to drought tolerance prior to yield testing.

Chickpea genotypes were obtained from the Principal Scientist, Pulses Improvement Project, MPKV, Rahuri. The thirty genotypes were screened for moisture stress induced by five different osmotic potential levels (0, -0.2, -0.4, -0.6 and -0.8 MPa) of polyethylene glycol 6000 (PEG-6000). Seeds of each cultivar were treated with a 5 % solution of sodium hypochlorite for 5 minutes for surface sterilization, and then residual chlorine was eliminated by washing of the seeds with distilled water. After then presoaked (16 hr) seeds were planted in between filter paper moistened with solution of five osmotic potential. Germination percentage, root and shoot length were measured on 16th day. On the basis of root, shoot ratio, vigour

index and proline content five susceptible and five tolerant genotypes were selected for pot and field experiment.

3.1.1 Germination (%)

Two replications of 40 seeds from different treatment combinations were germinated using between paper methods (BP) at $25 \pm 2^\circ\text{C}$ in germinator for 16 days. The germination percentage was computed on the basis of normal seedlings only.

$$\text{Germination \%} = \frac{\text{Total number of normal seedlings counted after germination test}}{\text{Total number of seeds kept for germination test}} \times 100$$

3.1.2 Shoot length (cm)

Ten normal seedlings from each of replication from different treatment combinations of germination test were selected randomly for shoot length and were measured in centimeter. The average of ten seedlings length was calculated and recorded in cm.

3.1.3 Root length (cm)

Ten normal seedlings selected from each of replication from different treatment combinations of germination test were used for root length and was measured in centimeter. The average of ten seedlings length was calculated and recorded in cm.

3.1.4 Vigour Index

The seedlings which were used to record the seedling length were used to know the vigour index. The ten normal seedlings from each replication was selected for calculation of vigour index (Abdul-Baki and Anderson, 1973) and was calculated as under

$$\text{Vigour Index} = \text{Average Seedling length (cm)} \times \text{Average germination percentage}$$

3.2 Experiment 2 : “Physiological and biochemical basis for moisture stress tolerance in chickpea under pot study.”

The selected ten genotypes (5 susceptible & 5 tolerant) were grown in pots. After 35 days of seedling growth (before anthesis), plants were divided into three groups control, moderate stress (-0.4 MPa) and severe stress (-1.2 MPa). The moisture stress was induced by irrigating the pot with PEG-6000 solution. The osmotic potential of the solution of treatment was decreased gradually at the rate -0.1 MPa/ alternate two days and -0.3 MPa/alternate two days until the stress level reach -0.4 MPa (moderate stress) and -1.2 MPa (severe stress).

The comparative studies were performed in control and treatment group of same age plants. Healthy leaves free of any diseases from control group and stress induced leaves were used for various physiological and biochemical assays. The concentration of PEG 6000 (g/l of water) for each water stress was determined by using the equation of Michel and Kaufman, (1973).

Experimental details :

1. Design of experiment	FCRD
2. Number of replications	Two
3. Conditions	1. Control 2. Moderate Stress 3. Severe Stress

3.3 Experiment 3 : “Physiological and biochemical basis for moisture stress tolerance in chickpea under rain out shelter.”

The selected ten genotypes (5 susceptible and 5 tolerant) were grown in rain out shelter during *rabi* 2012-13 and *rabi* 2013-14 under rain out shelter at Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist.: Ahmednagar (MS). The details are as below

3.3.1 Climate

Rahuri region comes under semi-arid tropics (Scarcity zone) with climate where the winters are short and mild. The rainfall was erratic and unevenly distributed in 15-45 days in different years. The annual rainfall received varies from 307 to 619 mm (average rainfall 525 mm). The average maximum and minimum temperatures are 32.1 and 16.6°C, respectively. The average relative humidity at 7.30 hrs (RH-I) is 72 per cent while at 14.00 hrs (RH-II) is 37 per cent. The annual average wind speed is 7.95 km hr⁻¹. The annual bright sunshine hours were 7.79 hr per day.

Table 1. Metrological data recorded during the crop growth period.

2012-13

Month	Metro. week	Temperature (°C)		Rainfall (mm)	Rainy days (No.)	R.H. (%)	
		Max.	Min.			Morning	Evening
1	2	3	4	5	6	7	8
Oct., 12	40	31.4	21.7	118.6	5	88	62
	41	32.1	19.7	00	-	79	41
	42	32.2	16.9	00	-	74	32
	43	31.4	19.5	18.6	1	76	46
	44	30.3	15.6	00	-	63	40
Nov., 12	45	31.7	14.9	00	-	62	34
	46	30.2	12.7	00	-	56	29
	47	30.0	11.3	00	-	52	29
	48	31.8	13.6	00	-	56	31
Dec., 12	49	31.3	12.6	00	-	52	30
	50	31.4	12.4	00	-	60	27
	51	29.9	11.2	00	-	55	28
	52	32.4	20.5	00	-	62	54
Jan., 13	1	31.3	11.2	00	-	56	31
	2	29.1	10.1	00	-	56	27
	3	30.4	11.7	00	-	50	32
	4	30.2	13.5	00	-	51	36
	5	31.7	15.9	00	-	68	33
Feb., 13	6	30.9	15.2	00	-	59	35
	7	31.9	15.2	00	-	59	35
	8	33.4	12.5	00	-	53	20
	9	33.8	13.6	00	-	40	26
Total				137.20	6.00		
Av.		31.31	14.61			60.32	34.45

2013-14

Month	Metero. week	Temperature (°c)		Rainfall (mm)	Rainy days (No.)	R.H. (%)	
		Max.	Min.			Morning	Evening
1	2	3	4	5	6	7	8
Oct-13	40	31.9	21.2	28.2	1	82	58
	41	31.1	20.0	65.8	3	77	57
	42	32.0	20.1	00.0	0	75	46
	43	31.2	19.6	05.2	1	68	56
	44	31.4	16.8	00.0	0	63	48
Nov-13	45	32.4	14.3	00.0	0	65	28
	46	31.8	13.5	00.0	0	69	27
	47	30.7	11.3	41.8	1	66	28
	48	31.2	15.3	00.0	0	72	36
Dec-13	49	29.0	13.5	00.0	0	72	38
	50	28.7	07.4	00.0	0	74	25
	51	29.5	09.6	00.0	0	55	28
	52	29.1	13.9	00.0	0	67	34
Jan-14	1	28.8	12.2	00.0	0	66	37
	2	29.5	12.3	00.0	0	59	34
	3	29.7	13.6	00.0	0	54	34
	4	29.0	14.8	00.0	0	67	39
	5	28.5	11.2	00.0	0	59	31
Feb-14	6	31.8	11.2	00.0	0	54	20
	7	28.0	10.3	00.0	0	54	27
	8	31.1	15.2	00.0	0	56	30
	9	30.8	13.6	00.6	0	51	33
Total				141.60	6.00		
Av.		29.98	12.54			64.77	36.09

Weather during the experimental period

Weather data during the crop growth period during 2012-13 the maximum temperature ranged from 33.8 to 29.9°C with an average temperature of 31.31°C. The minimum temperature ranged 21.7 to 10.1°C with an average of 14.61°C. During 2013-14, the maximum temperature ranged from 32.4 to 28.0°C with an average of temperature 29.48 °C, similarly the minimum temperature ranged from 21.2 °C to 7.4°C with an average of 12.54 °C. Mean relative humidity during 2012 -13 at 7.30 hr (RH – I) ranged between 88

and 40 per cent with mean 60.32 per cent while mean relative humidity at 4.30 hr (RH – II) ranged between 62 and 20 per cent with mean of 34.45 per cent.

The relative humidity during 2013-14 at 7.30 hr (RH- I) ranged between 82 and 51 per cent mean 64.77 per cent while at 4.30 hr (RH -II) ranged between 58 and 20 per cent mean 36.09 per cent.

The total rainfall received during crop growth period 2012-13 was 137.20 mm in 6 days in 40 and 43 MW afterwards there was absolutely no rain during crop growth period. While during crop growth period 2013-14, total rainfall received was 141.60 mm in 6 days.

Details of field Experimental

Season	:	Rabi 2012-13 and 2013-14
Design of experiment	:	Split Plot Design
Sowing date	:	I st year : 19/11/2012 II nd year : 24/10/2013
Sowing method	:	Dibbling
Fertilizer dose	:	25:50:30 NPK kg ha ⁻¹
Plot Size	:	Gross : 2.00 x 0.60 m
		Net : 2.00 x 0.60 m
Spacing	:	30 cm
Conditions	:	1. Irrigated 2. Early Drought Stress 3. Late Drought Stress

Drought Stress Application

Each genotype was grouped in three sets *viz.*, Irrigated, Early Drought Stress and Late Drought Stress. Water stress treatment was imposed at 40 DAS. Normal plants received three irrigations (at 20, 40 and 60 DAS) from the date of sowing to maturity. Early drought stress or pre-anthesis stressed plants (EDS) received two irrigations (20 and 60 DAS) whereas post-anthesis drought stressed plants (LDS) received two irrigations at 20 and 40 DAS. Stress cycle of seven days was given in both the conditions.

3.3.2 Intercultural operations

Gap filling was carried out seven day after sowing. Thinning was done by keeping only one healthy plant per hill.

3.3.3 Fertilizer Application

Total dose of 25 kg N ha⁻¹, 50 kg P₂O₅ ha⁻¹ and 30 kg K ha⁻¹ was given at the time of sowing

3.3.4 Phenological observations

3.3.4.1 Days to 50 per cent flowering

Numbers of days required for 50 per cent of the plants to flower were counted from date of sowing and mean was recorded.

3.3.4.2 Days to physiological maturity

Numbers of days required for physiological maturity from date of sowing were counted and recorded.

3.3.5 Physiological parameters

3.3.5.1 Observations recorded by IRGA

The net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were measured using Infra-Red Gas Analyzer (IRGA;

Model Portable Photosynthesis System LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA). The transpiration rate and stomatal conductance were measured continuously monitoring H₂O of the air entering and leaving in the IRGA headspace chamber and the measurements were made at mid day, between 11:30 and 12:00 eastern day time (1400–1800 mmolm⁻² s⁻¹ PPFD), on top fully expanded leaf . The flow-rate of air in the sample line was adjusted to 500 µmol s⁻¹.

3.3.5.2 SPAD Chlorophyll meter

Chlorophyll concentration was assessed using chlorophyll meter (SPAD-502 plus, Minolta). Measurements were taken at three points (upper, middle and lower parts). Average of these three readings was considered as SPAD reading of the leaf. SPAD reading was carried out at 50 % flowering and grain filling stage. The mean SCMR reading was taken out in the end and presented as average SPAD value.

3.3.5.3 Relative leaf water content (RLWC)

Relative leaf water content (RLWC) was measured by the method described by Henderson and Davies-Jr. (1990).

Protocol

The leaves from the top of the main stem was detached from 5 randomly selected plants and kept in sealable plastic bag in an ice box. The leaf samples were brought to a laboratory where fresh weight was recorded immediately. The leaf samples were then immediately hydrated to full turgidity for 2 hours by floating on de-ionized water in a close petri-dish under room temperature. After 2 hours the samples were taken out of water and were well dried with a filter paper. They were immediately weighted to obtain fully turgid weight (TW). Samples were then dried at 80 °C for 36 h and dry

weight (DW) was determined. The RLWC was calculated by using the following formula.

$$\text{RLWC, \%} = [(FW - DW) / (TW - DW)] \times 100$$

where, FW = fresh weight, TW = turgid weight, DW = dry weight

3.3.5.4 Photo synthetically Active Radiation (PAR)

LI-191 SA. Line quantum sensor ($\mu \text{ mol/m}^2/\text{sec}$)

The line quantum sensor (LI 191 SA) is the instrument designed for measuring PAR (Photo synthetically Active Radiation) through plant canopy.

Theory of operation

The LI-191 SA line quantum sensor, which spatially averages radiation over its one-meter length can, minimizes the error in measurement. The sensor should keep in horizontal position to the surface. Line quantum sensor was connected to the data logger (LI1000) to record the reading firstly; sensor surface should held above the crop canopy facing upward to measure incident radiation through polythene film. Subsequently, line quantum sensor was kept inverted over crop canopy for measuring reflected radiation. Then it was kept under the canopy facing upward to record transmitted radiation.

Measurement of incident PAR (PAR), $\mu \text{ mol m}^{-2} \text{ S}^{-1}$

For measurement of incoming PAR, line quantum sensor was positioned facing up 1 foot above the top of the canopy and value was recorded for incoming PAR.

Measurement of transmitted PAR (TPAR), $\mu \text{ mol m}^{-2} \text{ S}^{-1}$

Line quantum sensor was placed above the ground across the rows and value was recorded for TPAR.

Measurement of reflected PAR (RPAR), $\mu \text{ mol m}^{-2} \text{ S}^{-1}$

For measurement of reflected radiation line quantum

sensor was inverted 1 feet above the crop canopy and value was recorded for reflected radiation

Determination of absorbed PAR (APAR), $\mu \text{ mol m}^{-2} \text{ s}^{-1}$

APAR was worked out by adopting the equation given below. Incident radiation = Transmitted radiation + Reflected radiation + Absorbed radiation

Therefore,

$$\text{Absorbed radiation} = \text{Incident radiation} - (\text{Transmitted radiation} + \text{Reflected radiation})$$

The efficient absorbance of Photosynthetically active radiation was measured by Line quantum sensor at 50% flowering and grain filling stages of the crop. Reflected PAR by soil and Absorbed PAR (%) recorded with help of Line quantum sensor under moisture stress and non-stress conditions and calculated. The efficient PAR absorbance are used for the stress tolerance

3.3.6 Morphological observations

3.3.6.1 Plant Height (cm)

Plant height of five randomly selected plants from each plot was recorded in cm by measuring length from the base of the near the ground up to the growing point of the plant and its mean was calculated at harvesting.

3.3.6.2 Number of secondary branches per plant

Total number of secondary branches of 5 plants from each plot and replications were recorded and then the average numbers of secondary branches were computed.

3.3.7 Yield contributing observations

3.3.7.1 Number of pods per plant

Total numbers of pods of 5 uprooted plants from each plot were recorded and then the average numbers of pods per plant were computed.

3.3.7.2 Grain weight per plant (g)

The pods harvested from five observation plants were threshed separately and grain yield was recorded and average was worked out.

3.3.7.3 Harvest index (%)

The harvest index expressed in % was calculated by using the formula by Donald and Hamblin (1976).

$$\text{Harvest index} = \frac{\text{Economic Yield (g/plant)}}{\text{Biological Yield (g/plant)}} \times 100$$

3.3.7.4 Test weight (g)

The weight of 100 seeds taken from the net plot of each treatment was recorded separately.

3.3.8 Biochemical parameters

3.3.8.1 Proline

Proline content in leaf tissues of both control and drought stress chickpea at 50% flowering was determined using the acid ninhydrin reagent as per the method described by Bates *et al.* (1973). The proline content was expressed as μmoles per gram fresh weight.

Reagents

1. Acid ninhydrin: Ninhydrin (1.25 g) was dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid with agitation. The reagent was stored at 4° C and used within 24h.
2. Sulphosalicylic acid 3 % (w/v): Sulphosalicylic acid (3 g) was dissolved in distilled water and the volume made to 100 ml.
3. Stock solution of L-proline (100 mM): Standard L-proline (1.151 g) was dissolved in distilled water and the final volume made to 100 ml.
4. Working standard solution of L-proline (1 mM): From the stock solution, 1 ml was again diluted to 100 ml with distilled water. This diluted working solution contained 1 μ mole of proline per ml.

Protocol

Leaf samples (0.2 g) were homogenized with 2 ml of 3 per cent (w/v) aqueous solution of sulphosalicylic acid and the homogenate centrifuged at 10,000 xg for 10min. A suitable (in experiment I: 0.2ml control and 0.1ml for drought stress and in experiment II: 0.2 ml for control and drought stress) volume of the filtrate was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hr at 100° C. The reaction was terminated by placing the tubes in ice. The reaction mixture was extracted with 4 ml toluene and mixed vigorously for 15 to 20 seconds. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance read at 520 nm using toluene as

a blank. The proline concentration was determined from the standard curve and calculated on a fresh weight basis.

Calibration of a standard curve

In a triplicate set of test tubes, the working standard solution of proline was pipetted as 0, 50, 100, 150, 200, 250 and 300 μ l having the corresponding proline concentration of 0, 50, 100, 150, 200 and 300 nmoles. The volume was made to 1 ml with distilled water. The colour was developed as described above. The absorbance was recorded at 520 nm and a standard graph of absorbance against concentration of proline (nmoles) was plotted (Fig.1).

3.3.8.2 Glycine betaine

Glycine betaine content in leaves of both the unstressed and stressed seedlings was determined by using the Dragendorff reagent as per the method described by Stumpf (1984). The glycine betaine content was expressed as μ moles g^{-1} FW

Reagents

1. Dragendorff reagent: This reagent was prepared by mixing the equal volume of 0.35 M bismuth nitrate in 20% acetic acid (v/v) and 2.45 M sodium iodide (NaI) in distilled water.
2. Bismuth nitrate solution (0.35 M): The solution was prepared by dissolving 4.24g bismuth nitrate in distilled water and 20 per cent acetic acid. The volume was made to 25 ml with distilled water.
3. Sodium iodide solution (2.45 M): The solution was prepared by dissolving 9.180 g sodium iodide in distilled water and the volume was made to 25 ml.

4. Working solution of sodium iodide (0.49 M): Five ml of 2.45 M NaI solution was diluted in distilled water and the volume made to 25 ml.
4. Betaine aldehyde solution: The solution was prepared by dissolving 0.5 g of betaine aldehyde in 1 ml distilled water ($500\mu\text{g } \mu\text{l}^{-1}$).

Protocol

Leaf sample (0.2 g) was homogenized in a mortar and pestle with 2 ml of 80 per cent (v/v) ethanol. It was transferred into the microcentrifuge tube which was kept in the hot water bath for 20 min. The tubes were then removed from water bath and cooled at room temperature. It was centrifuged at 10,000 xg for 20 min and the supernatant was collected in clean microcentrifuge tube.

The supernatant (0.5 ml) was pipetted in the microcentrifuge tube to which 100 μl Dragendroff reagent was added. The solution was then centrifuged at 7000 xg for one min. After centrifugation, the supernatant was drawn with the help of a syringe and the orange pellet was dried. The pellet was dissolved in one ml solution of 2.45 M NaI. From this 500 μl aliquot was pipetted in test tubes containing 3 ml diluted 0.49 M NaI solution and mixed. The absorbance was read at 467 nm using 0.49 M NaI solution as a blank.

Calibration of a standard curve

In a set of microcentrifuge tubes, the standard solution of betaine aldehyde ($500\mu\text{g } \mu\text{l}^{-1}$) was pipetted as 0, 5, 10, 15, 20 and 25 μl to which 100 μl of Dragendorf reagent was added causing the precipitation of the QAC. It was then centrifuged at 7000 xg for one min. The supernatant was completely removed. The pellet was

dissolved in 1 ml of 2.45 M NaI solution. A known quantity of aliquot (10 μ l) was then added to three ml of 0.49 M NaI solution (0, 5, 10, 15, 20 and 25 μ l betaine aldehyde solution after dissolving pellet in 1ml and volume taken for colour development having the corresponding concentrations of 0, 25, 50, 75, 100 and 125 μ g). The absorbance was read at 467 nm using 0.49 M NaI solution as a blank and a standard curve of absorbance vs concentration of glycine betaine was plotted (Fig. 2).

3.3.8.3 Soluble protein

Soluble protein content of the enzyme extract was estimated as per the method described by Lowry *et al.* (1951).

Reagents

1. Solution A: 2 % Na_2CO_3 in 0.1 N NaOH: Twenty g sodium carbonate and 4 g sodium hydroxide was dissolved in distilled water and the volume made to 1 liter.
2. Solution B: It was prepared just prior to its use by mixing equal volume of
 - a) 1 % $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution: One g of copper sulphate was dissolved in 100 ml of distilled water.
 - b) 2 % Potassium sodium tartrate solution: Two g of potassium sodium tartrate was dissolved in 100 ml distilled water.
3. Solution C (Alkaline cupric tartarate solution): It was prepared just prior to its use by mixing 50 ml of solution A and 1 ml of solution of B.
4. Solution D [Folin – Ciocalteu reagent (1 N)]: Generally Folin–Ciocalteu reagent (phosphomolybdate and phosphotungstate) available commercially is two normal.

It was diluted by adding equal volume of water to get 1 N reagent and it prepared prior to its use. The reagent was stored in dark amber coloured bottle in cold.

5. Standard protein stock solution (1.25 mg per ml): BSA (125 mg) was dissolved in distilled water and the volume made to 100 ml.
6. Working standard protein stock solution: (250 μ g per ml): Five ml of stock solution of standard protein was diluted in distilled water and the volume made to 25 ml.

Protocol

a. Extraction of protein from plant material

Leaf material was cut into small pieces. After thorough mixing, representative leaf sample (0.2 g) was extracted with 2 ml of 0.1 M potassium phosphate buffer, pH 7.5 using pestle and mortar at 4°C. Extracted material was transferred to centrifuge tube. The homogenate was centrifuged for 15 min at 10,000 rpm at 4°C. The supernatant was decanted and the residue was discarded. The supernatant was diluted to 2 ml volume with the extraction buffer.

b. Estimation of soluble protein

The supernatant of leaf extract (0.1 ml) was taken in test tube. The volume was made to 1 ml with distilled water. A tube containing 1 ml distilled water served as a blank. Five ml of solution C was added and mixed well by vortexing and kept at room temperature for 10 min. Folin reagent (Solution D) (0.5 ml of 1 N) was added and was mixed well using a vortex mixer. Blue colour was developed immediately. All tubes were kept at room

temperature for 30 min. The absorbance of the blue colour was read at 660 nm using spectrophotometer. The protein content in the sample was calculated by using the standard curve, and results were expressed as $\mu\text{g protein ml}^{-1}$ sample solution.

c. Preparation of standard curve

In a set of test tubes, the working standard protein solution (250 $\mu\text{g per ml}$) was pipetted as 0, 0.1, 0.2, 0.3, 0.4 and 0.5 ml and volume was made to 1 ml with distilled water gave the corresponding concentrations of 0, 25, 50, 75, 100 and 125 μg of BSA. A tube containing 1 ml distilled water was served as a blank. Five ml of solution C was added and mixed well by vortexing and kept at room temperature for 10 min. Folin reagent (Solution D) (0.5 ml of 1 N) was added and was mixed well using a vortex mixer. Blue colour was developed immediately. All tubes were kept at room temperature for 30 min. The absorbance of the blue colour was read at 660 nm using spectrophotometer. Then the absorbance readings were plotted on a graph paper by taking BSA concentration on X-axis and absorbance on Y-axis (Fig. 3).

3.3.8.4 Enzymes extraction

Antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (POX) were extracted from leaf tissue by using the method of Coasta *et al.* (2002).

Preparation of crude enzyme extract

Reagent

1. Potassium phosphate buffer (0.1mM, pH 7.5) containing 1mM EDTA, 1mM PMSF and 5% (w/v) PVPP: It was prepared by mixing 16 ml of 0.2 M KH_2PO_4 and 84 ml of

0.2 M K_2HPO_4 . The pH of the buffer was adjusted to 7.5 and final volume made to 200 ml with distilled water. At the time of extraction of enzyme, 1 mM EDTA (0.058 g), 1mM PMSF (0.035 g) and 5% (w/v) PVPP (10 g) dissolved in 200 ml potassium phosphate buffer (0.1 mM, pH 7.5).

Protocol

For assays of SOD, CAT, APX and GPX leaf samples 200 mg were homogenized with a pestle in a chilled mortar with 2 ml of an ice-cold 0.1 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 1 mM PMSF and 5 % (w/v) PVPP. The homogenates were filtered through four layers of cheesecloth and then centrifuged at 4° C for 20 min at 15,000xg. The supernatant fraction was used as crude extract for enzyme activity assays.

3.3.8.5 Superoxide dismutase

Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium using the method described by Dhindsa *et al.* (1981).

Reagents

1. Potassium phosphate buffer (100 mM, pH 7.8): It was prepared by mixing 8.5 ml of 0.2 M KH_2PO_4 and 91.5 ml of 0.2 M K_2HPO_4 and final volume made to 200 ml with distilled water.
2. Methionine (200 mM): It was prepared by dissolving 1.492 g L-methionine in 50 ml distilled water.
3. Nitroblue tetrazolium (2.25 mM): It was prepared by dissolving 0.092 g nitroblue tetrazolium in 50 ml distilled water.

4. Ethylenediaminetetra acetic acid (3 mM): It was prepared by dissolving 0.112 g EDTA in 100 ml distilled water.
5. Sodium carbonate (1.5 M): It was prepared by dissolving 7.9 g Sodium carbonate in 50 ml distilled water.
6. Riboflavin (60 μ M): It was prepared by dissolving 0.047 g riboflavin (5 mM) in 25 ml of 0.1 N NaOH and further 0.3ml diluted to 25 ml with distilled water.

Protocol

Three ml enzyme reaction mixture contained: 50 mM phosphate buffer (pH 7.8) (1.5 ml of 100 mM), 13.33 mM methionine (0.2 ml of 200 mM), 75 μ M NBT (0.1 ml of 2.25 mM), 0.1mM EDTA (0.1 ml of 3 mM), 50 mM sodium carbonate (0.1 ml of 1.5 M), 100 μ l enzyme extract, 0.8 ml of distilled water and 2 μ M riboflavin (0.1 ml of 60 μ M).

The reaction was started by adding 2 μ M riboflavin and placing the tubes under two 15 w fluorescent bulbs for 15 min. A complete reaction mixture without enzyme extract, which gave the maximal colour, served as irradiated control. After 15 min, the reaction was terminated by switching off light and covering the tubes with black cloth. A complete reaction mixture without enzyme extract kept in dark served as non-irradiated blank. The reaction was started by switching on the light and was allowed to run for 10 min. The reaction was stopped by switching off the light and the tubes were covered with a black cloth. The absorbance of the reaction mixture was read at 560 nm. One unit of SOD was defined as the amount of enzyme required to cause 50 per cent inhibition of NBT reduction per min at 560 nm.

3.3.8.6 Catalase

Catalase activity was measured immediately in fresh extract and the assay employed was the one described by Aebi (1984). The hydrogen peroxide dependent oxidation was estimated by the decrease in absorbance at 240nm.

Reagents

1. Hydrogen peroxide (75 mM): Hydrogen peroxide, 775 μ l of 30 % (v/v) was dissolved in distilled water and the final volume made to 100 ml.
2. Potassium phosphate buffer (100 mM, pH 7.0): It was prepared by mixing 39 ml of 0.2 M KH_2PO_4 and 61 ml of 0.2 M K_2HPO_4 and the final volume made to 200ml with distilled water.

Protocol

Three ml enzyme reaction mixture contained: 50 mM potassium phosphate buffer (pH 7.0) (1.5 ml of 100 mM), 200 μ l enzyme extract, 1 ml of distilled water and 12.5 mM hydrogen peroxide (0.5 ml of 75 mM).

The reaction was initiated with addition of 0.5 ml of 75 mM H_2O_2 . For measurement of catalase enzyme activity, the decline in absorbance was recorded at 240 nm for three min at an interval of 30 sec. The amount of hydrogen peroxide decomposed was determined from molar extinction co-efficient (ϵ 36 $\text{M}^{-1} \text{cm}^{-1}$). The enzyme activity was expressed as μ moles of H_2O_2 decomposed $\text{mg}^{-1} \text{protein min}^{-1}$.

3.3.8.7 Ascorbate peroxidase

Ascorbate peroxidase activity was measured immediately in fresh extract which was assayed as per the method described by Nakano and Asada (1981).

Reagents

1. Potassium phosphate buffer (100 mM, pH 7.0): It was prepared by mixing 39 ml of 0.2 M KH_2PO_4 and 61 ml of 0.2 M K_2HPO_4 and the final volume made to 200 ml with distilled water.
2. Ascorbic acid (3 mM): It was prepared by dissolving 0.053g of ascorbic acid and the final volume made to 100 ml with distilled water.
3. Hydrogen peroxide (3 mM): It was prepared by diluting 1ml of 75 mM hydrogen peroxide to 25 ml with distilled water.
4. Ethylenediaminetetra acetic acid (3 mM): It was prepared by dissolving 0.112 g EDTA in 100 ml distilled water.

Protocol

Three milliliter of enzyme reaction mixture contained: 50 mM potassium phosphate buffer (pH 7.0) (1.5 ml of 100 mM), 0.5 mM ascorbic acid (0.5 ml of 3 mM), 0.1 mM EDTA (0.1 ml of 3 mM), 100 μl enzyme extract, 0.7 ml of distilled water and 0.1 mM hydrogen peroxide (0.1 ml of 3 mM).

The reaction was initiated by the addition of 0.1 ml of 3 mM H_2O_2 . The hydrogen peroxide dependent oxidation of ascorbic acid was followed by a decrease in the absorbance measured at 290 nm for three min at the interval of 30 sec. The amount of ascorbate oxidized was determined from molar extinction coefficient (ϵ 2.8 $\text{mM}^{-1} \text{cm}^{-1}$). The enzyme activity was expressed as nmoles of ascorbate oxidized $\text{mg}^{-1} \text{protein min}^{-1}$.

4. EXPERIMENTAL RESULTS

Plants are subjected to various abiotic stresses due to unfavorable environmental conditions that affect their growth, metabolism and yield. Drought is one of the major abiotic stress which limit the crop production in arid and semi arid tropics like India.

Chickpea yield is reputed to be most responsible when irrigated at flowering and pod filling. Water stress causes various physiological and biochemical effects on plants such as reduction of growth, decrease in chlorophyll, increase in hydrogen peroxide, increase in accumulation of osmolytes and levels of antioxidative enzymes.

One of the biochemical changes occurring when plants are subjected to stress condition is the accumulation of reactive oxygen species which are inevitable by products of normal cell. Plants have evaluated several mechanisms that allow perceiving the stress and rapidly regulating their physiological and metabolism to cope them. The antioxidant defense mechanism provides a strategy to enhance drought tolerance by increase the rate of ROS via enhanced electrolyte leakage in chloroplast and mitochondria. Plants with high levels of antioxidants either constitutive or induced have been reported to have great resistance to the oxidative damage.

In present investigation laboratory, pot and field studies were conducted and compared for various physiological

and biochemical aspects governing drought tolerance in chickpea crops. The results obtained in the present investigation are presented below

4.1. Screening of chickpea genotypes under polyethylene glycol (PEG 6000) induced osmotic stress

4.1.1 Seed germination

The effect of osmotic potential on germination percentage of chickpea genotypes is shown in Table 2 (a). The germination percentage was significantly affected by osmotic potential, resulting decrease in the osmotic potential from 0 to -0.8 MPa led to significant reduction in germination percentage and also the germination in all genotypes stopped completely in -0.8 MPa. The results indicated that genotypes responded differently to the osmotic potentials.

The per cent germination at control conduction ranged between 80 to 97.5 per cent. The genotypes Phule-G-06102 recorded the lowest germination (80 per cent) under control condition. The per cent germination at -0.2 MPa and -0.4 MPa were ranged between 45 to 95 per cent and 10 to 82.5 per cent, respectively. There was a considerable reduction in germination percentage at -0.6 MPa, only thirteen genotypes showed resistance to stress and germination ranged from 10 to 47.5 per cent. At osmotic potential of 0 (control), -0.2 MPa, -0.4 MPa and -0.6 MPa, the highest germination percentage was attained by Digvijay (97.5, 95.0, 82.5 and 47.5 per cent, respectively).

4.1.2 Root length (cm)

The root length was significantly decreased with decreasing osmotic potential in all the genotypes. The mean root length was 20.07, 13.99, 11.09 and 6.91 cm under 0, -0.2 MPa, -0.4 MPa and -0.6 MPa, respectively. As shown Table 2 (a), root length varied between 25.40 cm and 15.50 cm at control condition and 17.60 and 9.15 cm at -0.2 MPa osmotic stress.

There was a considerable reduction in root length at -0.4 MPa, which varied between 15.90 and 4.15 cm and 11.55 to 0 cm for -0.6 MPa. The genotype Phule-G 511-43-2 exhibited highest root length 15.90 and 11.15 cm at -0.4 MPa and -0.6 MPa, respectively. The lowest root length (4.15 cm) at -0.4 MPa osmotic stress was recorded by the genotype ICCV 11 117.

4.1.3 Shoot length (cm)

Shoot length was decreased significantly with decreasing osmotic potential as seen in Table 2 (a). The mean shoot length was progressively reduced from 17.36 cm at control to 7.61, 5.01 and 0.79 cm with decreasing osmotic potential -0.2 MPa, -0.4 MPa and -0.6 MPa, respectively. The highest shoot length was recorded by genotype ICC 4958 (11.65 and 8.05 cm) at -0.2 MPa and -0.4 MPa while the genotype Vijay recorded the highest shoot length (2.50 cm) at -0.6 MPa osmotic stress. Whereas, the genotypes ICCV 1117 (4.85 cm) and Phule-G-09103 (0.75 cm) recorded the lowest root length at -0.2 MPa and -0.4 MPa osmotic stress, respectively.

Table 2 a. Effect of PEG-6000-induced osmotic stress on germination (%), root and shoot length (cm) in Chickpea genotypes

SN	Genotype	Germination (%)				Root Length (cm)				Shoot Length (cm)			
		Control	-0.2 MPa	-0.4 MPa	-0.6 MPa	Control	-0.2 MPa	-0.4 MPa	-0.6 MPa	Control	-0.2 MPa	-0.4 MPa	-0.6 MPa
1.	RVSSG-10	85.0(67.2)	47.5(43.5)	25.0(30.0)	0	22.70	12.85	7.80	6.75	17.65	5.90	4.25	0
2.	JG 25	82.5(65.3)	70.0(56.7)	50.0(45.0)	0	23.90	14.70	11.00	7.10	20.75	5.65	4.20	0
3.	BG D 1069	95.0(63.6)	80.0(63.6)	60.0(50.7)	0	22.70	17.45	13.75	8.30	19.95	8.50	4.40	0
4.	JG-216	90.0(72.1)	77.5(61.7)	65.0(53.7)	0	22.15	9.15	9.00	8.80	20.05	5.80	4.45	0
5.	BGD 1070	90.0(72.1)	80.0(63.6)	67.5(55.2)	22.5(28.2)	21.65	12.60	12.25	6.85	19.50	7.90	3.75	1.40
6.	GJG 0814	95.0(77.0)	72.5(58.3)	65.0(53.7)	0	21.95	13.15	10.25	4.05	21.60	8.05	4.50	0
7.	GJG 0904	87.5(69.3)	60.0(50.8)	60.0(50.7)	0	18.75	14.40	12.25	7.30	16.25	6.10	4.65	0
8.	Phule G-0204-4	82.5(65.3)	77.5(61.7)	60.0(50.7)	0	20.65	14.85	12.50	7.20	18.30	8.35	6.85	0
9.	Phule G-0302-10	87.5(52.2)	72.5(58.3)	62.5(52.2)	0	19.65	13.60	9.90	4.30	16.60	10.15	5.75	0
10.	Phule G-0305-3	90.0(72.1)	72.5(58.3)	30.0(33.2)	0	16.05	13.25	11.50	5.40	12.85	7.25	5.85	0
11.	Phule G-0405-44-2	82.5(65.3)	67.5(55.2)	32.5(34.7)	0	21.00	13.10	6.50	5.55	18.60	6.65	2.40	0
12.	Phule G-0408-11-3	90.0(71.5)	82.5(65.3)	65.0(53.7)	15.0(22.5)	21.55	13.25	11.15	8.50	19.40	8.25	7.05	1.40
13.	Phule G-0408-14-1	95.0(77.0)	87.5 (69.3)	72.5(58.3)	32.5(34.7)	21.90	15.30	10.90	9.95	18.70	11.55	7.50	1.30
14.	Phule G-0511-43-2	95.0(77.0)	90.0(71.5)	75.0(60.1)	40.0(39.2)	21.75	16.60	15.90	11.15	18.50	8.50	6.50	2.00
15.	Phule G-0752	92.5(78.6)	92.5(74.3)	82.5(65.3)	37.5(37.7)	23.30	16.80	14.50	8.35	20.40	10.00	6.60	1.70
16.	Phule G-06102	80.0(63.4)	70.0(56.8)	45.0(42.1)	0	18.40	13.50	10.25	9.30	16.60	7.65	4.15	0
17.	Phule G-07101	82.5(65.3)	77.5(61.7)	52.5(46.4)	10.0(18.4)	15.65	13.65	13.50	6.65	13.90	8.40	2.95	0.60
18.	Phule G-07102	92.5(74.3)	85.0(67.2)	57.5(49.3)	0	17.80	14.15	12.90	6.15	16.40	5.75	3.80	0
19.	Phule G-08108	85.0(67.2)	47.5(43.5)	32.5(34.7)	0	17.65	13.75	10.50	4.00	15.50	7.00	3.50	0
20.	Phule G-09103	95.0(77.0)	60.0(50.7)	10.0(18.4)	0	19.90	14.25	6.70	0	18.50	7.75	0.75	0
21.	Phule G-09758	87.5(69.3)	70.0(56.8)	67.5(55.2)	12.5(20.6)	21.15	13.60	9.30	6.00	20.10	5.85	5.20	1.25
22.	Phule G-10114	85.0(67.2)	65.0(53.7)	47.5(43.5)	17.5(26.6)	17.75	12.50	8.25	6.55	16.15	6.90	4.25	1.80
23.	Phule G-02007-10	82.5(65.3)	45.0(42.1)	37.5(37.7)	0	21.25	15.35	11.05	7.30	18.45	6.85	6.40	0
24.	ICC 4958	92.5(74.3)	82.5(65.3)	67.5(55.2)	45.0(42.1)	18.15	17.60	14.20	7.40	16.20	11.65	8.05	1.35
25.	ICCV 11112	82.5(65.3)	62.5(52.2)	32.5(34.7)	0	22.35	14.15	10.70	0.00	17.00	6.95	3.85	0
26.	ICCV 11117	82.5(65.3)	65.0(53.7)	32.5(34.7)	0	15.55	10.45	4.15	1.85	13.55	4.85	2.79	0
27.	Vijay	95.0(77.0)	85.0(67.5)	67.5(55.2)	35.0(36.2)	18.55	15.20	13.60	8.35	17.05	7.70	7.10	2.50
28.	Vishal	85.0(67.2)	82.5(65.3)	72.5(58.3)	22.5(28.2)	25.40	16.00	14.00	11.55	17.40	5.50	7.20	1.20
29.	Digvijay	97.5(83.5)	95.0(77.0)	82.5(65.3)	47.5(43.5)	15.50	13.30	12.60	10.95	14.00	10.90	7.40	1.56
30.	Virat	82.5(65.3)	67.5(55.2)	65.0(53.7)	10.0(18.4)	17.50	11.25	11.90	5.15	10.40	6.00	4.25	1.60
	GM	88.0(70.5)	72.9(59.6)	54.8(47.8)	11.4(13.0)	20.07	13.99	11.09	6.91	17.36	7.61	5.01	0.79
	SE ±	3.20	1.95	1.87	1.42	0.53	0.40	0.36	0.28	0.65	0.26	0.21	0.52
	CD at (5%)	9.24	5.63	5.39	5.55	1.52	1.17	1.04	0.80	1.89	0.75	0.61	0.34
	CV (%)	6.41	4.62	5.45	15.52	3.71	4.09	4.60	5.64	5.33	4.80	5.99	8.8

*Figures in parenthesis indicates arc sine values

4.1.4 Root: shoot ratio

As the osmotic potential was decreased from 0 to -0.6 MPa, the root shoot ratio was increased (Table 2b). It varied between 1.02 and 1.68 at control, 1.32 and 2.91 at -0.2 MPa and 1.45 and 4.58 at -0.4 MPa osmotic potential. In higher osmotic potential (-0.6 MPa), it varied between 0 and 11.44. The genotype Phule G-7101 recorded the highest (4.58 and 11.44) root : shoot ratio at -0.4 MPa and -0.6 MPa osmotic potential.

4.1.5 Vigour index

In the study, the effect of osmotic potential on vigour index of genotypes was significant. Decreasing the osmotic potential caused remarkable decrease in vigour index. The mean vigour index was 32.96 at control and reduced to 1.18 at -0.16 MPa osmotic potential. The genotype Phule G-0752 demonstrated the highest vigour index (24.77, 17.42 and 4.16) followed by ICC 4958 (24.15, 15.03 and 3.93) and Vijay (19.44, 13.98 and 3.80) at -0.2 MPa, -0.4 MPa and -0.6 MPa osmotic potential, respectively. At -0.6 MPa osmotic stress, the genotype Phule G 0511-43 2 (5.20) recorded the highest vigour index.

4.1.6. Proline

The accumulation of proline was increased significantly in all the seedlings as the osmotic potential decreased from 0 to -0.6 MPa. The mean proline content 4.52 $\mu\text{moles g}^{-1}$ FW at control were increased to 6.02, 10.96 and 12.41 $\mu\text{moles g}^{-1}$ FW at -0.2, -0.4 and -0.6 MPa osmotic potential. The genotype Phule-G-0752 (14.09 $\mu\text{moles g}^{-1}$ FW) recorded the highest proline content at -0.4 MPa osmotic potential followed by Phule-G-0511-43-2

Table 2 b. Effect of PEG-6000-induced osmotic stress on root: shoot ratio, vigour index and proline content in Chickpea genotypes

SN	Genotype	Root : Shoot Ratio				Vigour Index				Proline (μ moles g ⁻¹ fw)			
		Control	-0.2 MPa	-0.4 MPa	-0.6 MPa	Control	-0.2 MPa	-0.4 MPa	-0.6 MPa	Control	-0.2 MPa	-0.4 MPa	-0.6 MPa
1.	RVSSG-10	1.29	2.18	1.84	-	34.30	8.88	3.01	-	4.66	5.56	9.43	-
2.	JG 25	1.15	2.60	2.62	-	36.83	14.25	7.60	-	3.77	6.47	10.45	-
3.	BG D 1069	1.14	2.05	3.13	-	40.52	20.78	10.89	-	4.96	8.25	9.80	-
4.	JG-216	1.10	1.58	2.02	-	37.94	11.59	8.76	-	5.85	8.13	11.89	-
5.	BGD 1070	1.25	1.83	3.27	4.93	26.02	16.40	5.21	1.86	5.11	6.27	10.00	12.69
6.	GJG 0814	1.02	1.63	2.28	-	41.37	15.39	9.58	-	3.61	4.14	10.55	-
7.	GJG 0904	1.15	2.36	2.63	-	30.60	12.31	10.14	-	4.02	4.81	8.15	-
8.	Phule G-0204-4	1.13	1.78	1.82	-	32.11	13.92	14.98	-	3.16	5.38	11.68	-
9.	Phule G-0302-10	1.18	1.34	1.72	-	31.71	14.83	11.34	-	3.56	5.73	10.73	-
10.	Phule G-0305-3	1.11	1.59	1.97	-	37.03	14.85	6.80	-	4.33	4.92	9.88	-
11.	Phule G-0405-44-2	1.10	1.81	2.71	-	28.01	13.10	5.94	-	3.54	6.72	7.34	-
12.	Phule G-0408-11-3	1.11	1.61	1.58	6.20	36.86	19.35	11.85	1.48	4.19	5.78	12.20	14.71
13.	Phule G-0408-14-1	1.14	1.32	1.45	8.04	35.52	22.17	13.36	3.54	4.28	4.77	11.61	14.57
14.	Phule G-0511-43-2	1.18	1.95	2.45	5.65	38.24	22.59	16.81	5.20	3.74	5.00	13.81	14.09
15.	Phule G-0752	1.14	1.68	2.20	4.94	40.36	24.77	17.42	4.16	4.21	4.80	14.09	17.21
16.	Phule G-06102	1.11	1.76	2.47	-	28.00	14.83	6.51	-	5.28	5.77	13.67	-
17.	Phule G-07101	1.13	1.63	4.58	11.44	24.36	17.12	8.62	0.73	3.52	5.27	8.90	11.13
18.	Phule G-07102	1.09	2.46	3.39	-	31.62	16.92	9.58	-	4.48	4.94	10.84	-
19.	Phule G-08108	1.14	1.96	3.00	-	28.18	9.86	4.55	-	4.57	5.94	11.75	-
20.	Phule G-09103	1.08	1.84	2.93	-	36.48	13.20	0.69	-	4.55	5.20	9.15	-
21.	Phule G-09758	1.05	2.32	1.79	4.80	36.06	13.59	9.79	0.91	5.58	6.32	11.56	12.74
22.	Phule G-10114	1.13	1.97	1.94	3.77	33.66	12.84	2.91	1.44	5.37	6.33	10.52	-
23.	Phule G-02007-10	1.15	2.24	1.73	-	32.74	9.99	6.55	-	5.38	5.76	11.85	-
24.	ICC 4958	1.12	1.51	1.76	5.48	31.76	24.15	15.03	3.93	5.13	9.21	13.10	17.13
25.	ICCV 11112	1.31	2.04	2.78	-	32.50	13.20	4.73	-	4.65	5.89	9.18	-
26.	ICCV 11117	1.15	2.15	1.49	-	24.00	9.95	2.25	-	4.32	4.95	7.98	-
27.	Vijay	1.09	1.97	1.92	3.34	33.82	19.44	13.98	3.80	4.57	9.26	12.67	18.84
28.	Vishal	1.46	2.91	1.94	6.42	36.38	17.73	15.37	2.46	5.30	6.75	11.73	14.71
29.	Digvijay	1.11	1.22	1.70	7.02	28.79	22.99	16.51	5.02	4.76	6.09	12.60	15.08
30.	Virat	1.68	1.88	2.80	3.22	23.04	11.63	10.50	0.68	5.38	6.16	11.69	11.97
	GM	1.17	1.91	2.33	3.00	32.96	15.75	9.37	1.18	4.52	6.02	10.96	12.41
	SE +	0.04	0.08	0.08	0.16	1.18	0.74	0.53	0.22	0.14	0.11	0.27	0.14
	CD at (5%)	0.13	0.22	0.24	0.47	3.41	2.13	1.53	0.65	0.41	0.31	0.77	0.39

(13.81 $\mu\text{moles g}^{-1}\text{FW}$). At -0.6 MPa osmotic potential, the genotypes Vijay (18.84 $\mu\text{moles g}^{-1}\text{FW}$) accumulated the highest proline content followed by the genotype Phule-G-0752 (17.21 $\mu\text{moles g}^{-1}\text{FW}$).

4.2 Pot Study

4.2.1 Effect of PEG-6000 induced osmotic stress on physiological parameters

4.2.1.1 Rate of photosynthesis

The rate of photosynthesis was recorded by using IRGA. The data on rate of photosynthesis under PEG-6000 induced osmotic stress at 50 per cent flowering in chickpea genotypes are presented in Table 3. The plants were subjected to extensive level of water stress of -0.4 MPa and -1.2 MPa, induced by polyethylene glycol-6000. PEG induced drought stress imposed to plants, significantly decreased rate as photosynthesis at stress level.

The genotype ICC 4958 ($14.31 \mu\text{mol m}^{-2} \text{s}^{-1}$) recorded the maximum rate of photosynthesis under control condition followed by ICCV 11112 ($13.49 \mu\text{mol m}^{-2} \text{s}^{-1}$). The genotype ICC 4958 recorded significantly highest rate of photosynthesis ($10.73 \mu\text{mol m}^{-2} \text{s}^{-1}$) under moderate stress followed by Phule G 0511-43-2 ($9.37 \mu\text{mol m}^{-2} \text{s}^{-1}$). The genotype ICCV 11112 recorded the lowest rate of photosynthesis ($1.27 \mu\text{mol m}^{-2} \text{s}^{-1}$) while the genotype ICC 4958 recorded the highest rate of photosynthesis ($3.59 \mu\text{mol m}^{-2} \text{s}^{-1}$) followed by Digvijay ($3.40 \mu\text{mol m}^{-2} \text{s}^{-1}$) under severe stress (-1.2 MPa).

Table 3. Effect of PEG-6000-induced osmotic stress on rate of photosynthesis ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$) at 50% flowering in susceptible and tolerant chickpea genotypes

Sr. No.	Genotypes	Rate of photosynthesis ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$)			
		Control	Moderate Stress (-0.4MPa)	Severe Stress (-1.2 MPa)	Mean
1	Phule G-0305-3	12.44	5.46	1.64	6.51
2	Phule G 0405-44-2	11.68	5.55	1.32	6.18
3	Phule G 09103	11.75	4.80	1.43	5.99
4	ICCV 11112	13.49	6.16	1.27	6.97
5	ICCV 11117	12.20	5.45	1.54	6.40
	Susceptible mean	12.31	5.48	1.44	6.41
6	Phule G 0511-43-2	13.24	9.37	2.95	8.52
7	Phule G 0752	12.08	8.18	2.66	7.64
8	ICC 4958	14.31	10.73	3.59	9.54
9	Vijay	12.19	8.71	3.28	8.06
10	Digvijay	13.22	9.06	3.40	8.56
	Tolerant mean	13.01	9.21	3.17	8.46
	Mean	12.66	7.35	2.31	7.44
			SE₊	CD at 5%	
	Condition		0.08	0.21	
	Genotype		0.04	0.13	
	Condition X Genotype		0.24	0.67	
	CV %			5.54	

4.2.1.2 Transpiration

The rate of transpiration under PEG-6000 induced osmotic stress recorded by IRGA are presented in Table 4. The significant variation in rate of transpiration among all the genotypes was observed under stress conditions as compared to control condition. As far as genotype is concerned, the tolerant genotype ICC 4958 recorded the lowest mean rate of transpiration ($1.69 \text{ m mol m}^{-2}\text{s}^{-1}$) followed by the Vijay ($1.77 \text{ m mol m}^{-2}\text{s}^{-1}$).

The genotype ICC 11117 ($5.84 \text{ m mol m}^{-2}\text{s}^{-1}$) recorded significantly highest rate of transpiration under control condition. The genotype ICC 4958 recorded the lowest rate of transpiration ($3.26 \text{ m mol m}^{-2}\text{s}^{-1}$) followed by the genotypes Vijay ($3.35 \text{ m mol m}^{-2} \text{ s}^{-1}$) under control condition. The genotype ICCV 1117 recorded the highest rate of transpiration ($2.36 \text{ m mol m}^{-2}\text{s}^{-1}$) followed by Phule G -09103 ($2.21 \text{ m mol m}^{-2}\text{s}^{-1}$) under moderate stress (-0.4 MPa). While lowest rate of transpiration under moderate stress was recorded by the genotype Phule G 0752 ($1.29 \text{ m mol m}^{-2} \text{ S}^{-1}$) followed by ICC 4958 ($1.39 \text{ m mol m}^{-2} \text{ s}^{-1}$). Under severe stress the lowest rate of transpiration was recorded by the genotype ICC 4958 ($0.43 \text{ m mol m}^{-2}\text{s}^{-1}$) followed by the genotype Vijay ($0.53 \text{ m mol m}^{-2}\text{s}^{-1}$).

4.2.1.3 Stomatal conductance

The stomatal conductance was recorded by IRGA at 50 % flowering are presented in Table 5. It was observed that, there was a considerable variation in stomatal conductance among all the genotype studied.

Table 4. Effect of PEG-6000-induced osmotic stress on mean rate of transpiration rate ($\text{m mol m}^{-2}\text{s}^{-1}$) at 50% flowering in susceptible and tolerant chickpea genotypes

Sr. No.	Genotypes	Transpiration rate ($\text{m mol m}^{-2}\text{s}^{-1}$)			
		Control	Moderate Stress (-0.4 MPa)	Severe Stress (-1.2 MPa)	Mean
1	Phule G-0305-3	4.87	2.06	1.17	2.70
2	Phule G 0405-44-2	4.77	1.90	1.08	2.58
3	Phule G 09103	4.89	2.21	1.01	2.70
4	ICCV 11112	3.26	1.74	0.94	1.98
5	ICCV 11117	5.84	2.36	1.20	3.13
	Susceptible mean	4.73	2.05	1.08	2.62
6	Phule G 0511-43-2	4.08	1.54	0.68	2.10
7	Phule G 0752	3.65	1.29	0.59	1.84
8	ICC 4958	3.26	1.39	0.43	1.69
9	Vijay	3.35	1.45	0.53	1.77
10	Digvijay	3.57	1.50	0.58	1.88
	Tolerant mean	3.58	1.43	0.56	1.86
	Mean	4.15	1.74	0.82	2.24
			SE₊	CD at 5%	
	Condition		0.025	0.070	
	Genotype		0.015	0.043	
	Condition X Genotype		0.078	0.22	
	CV %			6.05	

Table 5 . Effect of PEG-6000-induced osmotic stress on Stomatal conductance ($\text{mol m}^{-2}\text{sec}^{-1}$) at 50% flowering in susceptible and tolerant chickpea genotypes

Sr. No.	Genotypes	Stomatal conductance ($\text{mol m}^{-2}\text{sec}^{-1}$)			
		Control	Moderate Stress (-0.4 MPa)	Severe Stress (-1.2 MPa)	Mean
1	Phule G-0305-3	0.13	0.09	0.07	0.10
2	Phule G 0405-44-2	0.17	0.11	0.07	0.11
3	Phule G 09103	0.16	0.09	0.05	0.10
4	ICCV 11112	0.18	0.11	0.06	0.12
5	ICCV 11117	0.18	0.10	0.05	0.11
	Susceptible mean	0.16	0.10	0.06	0.11
6	Phule G 0511-43-2	0.15	0.09	0.08	0.11
7	Phule G 0752	0.18	0.12	0.09	0.13
8	ICC 4958	0.21	0.15	0.08	0.15
9	Vijay	0.22	0.15	0.11	0.16
10	Digvijay	0.19	0.15	0.08	0.14
	Tolerant mean	0.19	0.13	0.09	0.14
	Mean	0.18	0.12	0.07	0.12
			SE₊	CD at 5%	
	Condition			0.002	0.004
	Genotype			0.001	0.003
	Condition X Genotype			0.005	0.014
	CV %			6.81	

The genotype Vijay ($0.22 \text{ m mol m}^{-2}\text{s}^{-1}$) recorded the highest stomatal conductance followed by the genotype ICC 4958 ($0.21 \text{ m mol m}^{-2}\text{s}^{-1}$) under control condition. PEG 6000 induced water stress significantly which resulted in decrease stomatal conductance under stress conditions.

The highest stomatal conductance ($0.15 \text{ mol m}^{-2}\text{s}^{-1}$) under moderate stress was recorded by the genotypes ICC 4958, Vijay and Digvijay. The significantly highest stomatal conductance was recorded by the genotype Vijay ($0.11 \text{ m mol m}^{-2}\text{s}^{-1}$) under severe stress followed by the genotype Phule-G-0752 ($0.09 \text{ mol m}^{-2}\text{s}^{-1}$) while the lowest stomatal conductance ($0.05 \text{ mol m}^{-2}\text{s}^{-1}$) was recorded by the genotypes Phule G 09103 and ICCV 11117.

4.2.1.4 SCMR value or SPAD reading

The SCMR value or SPAD reading assessed by using chlorophyll meter (SPAD-502 plus, Minolta) and data are presented in Table 6. PEG induced drought stress to plants, which showed significantly decreased SCMR value at stress levels. The highest SCMR value were observed in all genotypes under control condition while both progressive stress of -0.04 MPa and -1.2 MPa caused significant reduction in SCMR value.

The genotype ICC 4958 recorded the maximum SCMR value (58.56) followed by Vijay (57.60) under control condition, whereas the genotype Phule G 0305-3 recorded the lowest (51.55) SCMR value. The tolerant genotype Vijay recorded the highest SCMR value (47.25 and 40.58) followed by the genotype ICC 4958 (46.64 and 38.87) under moderate (-0.4 MPa) and severe stress

Table 6 . Effect of PEG-6000 induced osmotic stress on SCMR Value at 50% flowering in the leaves of susceptible and tolerant chickpea genotypes

Sr. No.	Genotypes	SCMR value			
		Control	Moderate Stress (-0.4 MPa)	Severe Stress (-1.2 MPa)	Mean
1	Phule G-0305-3	51.65	36.09	29.41	39.05
2	Phule G 0405-44-2	54.61	38.81	32.99	42.14
3	Phule G 09103	52.05	35.17	26.90	38.04
4	ICCV 11112	55.41	41.84	29.14	42.13
5	ICCV 11117	54.20	39.44	33.52	42.39
	Susceptible mean	53.58	38.27	30.39	40.75
6	Phule G 0511-43-2	57.05	44.33	37.65	46.34
7	Phule G 0752	56.37	44.36	38.78	46.50
8	ICC 4958	58.56	46.64	38.87	48.02
9	Vijay	57.60	47.25	40.48	48.44
10	Digvijay	54.26	43.72	37.45	45.14
	Tolerant mean	56.77	45.26	38.65	46.89
	Mean	55.18	41.76	34.52	43.82
			SE₊	CD at 5%	
	Condition	0.36		1.01	
	Genotype	0.21		0.62	
	Condition X Genotype	1.13		3.22	
	CV %			4.50	

(-1.2 MPa), respectively. The lowest SCMR value was recorded by the susceptible genotype Phule G 09103 (26.90) under severe stress.

4.2.1.5 Relative leaf water content

The data on per cent relative leaf water content influenced by PEG 6000 induced osmotic stress in chickpea genotypes are presented in Table 7. The significant decrease in RLWC was observed in stressed leaves as compared to control.

The tolerant genotype Vijay recorded the highest (70.15 %) leaf water content followed by the genotype Digvijay (69.24 %) under control condition. The lowest relative leaf water content was recorded by genotype Phule G 0305-3 (53.53 %) while the genotype Vijay (64.38 %) recorded the highest RLWC under moderate stress. The sharp decrease in RLWC was noted under severe stress (-1.2 MPa). The lowest RLWC was observed in susceptible genotype Phule G-09103 (30.58 %). The tolerant genotype ICC 4958 (56.58%) recorded the highest RLWC followed by genotype Digvijay (53.35) under the severe stress (-1.2 MPa).

4.2.2 Effect of PEG-6000 induced osmotic stress on accumulation of osmolytes

4.2.2.1 Proline

The data on proline content in the leaves of chickpea genotypes under control, mild and severe osmotic stress induced by PEG 6000 are depicted in Table 8. Proline accumulation in the leaves were significantly increased under moderate (-0.4 MPa) and severe (-1.2 MPa) stress in all genotypes.

Table 7. Effect of PEG-6000 induced osmotic stress on Relative Leaf Water Content in the leaves of chickpea genotypes

Sr. No.	Genotypes	Relative Leaf Water Content (%)			
		Control	Moderate Stress (-0.4 MPa)	Severe Stress (-1.2 MPa)	Mean
1	Phule G-0305-3	64.24	53.53	36.61	51.46
2	Phule G 0405-44-2	65.61	57.07	33.85	52.18
3	Phule G 09103	68.32	56.87	30.58	51.93
4	ICCV 11112	65.56	57.41	34.59	52.52
5	ICCV 11117	64.63	54.96	39.34	52.98
	Susceptible mean	65.67	55.97	34.99	52.21
6	Phule G 0511-43-2	67.95	59.23	51.56	59.58
7	Phule G 0752	68.08	61.99	52.26	60.77
8	ICC 4958	69.11	62.05	56.58	62.58
9	Vijay	70.15	64.38	51.26	61.93
10	Digvijay	69.24	62.52	53.35	61.70
	Tolerant mean	68.91	62.03	53.00	61.31
	Mean	67.29	59.00	44.00	56.76
			SE_±	CD at 5%	
	Condition			0.42	1.18
	Genotype			0.25	0.72
	Condition X Genotype			1.32	3.73
	CV %			4.02	

Table 8. Effect of PEG-6000 induced osmotic stress on proline content in the leaves of susceptible and tolerant chickpea genotypes

Sr. No.	Genotypes	Proline (μ moles g^{-1} FW)			
		Control	Moderate Stress (-0.4 MPa)	Severe Stress (-1.2 MPa)	Mean
1	Phule G-0305-3	4.02	5.06	11.75	6.94
2	Phule G 0405-44-2	5.58	7.48	14.86	9.31
3	Phule G 09103	5.08	6.47	12.88	8.14
4	ICCV 11112	5.65	6.52	15.71	9.29
5	ICCV 11117	3.53	6.02	12.10	7.21
	Susceptible mean	4.77	6.31	13.46	8.18
6	Phule G 0511-43-2	6.04	9.94	18.10	11.36
7	Phule G 0752	6.02	9.77	20.44	12.07
8	ICC 4958	5.39	9.63	21.50	12.18
9	Vijay	4.59	9.86	22.98	12.47
10	Digvijay	5.30	9.60	21.15	12.02
	Tolerant mean	5.468	9.76	20.83	12.02
	Mean	5.12	8.03	17.15	10.10
			SE₊	CD at 5%	
	Condition			0.037	0.101
	Genotype			0.022	0.063
	Condition X Genotype			0.11	0.33
	CV %			1.99	

The tolerant genotype Phule G-0511-43-2 (9.94 $\mu\text{moles g}^{-1}$ FW) recorded highest proline accumulation followed by Vijay (9.86 $\mu\text{moles g}^{-1}$ FW) under moderate stress. The susceptible genotype Phule G 0305-3 recorded significantly lowest (5.06 and 11.75 $\mu\text{moles g}^{-1}$ FW) proline accumulation under moderate and severe stress, respectively. The significantly highest proline accumulation in the leaves was recorded by the tolerant genotype Vijay (22.98 $\mu\text{moles g}^{-1}$ FW) followed by the genotype ICC 4958 (21.50 $\mu\text{moles g}^{-1}$ FW) than rest of genotypes under severe stress (-1.2 MPa).

4.2.2.2 Glycine betaine

The data on glycine betaine content in the leaves of chickpea genotypes under control, mild and severe osmotic stress induced by PEG 6000 are depicted in Table 9. As the osmotic potential was decreased the glycine betaine content was significantly increased in moderate (-0.4 MPa) and severe stress (-1.2 MPa).

The significantly highest glycine betaine content was recorded by the tolerant genotype Phule-G-0511-43-2 (8.52 $\mu\text{moles g}^{-1}$ FW) under control condition while the susceptible genotype Phule-G-09103 recorded significantly lowest (4.09 $\mu\text{moles g}^{-1}$ FW) glycine betaine content under control condition. Under moderate stress (-0.04 MPa) the tolerant genotype Vijay (15.42 $\mu\text{moles g}^{-1}$ FW) recorded the significantly highest glycine betaine. The susceptible genotypes Phule-G-09103 accumulate significantly lowest (6.09 and 7.64 $\mu\text{moles g}^{-1}$ FW) glycine betaine under moderate and severe stress, respectively.

Table 9. Effect of PEG-6000 induced osmotic stress on glycine betaine content in the leaves of tolerant and susceptible chickpea genotypes

Sr. No.	Genotypes	Glycine betaine ($\mu\text{moles g}^{-1}$ FW)			
		Control	Moderate Stress (-0.4 MPa)	Severe Stress (-1.2 MPa)	Mean
1	Phule G-0305-3	4.94	7.22	10.16	7.44
2	Phule G 0405-44-2	4.77	6.56	9.30	6.88
3	Phule G 09103	4.09	6.09	7.64	5.94
4	ICCV 11112	5.83	8.35	10.26	8.14
5	ICCV 11117	6.14	8.30	12.43	8.96
	Susceptible mean	5.15	7.30	9.96	7.47
6	Phule G 0511-43-2	8.52	14.30	22.00	14.94
7	Phule G 0752	6.39	10.39	18.09	11.62
8	ICC 4958	7.39	12.65	25.16	15.07
9	Vijay	6.91	15.42	24.61	16.31
10	Digvijay	7.68	14.23	22.96	14.96
	Tolerant mean	7.78	13.40	22.56	14.58
	Mean	6.27	10.35	16.26	10.96
			SE₊	CD at 5%	
	Condition			0.06	0.16
	Genotype			0.04	0.10
	Condition X Genotype			0.18	0.51
	CV %			2.75	

The significantly highest glycine betaine content was recorded by tolerant genotype ICC 4958 (25.16 $\mu\text{moles g}^{-1}$ FW) under severe stress followed by the genotype Vijay (24.61 $\mu\text{moles g}^{-1}$ FW).

4.2.3 Effect of PEG-6000 -induced osmotic stress on antioxidative enzymes

4.2.3.1 Super oxide dismutase

The data on super oxide dismutase assay as influenced by PEG 6000 induced osmotic stress in chickpea genotypes are presented in Table 10. The SOD activity was increased as osmotic stress induced from moderate to severe stress. As far as genotype is concerned the tolerant genotype Digvijay recorded significantly highest mean SOD activity (142.65 units mg^{-1} protein) followed by the genotype Vijay (135.36 units mg^{-1} protein). The susceptible genotype ICCV 11 112 recorded the lowest (113.36 units mg^{-1} protein) SOD activity followed by Phule G-0305-3 (114.10 units mg^{-1} protein) under moderate stress condition. The SOD activity was significantly increased as PEG induced osmotic stress increased from moderate to severe stress. The lowest SOD activity was recorded by the susceptible genotype Phule G-09103 (163.89 units mg^{-1} protein) under severe stress. The tolerant genotype Digvijay recorded the significantly highest SOD activity (142.65 and 226.03 units mg^{-1} protein) under moderate (-0.4 MPa) and severe (-1.2 MPa) stress, respectively.

Table 10 .Effect of PEG-6000-induced osmotic stress on superoxide dismutase in the leaves of susceptible and tolerant chickpea genotypes

Sr. No.	Genotypes	Superoxide dismutase (units mg ⁻¹ protein)			
		Control	Moderate Stress (-0.4 MPa)	Severe Stress (-1.2 MPa)	Mean
1	Phule G-0305-3	72.24	114.10	167.97	118.10
2	Phule G 0405-44-2	71.25	119.74	172.53	121.17
3	Phule G 09103	69.06	121.47	163.89	118.14
4	ICCV 11112	63.49	113.36	165.56	114.14
5	ICCV 11117	70.63	117.50	174.14	120.76
	Susceptible mean	69.33	117.23	168.82	118.46
6	Phule G 0511-43-2	66.40	125.91	186.13	126.15
7	Phule G 0752	74.31	132.19	182.78	129.76
8	ICC 4958	67.71	128.70	206.88	134.43
9	Vijay	73.60	135.36	200.30	136.42
10	Digvijay	70.00	142.65	226.03	146.23
	Tolerant mean	70.4	132.96	200.42	134.6
	Mean	69.87	125.10	184.62	126.53
			SE_±	CD at 5%	
	Condition		0.39	1.11	
	Genotype		0.23	0.68	
	Condition X Genotype		1.24	3.52	
	CV %			1.70	

4.2.3.2 Catalase activity

Effect of PEG 6000 osmotic stress on catalase activity on the chickpea genotypes is depicted in Table 11. As the PEG induced osmotic stress increased from moderate to severe stress, the catalase activity in leaves of all genotypes was increased significantly.

The tolerant genotype Phule G 05111-43-2 recorded the significantly highest ($8.67 \mu \text{ mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$) under control condition. The susceptible genotype Phule G-0305 ($8.89 \mu \text{ mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$) recorded significantly lowest catalase activity in leaves under moderate stress. The significantly highest CAT activity was recorded by tolerant genotype Vijay ($15.36 \mu \text{ mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$) followed by Digvijay ($14.66 \mu \text{ mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$) under moderate stress. Under severe stress, the tolerant genotype ICC 4958 ($36.02 \mu \text{ mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$) recorded the significantly highest CAT activity in leaves followed by Vijay ($34.27 \mu \text{ mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$)

4.2.3.3 Ascorbate peroxidase activity

The ascorbate peroxidase activity influenced by PEG 6000 induced osmotic stress in chickpea leaves is shown in Table 12. It is observed from data that the ascorbate peroxidase activity was significantly increased under moderate and severe stress in all genotypes. Under control condition the genotype Phule-G-0752 ($345.16 \mu \text{ moles of ascorbate oxidized mg}^{-1} \text{ protein min}^{-1}$)

Table 11. Effect of PEG-6000-induced osmotic stress on catalase activity in the leaves of susceptible and tolerant chickpea genotypes

Sr. No.	Genotypes	Catalase (μ mole H_2O_2 decomposed mg^{-1} protein min^{-1})			
		Control	Moderate Stress (-0.4 MPa)	Severe Stress (-1.2 MPa)	Mean
1	Phule G-0305-3	6.32	8.89	20.71	11.97
2	Phule G 0405-44-2	6.37	9.76	17.87	11.33
3	Phule G 09103	7.75	10.05	22.29	13.36
4	ICCV 11112	6.87	10.58	25.03	14.16
5	ICCV 11117	7.52	11.66	24.76	14.65
	Susceptible mean	6.96	10.18	22.13	13.09
6	Phule G 0511-43-2	8.67	13.44	32.12	18.08
7	Phule G 0752	8.02	13.74	29.29	17.02
8	ICC 4958	7.79	12.88	36.02	18.90
9	Vijay	7.56	15.36	34.27	19.07
10	Digvijay	6.24	14.66	33.44	18.11
	Tolerant mean	7.66	14.02	33.03	18.24
	Mean	7.31	12.10	27.58	15.66
			SE₊	CD at 5%	
	Condition		0.046	0.13	
	Genotype		0.028	0.078	
	Condition X Genotype		0.14	0.41	
	CV %			1.59	

Table 12. Effect of PEG-6000 induced osmotic stress on ascorbate peroxidase in the leaves of susceptible and tolerant chickpea genotypes

Sr. No.	Genotypes	Ascorbate peroxidase (η moles of ascorbate oxidized mg^{-1} protein min^{-1})			
		Control	Moderate Stress (-0.4MPa)	Severe Stress (-1.2MPa)	Mean
1	Phule G-0305-3	226.78	459.32	589.03	425.04
2	Phule G 0405-44-2	151.04	321.30	459.41	310.58
3	Phule G 09103	233.28	452.12	598.19	427.86
4	ICCV 11112	281.70	431.33	740.47	484.50
5	ICCV 11117	198.49	304.05	577.54	360.03
	Susceptible mean	218.3	393.62	592.93	401.6
6	Phule G 0511-43-2	295.40	777.56	1122.79	731.92
7	Phule G 0752	345.16	1015.89	1264.63	875.23
8	ICC 4958	322.73	1015.51	1521.66	953.30
9	Vijay	289.70	888.83	1253.48	810.67
10	Digvijay	250.39	743.02	1048.95	680.79
	Tolerant mean	300.68	888.16	1242.30	810.38
	Mean	259.47	640.89	917.61	605.99
			SE₊	CD at 5%	
	Condition		2.06	5.82	
	Genotype		1.25	3.54	
	Condition X Genotype		6.51	18.40	
	CV %			1.85	

recorded significantly the highest APX activity. The susceptible genotype Phule-G-0405-44-2 recorded significantly lowest APX activity 151.04, 321.30 and 459.41 η moles of ascorbate oxidized mg^{-1} protein min^{-1} under control, moderate stress (- 0.4 MPa) and severe stress (-1.2 MPa), respectively.

The tolerant genotype Phule-G-0752 (1015.89 η moles of ascorbate oxidized mg^{-1} protein min^{-1}) recorded significantly highest APX activity than rest of other genotypes under moderate stress except Phule G-0752 (1015.51 η moles of ascorbate oxidized mg^{-1} protein min^{-1}). Under severe stress the significant highest APX activity was recorded by the genotype ICC 4958 (1521.66 η moles of ascorbate oxidized mg^{-1} protein min^{-1}).

4.3 Field Experiment

4.3.1 Effect of moisture stress on soil moisture status

The soil moisture content of the experiment plot at field capacity, permanent wilting point and available soil moisture was 41.69 and 17.84 and 23.85, respectively.

In field study each genotype was grouped in three sets *viz.*, irrigated, early drought stress and late drought stress. Normal plants were given three irrigators (at 20, 40 and 60 DAS) from the date of sowing to maturity. Early drought stressed or pre-anthesis drought stressed plants (EDS) received two irrigations (20 and 60 DAS) whereas late drought stress or post anthesis drought stressed plants (LDS) received two irrigations at 20 and 40 DAS. Under early drought stressed condition, there was a considerable depletion of available soil moisture at flowering

and as such the crop is exposed to pre-flowering stress. In late drought stress condition, the available moisture was reduced from flowering onwards and crop was exposed to the terminal drought.

The soil moisture status for the year 2012-13, under and year 2013-14 is presented in Table 13. Under normal condition, the scheduling irrigation was at critical growth stages and therefore the crop did not experienced moisture stress throughout the growth period. In the year 2012-13 under normal condition, the soil moisture content ranged between 23.50 to 34.50 per cent in 0-15 cm depth of soil, 24.20 to 36.30 per cent in 15-30 cm and 24.21 to 37.20 per cent in 30-45 cm depth of soil.

Under early drought stress condition, due to withholding of irrigation at 40 DAS, the soil moisture content at 45 DAS was declined to 19.10 per cent at 0-15 cm, soil depth, 21.40 and 22.30 per cent at 15-30 and 30-45 cm soil depth. Scheduled irrigation at 60 DAS, under EDS, increased the soil moisture content at 65 DAS and reached to 25.60, 29.80 and 31.20 per cent at 0-15, 15-20 and 30-45 cm depth of soil.

The late drought stressed plants received two irrigations at 20 and 40 DAS. The soil moisture content was 33.40, 35.60 and 39.50 per cent at 25 DAS at 0-15 cm, 15-30 and 30-45 cm soil depth, respectively. At 45 DAS, the soil moisture content was 33.20, 34.50 and 33.50 at 0-35, 15-30 and 30-45 cm depth of soil. Thereafter, it depleted and approached to 20.30, 21.80 and 23.40 at 65 DAS, respectively in the year 2012-13.

Table 13. Soil moisture status, available moisture and severity duration frequency (SDF)
Year : 2012-13

Stag- es	DAS	Soil Moisture Content (SMC %)			Available Moisture			Stress Duration Frequency (SDF)		
0-15 cm		Normal	EDS	LDS	Normal	EDS	LDS	Normal	EDS	LDS
1	25	34.50	33.60	33.40	16.66	15.76	15.56	0.17	0.19	0.20
2	45	32.30	19.10	33.20	14.46	1.26	15.36	0.23	0.54	0.20
3	65	29.30	25.60	20.30	11.46	7.76	2.46	0.30	0.39	0.51
4	100	23.50	23.20	18.10	5.66	5.36	0.26	0.44	0.44	0.57
15-30 cm										
1	25	36.30	34.60	35.60	18.46	16.76	17.76	0.13	0.17	0.15
2	45	35.20	21.40	34.50	17.36	3.56	16.66	0.16	0.49	0.17
3	65	30.20	29.80	21.80	12.36	11.96	3.96	0.28	0.29	0.48
4	100	24.20	22.80	18.80	6.36	4.96	0.96	0.42	0.45	0.55
30-45 cm										
1	25	37.20	38.40	39.50	19.36	20.56	21.66	0.11	0.08	0.05
2	45	36.50	22.30	33.50	18.66	4.46	15.66	0.12	0.47	0.20
3	65	34.80	31.20	23.40	16.96	13.36	5.56	0.17	0.25	0.44
4	100	24.21	23.4	19.2	6.36	5.56	1.36	0.42	0.44	0.54

Year : 2013-14

Stag- es	DAS	Soil Moisture Content (SMC %)			Available Moisture			Stress Duration Frequency (SDF)		
0-15 cm		Normal	EDS	LDS	Normal	EDS	LDS	Normal	EDS	LDS
1	25	33.60	32.50	32.40	15.76	14.66	14.56	0.19	0.22	0.22
2	45	31.50	18.70	31.80	13.66	0.86	13.96	0.24	0.55	0.24
3	65	29.20	26.40	18.80	11.36	8.56	0.96	0.30	0.37	0.55
4	100	22.50	22.20	17.96	4.66	4.36	0.12	0.46	0.47	0.57
15-30 cm										
1	25	34.50	33.30	33.40	16.66	15.46	15.56	0.17	0.20	0.20
2	45	34.50	20.20	32.90	16.66	2.36	15.06	0.17	0.52	0.21
3	65	30.40	27.20	19.70	12.56	9.36	1.86	0.27	0.35	0.53
4	100	23.50	23.20	18.40	5.66	5.36	0.56	0.44	0.44	0.56
30-45 cm										
1	25	36.40	35.52	36.60	18.56	17.68	18.76	0.13	0.15	0.12
2	45	35.60	22.40	33.40	17.76	4.56	15.56	0.15	0.46	0.20
3	65	34.30	29.80	22.20	16.46	11.96	4.36	0.18	0.29	0.47
4	100	24.10	23.30	18.90	6.26	5.46	1.06	0.42	0.44	0.55

EDS : Early Drought Stress

LDS : Late Drought Stress

Field Capacity : 41.69%

Permanent Wilting point : 17.84%

Under normal condition, the available moisture was ranged from 5.66 to 16.65, 6.36 to 18.46 and 6.36 to 19.36 at 0-15, 15-30 and 30-45 cm soil depth, respectively. Under early drought stress condition available moisture at 45 DAS was 1.26, 3.56 and 4.46 per cent at 0-15, 15-30 and 30-45 cm depth of soil. While, under late drought stress condition it was 2.46, 3.96 and 5.56 per cent at 65 DAS at 0-15, 15-30 and 30-45 cm soil depth, respectively.

Similar trend was observed during the year 2013-14 for the available moisture content. Water stress imposed at pre flowering stage by withholding of irrigation results in decline in available moisture to 0.86, 2.36 and 4.56 per cent at 45 DAS at 0-15, 15-30 and 30-45 cm soil depth, respectively. The SDF value in lower in normal condition ranged between 0.17 to 0.44 and 0.19 to 0.46 in 0-15 cm depth, 0.13 to 0.42 and 0.17 to 0.44 in 15-30 cm depth and 0.11 to 0.42 and 0.13 to 0.42 in 30-45 cm depth of soil, in the year 2012-13 and 2013-14, respectively. In early drought stress condition the SDF value are 0.54, 0.49 and 0.47 in 0-15, 15-30 and 30-45 cm soil depth at 45 DAS. While in late drought stress condition, the SDF values were 0.51, 0.48 and 0.44 and 0-15 cm, 15-30 cm and 30-45 cm depth of soil at 65 DAS in the year 2012-13. Similar trend in SDF value was observed during the year 2013-14.

4.3.2 Effect of moisture stress on phenological parameters

4.3.2.1 Days to 50 % flowering

The data on the days to 50 % flowering as influenced by moisture stress in chickpea genotypes are presented in Table 14. A considerable variation was observed for days to 50 % flowering among all the genotypes studied. The genotypes over the season and the interactions were statistically significant for both the year.

In the year 2012-13, the genotypes Phule-G-09103 required the minimum number of days (43.33) for 50 % flowering under irrigated condition followed by ICCV-11117 (44.00). The genotype Phule-G-09103 and Vijay required minimum number of days (37.67) to 50 % flowering under early drought stress condition.

In the year 2013-14, the genotype Vijay (44.33) required minimum number of days to 50 % flowering followed by the genotype Phule-G-0752 (44.67) under unstressed condition. Under early drought stress condition the genotype Vijay required minimum number of days (39.00) for 50 per cent flowering followed by genotype Phule-G-09103 (39.33).

On the basis of pooled analysis it is revealed that significantly minimum number of days were required for 50 % flowering in all genotypes under stress. Under early drought stress condition the genotype Vijay (38.33) required minimum number of days followed by Phule G 09103 (38.50).

Table 14: Days to 50% flowering as influenced by moisture stress in Chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	49.33	41.67	49.00	46.67	49.00	41.00	51.67	47.22	49.17	41.33	50.33	46.94
2	Phule G 0405-44-2	48.33	43.00	48.33	46.56	49.33	44.00	49.33	47.56	48.83	43.50	48.83	47.06
3	Phule G 09103	43.33	37.67	44.00	41.67	46.67	39.33	45.67	43.89	45.00	38.50	44.83	42.78
4	ICCV 11112	48.33	42.00	47.00	45.78	47.67	43.00	46.33	45.67	48.00	42.50	46.67	45.72
5	ICCV 11117	44.00	38.33	44.67	42.33	47.33	41.00	46.67	45.00	45.67	39.67	45.67	43.67
	Susceptible Mean	46.67	40.53	46.60	44.60	48.00	41.67	47.93	45.87	47.33	41.10	47.27	45.23
6	Phule G 0511-43-2	48.00	43.33	48.00	46.44	48.67	42.00	47.67	46.11	48.33	42.67	47.83	46.28
7	Phule G 0752	44.33	39.67	45.00	43.00	44.67	39.67	47.00	43.78	44.50	39.67	46.00	43.39
8	ICC 4958	48.33	42.33	48.33	46.33	50.67	44.00	49.00	47.89	49.50	43.17	48.67	47.11
9	Vijay	44.67	37.67	43.00	41.78	44.33	39.00	44.00	42.44	44.50	38.33	43.50	42.11
10	Digvijay	46.67	42.33	47.67	45.56	47.67	43.00	47.67	46.11	47.17	42.67	47.67	45.83
	Tolerant mean	46.40	41.07	46.40	44.62	47.20	41.53	47.07	45.27	46.80	41.30	46.73	44.94
	Mean	46.53	40.80	46.50	44.61	47.60	41.60	47.50	45.57	47.07	41.20	47.00	45.09
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		0.12	0.33			0.11	0.32			0.14	0.39	
	Genotypes (G)		0.25	0.64			0.24	0.68			0.29	0.84	
	Interaction (G X C)		0.42	1.21			0.42	1.19			0.52	1.45	

While the genotype Phule-G-0405-44-2 (48.83), required highest number of days to 50 % flowering under unstressed normal condition.

4.3.2.2 Days to maturity

The data pertaining to days to maturity as influenced by moisture stress in chickpea genotype are depicted in Table 15. The differences amongst the genotype over the season and their interactions were statistically significant for both the year 2012-13 and 2013-14.

In the year 2012-13, the genotype Phule G 0305-3 required minimum number of days to maturity (106 and 98) followed by the genotype Phule G-0752 (106.33 and 98.33) under normal and late drought stress conditions, respectively. The genotype ICCV 11112 and Vijay required maximum days (111) to maturity under normal condition.

The genotype Phule G 305-3 required minimum number of days to maturity (108) under normal condition while the genotype Phule G 09103 (113.33) required maximum number of days to maturity under unstress condition in the year 2013-14.

On the basis of pooled of two year the genotypes Phule G 0752 required minimum number of days to maturity (106.83) under normal condition, whereas the genotypes Phule G-0305-3 required minimum number of days to maturity (99.33) under late drought stress condition.

Table 15 : Days to maturity as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	106.00	105.67	98.00	103.22	108.00	109.00	100.67	105.89	107.00	107.33	99.33	104.56
2	Phule G 0405-44-2	109.00	107.33	99.00	105.11	112.00	110.33	105.67	109.33	110.50	108.83	102.33	107.22
3	Phule G 09103	109.67	110.67	101.00	107.11	113.33	112.33	105.00	110.22	111.50	111.50	103.00	108.67
4	ICCV 11112	111.00	110.00	102.00	107.67	109.00	102.33	100.00	103.78	110.00	106.17	101.00	105.72
5	ICCV 11117	107.33	105.33	101.00	104.56	111.67	109.00	103.00	107.89	109.50	107.17	102.00	106.22
	Susceptible Mean	108.60	107.80	100.20	105.53	110.80	108.60	102.87	107.42	109.70	108.20	101.53	106.48
6	Phule G 0511-43-2	107.00	106.00	99.00	104.00	109.67	107.67	103.00	106.78	108.33	106.83	101.00	105.39
7	Phule G 0752	106.33	101.67	98.33	102.11	107.33	107.00	102.33	105.56	106.83	104.33	100.33	103.83
8	ICC 4958	110.33	107.67	106.67	108.22	111.00	108.00	105.00	108.00	110.67	107.83	105.83	108.11
9	Vijay	111.00	109.33	107.00	109.11	111.33	112.00	104.67	109.33	111.17	110.67	105.83	109.22
10	Digvijay	110.33	112.33	102.00	108.22	110.33	109.33	104.33	108.00	110.33	110.83	103.17	108.11
	Tolerant mean	109.00	107.40	102.60	106.33	109.93	108.80	103.87	107.53	109.47	108.10	103.23	106.93
	Mean	108.80	107.60	101.40	105.93	110.37	108.70	103.37	107.48	109.58	108.15	102.38	106.71
			SE_±	CD(5%)			SE_±	CD(5%)	CV(%)		SE_±	CD(5%)	
	Conditions (C)		0.46	1.31			0.27	0.76			0.46	1.29	
	Genotypes (G)		0.61	1.72			0.55	1.57			0.71	1.99	
	Interaction (G X C)		1.05	2.98			0.46	2.72	1.54		1.23	NS	

4.3.3 Effect of moisture stress on physiological parameters

4.3.3.1 Photosynthetic rate at 50 per cent flowering

The results on rate of photosynthesis at 50 % flowering influenced by moisture stress in chickpea genotypes are depicted in Table 16. The rate of photosynthesis was decreased significantly at 50 % flowering in all genotypes under early drought stress, due to withholding of irrigation at 40 DAS.

The rate of photosynthesis at 50 % flowering was higher in genotype Digvijay ($18.71 \mu \text{ mol m}^{-2}\text{s}^{-1}$) followed by the genotype Phule G-0511-43-2 ($18.06 \mu \text{ mol m}^{-2}\text{s}^{-1}$) under normal condition, during the year 2012-13. The genotype Digvijay ($17.62 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded significantly highest rate of photosynthesis under early drought stress. The susceptible genotype, Phule G-09103 recorded the significantly lowest rate of photosynthesis ($10.46 \mu \text{ mol m}^{-2}\text{s}^{-1}$) under early drought stress condition except ICCV 11112 ($10.76 \mu \text{ mol m}^{-2}\text{s}^{-1}$).

During the year 2013-14, the genotype Phule G-0511-43-2 ($18.72 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest rate of photosynthesis followed by the genotype Digvijay ($18.63 \mu \text{ mol m}^{-2}\text{s}^{-1}$) under normal condition. The tolerant genotype Digvijay recorded significantly highest rate of photosynthesis ($17.73 \mu \text{ mol m}^{-2}\text{s}^{-1}$) under early drought stress followed by the genotype Phule G-0511-43-2 ($16.13 \mu \text{ mol m}^{-2}\text{s}^{-1}$).

Overall on the basis of pooled analysis it was observed that at 50 per cent flowering the tolerant genotype Digvijay

Table 16 : Mean rate of photosynthesis ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$) at 50 % flowering as influenced by moisture stress in Chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	17.41	12.63	16.52	15.52	16.32	10.54	13.50	13.45	16.87	11.59	15.01	14.49
2	Phule G 0405-44-2	17.41	12.99	16.55	15.65	16.63	11.56	14.82	14.33	17.02	12.27	15.68	14.99
3	Phule G 09103	15.50	10.46	14.74	13.57	14.74	12.01	16.42	14.39	15.12	11.24	15.58	13.98
4	ICCV 11112	17.15	10.76	15.32	14.41	17.64	12.21	17.04	15.63	17.39	11.48	16.18	15.02
5	ICCV 11117	15.97	12.29	14.25	14.17	17.39	10.70	15.40	14.49	16.68	11.50	14.83	14.33
	Susceptible Mean	16.69	11.83	15.48	14.66	16.54	11.40	15.43	14.46	16.62	11.62	15.46	14.56
6	Phule G 0511-43-2	18.06	14.61	17.41	16.69	18.72	16.13	17.54	17.47	18.39	15.37	17.48	17.08
7	Phule G 0752	17.49	13.34	17.21	16.01	16.59	14.20	16.32	15.70	17.04	13.77	16.77	15.86
8	ICC 4958	17.49	15.22	16.43	16.38	17.53	14.61	16.51	16.22	17.51	14.92	16.47	16.30
9	Vijay	16.78	15.75	18.89	17.14	16.36	14.68	16.75	15.93	16.57	15.22	17.82	16.53
10	Digvijay	18.71	17.62	20.97	19.10	18.63	17.73	19.31	18.56	18.67	17.68	20.14	18.83
	Tolerant mean	17.70	15.31	18.18	17.06	17.57	15.47	17.29	16.78	17.63	15.39	17.73	16.92
	Mean	17.20	13.57	16.83	15.86	17.05	13.44	16.36	15.62	17.13	13.50	16.59	15.74
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.22	0.62			0.15	0.43			0.23	0.65	
	Genotypes (G)		0.36	1.01			0.31	0.89			0.41	1.15	
	Interaction (G X C)		0.63	1.83			0.54	1.54			0.71	1.99	

recorded the highest (20.14 and 17.68 $\mu \text{ mol m}^{-2}\text{s}^{-1}$) under unstressed condition and early drought stress condition, respectively.

4.3.3.2 Photosynthesis at grain filling stage

The rate of photosynthesis at grain filling stage was influenced by moisture stress in chickpea genotypes are depicted in Table 17. The rate of photosynthesis was decreased significantly at grain filling stage in all genotypes under late drought stress, due to withholding irrigation at 60 DAS.

In the year 2012-13, the rate of photosynthesis at grain filling stage was highest in genotype ICC 4958 (13.38 $\mu \text{ mol m}^{-2}\text{s}^{-1}$) followed by the genotype Phule G 0511-43-2 (13.25 $\mu \text{ mol m}^{-2}\text{s}^{-1}$) under normal condition. The genotype Phule-G-0405-44-2 recorded lowest (8.35 and 4.45 $\mu \text{ mol m}^{-2}\text{s}^{-1}$) rate of photosynthesis at under early and late drought stress, respectively. While, the tolerant genotype Digvijay recorded the highest (10.54 and 7.00 $\mu \text{ mol m}^{-2}\text{s}^{-1}$) rate of photosynthesis at grain filling stage under early and late drought stress condition, respectively.

Similar trend was observed in the year 2013-14, the tolerant genotype Digvijay recorded the highest (13.23 and 6.58 $\mu \text{ mol m}^{-2}\text{s}^{-1}$) rate of photosynthesis under normal and late drought stress condition at grain filling stage. The lowest rate of photosynthesis was recorded by the susceptible genotype Phule G-0405-44-2 (10.07 and 4.02 $\mu \text{ mol m}^{-2}\text{s}^{-1}$) under normal and late drought stress condition, respectively. Under late drought stress

Table 17 : Mean rate of photosynthesis ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$) at grain filling stage as influenced by moisture stress in Chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	12.83	9.22	5.34	9.13	12.35	9.22	4.86	8.81	12.59	9.22	5.10	8.97
2	Phule G 0405-44-2	10.57	8.35	4.45	7.79	10.07	8.32	4.02	7.47	10.32	8.34	4.24	7.63
3	Phule G 09103	12.31	9.49	4.60	8.80	11.11	9.15	3.93	8.06	11.71	9.32	4.26	8.43
4	ICCV 11112	10.62	9.16	4.58	8.12	11.48	8.44	4.16	8.03	11.05	8.80	4.37	8.07
5	ICCV 11117	12.40	9.67	4.49	8.85	10.60	8.38	4.04	7.67	11.50	9.02	4.27	8.26
	Susceptible Mean	11.75	9.18	4.69	8.54	11.12	8.70	4.20	8.01	11.44	8.94	4.45	8.27
6	Phule G 0511-43-2	13.25	10.08	4.55	9.30	11.99	8.81	4.76	8.52	12.62	9.45	4.66	8.91
7	Phule G 0752	11.06	9.49	6.51	9.02	11.56	9.60	5.12	8.76	11.31	9.54	5.81	8.89
8	ICC 4958	13.38	10.37	6.15	9.97	13.09	9.46	5.11	9.22	13.23	9.92	5.63	9.59
9	Vijay	13.14	10.15	6.77	10.02	12.75	9.61	5.40	9.25	12.95	9.88	6.09	9.64
10	Digvijay	12.95	10.54	7.00	10.16	13.23	10.19	6.58	10.00	13.09	10.36	6.79	10.08
	Tolerant mean	12.76	10.13	6.20	9.69	12.52	9.53	5.39	9.15	12.64	9.83	5.80	9.42
		12.25	9.65	5.44	9.12	11.82	9.12	4.80	8.58	12.04	9.38	5.12	8.85
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		0.08	0.22			0.14	0.39			0.13	0.38	
	Genotypes (G)		0.17	0.48			0.15	0.42			0.19	0.55	
	Interaction (G X C)		0.28	0.83			0.25	0.73			0.28	0.89	

the susceptible genotype Phule G 09103 recorded the lowest rate of photosynthesis ($3.93 \mu \text{ mol m}^{-2}\text{s}^{-1}$) at grain filling stage under late drought stress (post flowering stress).

Overall on the basis of pooled analysis it was observed that at grain filling stage the tolerant genotype Digvijay recorded the highest (10.36 and $6.79 \mu \text{ mol m}^{-2}\text{s}^{-1}$) under early and late drought stress condition, respectively.

4.3.3.3 Transpiration rate at 50 per cent flowering

The rate of transpiration at 50 % flowering was influenced by moisture stress in chickpea genotypes is presented in Table 18. The rate transpiration was decreased significantly at 50 % flowering in all genotypes under early drought stress.

The genotype ICCV 11117 recorded significantly highest rate of transpiration ($7.13 \text{ m mol m}^{-2}\text{s}^{-1}$) at 50 % flowering than rest of genotypes studied under normal condition, during the year 2012-13. The lowest rate of transpiration was recorded by the genotype Phule-G-0405-44-2 ($4.14 \text{ m mol m}^{-2}\text{s}^{-1}$) at 50 per cent flowering under normal condition. At pre flowering stress or early drought stress the rate of transpiration was significantly reduced in all the genotypes. The tolerant genotype, Digvijay recorded the lowest rate of transpiration ($3.19 \text{ m mol m}^{-2}\text{s}^{-1}$) followed by the genotype Vijay ($3.60 \text{ m mol m}^{-2}\text{s}^{-1}$) under early drought stress condition.

During the year 2013-14, the genotype Phule G-0405 44-2 ($6.36 \text{ m mol m}^{-2}\text{s}^{-1}$) recorded significantly highest rate of transpiration under normal condition, while the tolerant genotype

Table 18 : Mean rate of transpiration ($\text{m mol m}^{-2}\text{s}^{-1}$) at 50 % flowering as influenced by moisture stress in Chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	5.25	4.43	4.70	4.79	5.51	4.88	4.84	5.08	5.38	4.66	4.77	4.93
2	Phule G 0405-44-2	4.14	3.92	4.94	4.33	6.36	4.77	5.43	5.52	5.25	4.34	5.19	4.93
3	Phule G 09103	4.85	4.75	5.46	5.02	5.00	4.90	4.98	4.96	4.92	4.83	5.22	4.99
4	ICCV 11112	5.62	4.98	5.73	5.44	5.18	3.89	5.45	4.84	5.40	4.44	5.59	5.14
5	ICCV 11117	7.13	5.01	6.05	6.06	5.79	4.96	6.16	5.64	6.46	4.99	6.11	5.85
	Susceptible Mean	5.40	4.62	5.38	5.13	5.57	4.68	5.37	5.21	5.48	4.65	5.38	5.17
6	Phule G 0511-43-2	4.47	4.44	4.48	4.47	4.45	3.58	4.77	4.27	4.46	4.01	4.63	4.37
7	Phule G 0752	5.23	4.23	4.25	4.57	5.18	3.65	5.21	4.68	5.20	3.94	4.73	4.63
8	ICC 4958	5.00	4.36	5.17	4.84	4.68	3.26	5.44	4.46	4.84	3.81	5.31	4.65
9	Vijay	4.66	3.60	4.53	4.26	4.58	3.35	5.56	4.50	4.62	3.48	5.05	4.38
10	Digvijay	4.99	3.19	4.48	4.22	3.69	2.95	3.60	3.41	4.34	3.07	4.04	3.81
	Tolerant mean	4.87	3.96	4.58	4.47	4.52	3.36	4.92	4.26	4.69	3.66	4.75	4.37
		5.13	4.29	4.98	4.80	5.04	4.02	5.15	4.74	5.09	4.15	5.06	4.77
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		0.03	0.06			0.08	0.22			0.07	0.19	
	Genotypes (G)		0.11	0.30			0.10	0.29			0.13	0.36	
	Interaction (G X C)		0.18	0.52			0.19	0.56			0.22	0.62	

Digvijay recorded the lowest ($3.69 \text{ m mol m}^{-2}\text{s}^{-1}$) rate of transpiration. Under early drought stress, the tolerant genotype Digvijay ($2.95 \text{ m mol m}^{-2}\text{s}^{-1}$) recorded the lowest rate of transpiration followed by the genotype ICC 4958 ($3.26 \text{ m mol m}^{-2}\text{s}^{-1}$).

On pooled basis, the tolerant genotype Digvijay recorded significantly lowest ($3.07 \text{ m mol m}^{-2}\text{s}^{-1}$) rate of transpiration under early drought stress condition. The genotype ICCV 11117 recorded the highest rate of transpiration ($4.99 \text{ m mol m}^{-2}\text{s}^{-1}$) under early drought stress condition

4.3.3.4 Transpiration rate at grain filling stage

The data on mean rate of transpiration at grain filling stage was influenced by moisture stress in chickpea genotypes are depicted in Table 19. The significant reduction in rate of transpiration was observed in all genotypes on both years under early and late drought stress condition as compared to normal condition.

The rate of transpiration at grain filling stage was significantly highest in genotype Phule-G-0305-3 ($4.85 \text{ m mol m}^{-2}\text{s}^{-1}$) while, the genotype ICC 4958 recorded the lowest ($3.35 \text{ m mol m}^{-2}\text{s}^{-1}$) rate of transpiration under normal condition during the year 2012-13. The tolerant genotype ICC 4958 recorded significantly lowest rate of ($2.41 \text{ m mol m}^{-2}\text{s}^{-1}$) except Phule G-0511-43-2 ($2.46 \text{ m mol m}^{-2}\text{s}^{-1}$) under early drought stress condition. The tolerant genotype Vijay ($1.64 \text{ m mol m}^{-2}\text{s}^{-1}$) recorded significantly lowest rate of transpiration at grain filling

Table 19 : Mean rate of transpiration ($\text{m mol m}^{-2}\text{s}^{-1}$) at grain filling stage as influenced by moisture stress in Chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	4.85	3.52	2.74	3.71	5.42	3.63	2.61	3.89	5.13	3.58	2.68	3.80
2	Phule G 0405-44-2	4.04	3.54	2.61	3.40	4.38	4.37	2.93	3.89	4.21	3.95	2.77	3.65
3	Phule G 09103	3.63	3.44	2.43	3.16	3.84	3.79	3.39	3.68	3.74	3.62	2.91	3.42
4	ICCV 11112	3.46	3.31	2.53	3.10	4.54	3.78	3.33	3.88	4.00	3.55	2.93	3.49
5	ICCV 11117	4.17	3.75	2.38	3.43	4.87	4.73	3.64	4.41	4.52	4.24	3.01	3.92
	Susceptible Mean	4.03	3.51	2.54	3.36	4.61	4.06	3.18	3.95	4.32	3.79	2.86	3.66
6	Phule G 0511-43-2	3.97	2.46	2.77	3.06	4.01	2.88	2.70	3.20	3.99	2.67	2.73	3.13
7	Phule G 0752	4.05	3.88	1.83	3.25	3.73	2.92	1.69	2.78	3.89	3.40	1.76	3.02
8	ICC 4958	3.35	2.41	2.00	2.59	3.19	3.73	2.02	2.98	3.27	3.07	2.01	2.78
9	Vijay	3.40	3.75	1.64	2.93	3.55	2.64	2.60	2.93	3.48	3.19	2.12	2.93
10	Digvijay	4.17	2.78	2.33	3.10	3.63	2.44	2.49	2.85	3.90	2.61	2.41	2.97
	Tolerant mean	3.79	3.06	2.11	2.99	3.62	2.92	2.30	2.95	3.71	2.99	2.21	2.97
		3.91	3.28	2.33	3.17	4.12	3.49	2.74	3.45	4.01	3.39	2.53	3.31
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.017	0.048			0.036	0.102			0.034	0.096	
	Genotypes (G)		0.072	0.206			0.073	0.207			0.089	0.250	
	Interaction (G X C)		0.13	0.36			0.14	0.40			0.16	0.46	

stage under late drought stress condition except the genotype Phule G 0752 ($1.83 \text{ m mol m}^{-2}\text{s}^{-1}$).

In the year, 2013-14, the susceptible genotype Phule-G-0305-3 recorded significantly highest ($5.42 \text{ m mol m}^{-2}\text{s}^{-1}$) rate of transpiration under normal condition, while the genotype ICC 4958 ($3.19 \text{ m mol m}^{-2}\text{s}^{-1}$) recorded the lowest rate of transpiration at grain filling stage. Under early drought stress condition the genotype Digvijay ($2.44 \text{ m mol m}^{-2}\text{s}^{-1}$) recorded the lowest rate of transpiration at grain filling stage followed by the genotype Vijay ($2.64 \text{ m mol m}^{-2}\text{s}^{-1}$). The tolerant genotype Phule G 0752 recorded the significantly lowest ($1.69 \text{ m mol m}^{-2}\text{s}^{-1}$) rate of transpiration at maturity except the genotype ICC 4958 ($2.02 \text{ m mol m}^{-2}\text{s}^{-1}$) under late drought (post flowering) stress condition at grain filling stage. On the basis of pooled data, on an average the tolerant genotype ICC 4958 ($2.78 \text{ m mol m}^{-2}\text{s}^{-1}$) recorded the lowest rate of transpiration at grain filling stage.

4.3.3.5 Stomatal conductance at 50 per cent flowering

The data on the stomatal conductance at 50 % flowering as influenced by moisture stress in chickpea genotype are depicted in Table 20. The difference among the genotypes over the years and their interaction were statistically significant for both the year 2012-13 and 2013-14.

In the year 2012-13, the genotype Digvijay ($0.26 \text{ mol m}^{-2}\text{s}^{-1}$) recorded the maximum stomatal conductance under normal condition at 50 % flowering followed by the genotype ICC 4958 ($0.25 \text{ mol m}^{-2}\text{s}^{-1}$). Under early drought stress the tolerant

Table 20 : Mean stomatal conductance (mol m⁻²s⁻¹) at 50 % flowering as influenced by moisture stress in Chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	0.24	0.16	0.26	0.22	0.24	0.16	0.23	0.21	0.24	0.16	0.24	0.21
2	Phule G 0405-44-2	0.23	0.19	0.23	0.21	0.21	0.14	0.23	0.19	0.22	0.17	0.23	0.20
3	Phule G 09103	0.20	0.15	0.21	0.19	0.22	0.16	0.24	0.21	0.21	0.16	0.23	0.20
4	ICCV 11112	0.23	0.18	0.22	0.21	0.21	0.15	0.22	0.19	0.22	0.16	0.22	0.20
5	ICCV 11117	0.22	0.19	0.22	0.21	0.21	0.16	0.22	0.20	0.22	0.17	0.22	0.20
	Susceptible Mean	0.23	0.17	0.23	0.21	0.22	0.15	0.23	0.20	0.22	0.16	0.23	0.20
6	Phule G 0511-43-2	0.24	0.20	0.22	0.22	0.26	0.21	0.25	0.24	0.25	0.20	0.24	0.23
7	Phule G 0752	0.23	0.20	0.22	0.22	0.24	0.19	0.24	0.22	0.23	0.19	0.23	0.22
8	ICC 4958	0.25	0.21	0.23	0.23	0.26	0.22	0.25	0.24	0.25	0.22	0.24	0.24
9	Vijay	0.24	0.21	0.25	0.23	0.25	0.20	0.25	0.23	0.25	0.21	0.25	0.23
10	Digvijay	0.26	0.21	0.25	0.24	0.23	0.20	0.23	0.22	0.25	0.21	0.24	0.23
	Tolerant mean	0.24	0.21	0.23	0.23	0.25	0.20	0.24	0.23	0.25	0.21	0.24	0.23
		0.23	0.19	0.23	0.22	0.23	0.18	0.24	0.22	0.23	0.18	0.23	0.22
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		0.005	0.013			0.003	0.008			0.005	0.013	
	Genotypes (G)		0.005	0.015			0.006	0.016			0.007	0.019	
	Interaction (G X C)		0.01	0.03			0.01	0.03			0.01	0.03	

genotypes ICC 4958, Vijay and Digvijay ($0.21 \text{ mol m}^{-2}\text{s}^{-1}$) maintained the maximum stomatal conductance.

In the year 2013-14, the tolerant genotypes Phule G-0511-43-2 and ICC 4958 recorded maximum stomatal conductance ($0.26 \text{ mol m}^{-2}\text{s}^{-1}$) under normal condition. The genotypes ICC 4958 exhibited maximum ($0.22 \text{ mol m}^{-2}\text{s}^{-1}$) stomatal conductance under early drought stress condition followed by the genotype Phule G-0511-43-2 ($0.21 \text{ mol m}^{-2}\text{s}^{-1}$).

On the basis of pooled analysis, it is observed that the stomatal conductance was significantly reduced in all the genotypes under early drought stress condition. Under early drought stress condition the tolerant genotype ICC 4958 ($0.22 \text{ mol m}^{-2}\text{s}^{-1}$) recorded the maximum stomatal conductance followed by the genotype Vijay and Digvijay ($0.21 \text{ mol m}^{-2}\text{s}^{-1}$). However, the susceptible genotype Phule G-0305-3 ($0.16 \text{ mol m}^{-2}\text{s}^{-1}$) recorded the lowest stomatal conductance as 50 % flowering under early drought stress condition.

4.3.3.6 Stomatal conductance at grain filling stage

The stomatal conductance was recorded by IRGA. The data on the stomatal conductance at grain filling stage as influenced by moisture stress in chickpea genotypes are presented in Table 21. The differences among the genotypes over the season and their interaction were statistically significant for both the years, 2012-13 and 2013-14.

The genotype Vijay ($0.17 \text{ mol m}^{-2}\text{s}^{-1}$) recorded significantly the highest stomatal conductance under normal

Table 21: Mean stomatal conductance (mol m⁻²s⁻¹) at grain filling stage as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	0.13	0.08	0.07	0.09	0.14	0.10	0.07	0.10	0.13	0.09	0.07	0.10
2	Phule G 0405-44-2	0.11	0.07	0.05	0.08	0.12	0.09	0.09	0.10	0.12	0.08	0.07	0.09
3	Phule G 09103	0.12	0.12	0.06	0.10	0.13	0.09	0.07	0.10	0.13	0.10	0.07	0.10
4	ICCV 11112	0.09	0.08	0.07	0.08	0.11	0.08	0.04	0.08	0.10	0.08	0.06	0.08
5	ICCV 11117	0.10	0.09	0.07	0.09	0.13	0.07	0.08	0.09	0.11	0.08	0.08	0.09
	Susceptible Mean	0.11	0.09	0.07	0.09	0.13	0.08	0.07	0.09	0.12	0.09	0.07	0.09
6	Phule G 0511-43-2	0.14	0.11	0.11	0.12	0.15	0.11	0.12	0.13	0.15	0.11	0.11	0.12
7	Phule G 0752	0.13	0.11	0.10	0.11	0.14	0.13	0.11	0.13	0.13	0.12	0.10	0.12
8	ICC 4958	0.14	0.12	0.12	0.13	0.13	0.11	0.10	0.11	0.14	0.11	0.11	0.12
9	Vijay	0.17	0.16	0.15	0.16	0.15	0.14	0.13	0.14	0.16	0.15	0.14	0.15
10	Digvijay	0.14	0.12	0.11	0.12	0.14	0.14	0.12	0.13	0.14	0.13	0.11	0.13
	Tolerant mean	0.14	0.12	0.12	0.13	0.14	0.13	0.11	0.13	0.14	0.12	0.12	0.13
		0.13	0.11	0.09	0.11	0.13	0.11	0.09	0.11	0.13	0.11	0.09	0.11
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		0.002	0.005			0.001	0.008			0.002	0.005	
	Genotypes (G)		0.003	0.008			0.003	0.008			0.003	0.010	
	Interaction (G X C)		0.005	0.013			0.005	0.014			0.006	0.017	

condition at grain filling stage in the year 2012-13. The same genotype Vijay (0.16 and $0.15 \text{ mol m}^{-2}\text{s}^{-1}$) recorded significantly highest stomatal conductance at grain filling stage under early and late drought stress condition, respectively. The genotype Phule G-0405-44-2 ($0.05 \text{ mol m}^{-2}\text{s}^{-1}$) recorded the lowest stomatal conductance under late drought stress condition.

In the year 2013-14, the genotype Phule-G-0511-42-3 and Vijay ($0.15 \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest stomatal conductance at grain filling stage under unstressed condition. Under early and late drought stress the genotype Vijay (0.14 and $0.13 \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest stomatal conductance at grain filling stage followed by the genotype Digvijay (0.14 and $0.12 \text{ mol m}^{-2}\text{s}^{-1}$).

On the basis of pooled analysis, at grain filling stage the tolerant genotype Vijay recorded the highest (0.16 , 0.15 and $0.14 \text{ mol m}^{-2}\text{s}^{-1}$) stomatal conductance at normal, early and late drought stress condition followed by the genotype Digvijay (0.14 , 0.13 and $0.11 \text{ mol m}^{-2}\text{s}^{-1}$). The susceptible genotype ICCV 11 112 ($0.06 \text{ mol m}^{-2}\text{s}^{-1}$) recorded the lowest stomatal conductance at grain filling stage under late drought stress condition.

4.3.3.7 SCMR value at 50 per cent flowering

The SPAD chlorophyll meter reading (SCMR) is an indicator of the photo-synthetically active transmittance characteristics of the leaf, which is dependent on the unit amount of chlorophyll per unit leaf area. Table 22 depicted that SCMR value at 50 per cent flowering was reduced by moisture stress in

Table 22 : Mean SCMR values at 50 % flowering as influenced by moisture stress in Chickpea Genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	57.68	41.37	59.33	52.79	56.06	42.16	56.24	51.49	56.87	41.77	57.79	52.14
2	Phule G 0405-44-2	60.35	37.80	61.81	53.32	58.45	43.89	57.09	53.14	59.40	40.84	59.45	53.23
3	Phule G 09103	53.33	36.10	52.33	47.26	54.33	38.36	55.56	49.42	53.83	37.23	53.95	48.34
4	ICCV 11112	59.84	43.88	57.83	53.85	61.66	41.00	58.50	53.72	60.75	42.44	58.17	53.79
5	ICCV 11117	58.12	42.29	55.77	52.06	62.28	44.95	60.62	55.95	60.20	43.62	58.19	54.00
	Susceptible Mean	57.87	40.29	57.41	51.86	58.56	42.07	57.60	52.74	58.21	41.18	57.51	52.30
6	Phule G 0511-43-2	63.46	44.03	64.48	57.32	60.80	46.04	61.49	56.11	62.13	45.04	62.98	56.72
7	Phule G 0752	61.16	50.57	61.53	57.75	61.89	49.00	60.47	57.12	61.52	49.78	61.00	57.44
8	ICC 4958	64.12	56.03	62.32	60.82	62.10	51.67	59.10	57.62	63.11	53.85	60.71	59.22
9	Vijay	62.30	57.07	62.73	60.70	60.54	50.50	60.28	57.11	61.42	53.79	61.51	58.90
10	Digvijay	65.18	58.66	63.70	62.51	64.00	58.05	63.00	61.68	64.59	58.35	63.35	62.10
	Tolerant mean	63.24	53.27	62.95	59.82	61.87	51.05	60.87	57.93	62.55	52.16	61.91	58.88
	Mean	60.55	46.78	60.18	55.84	60.21	46.56	59.24	55.34	60.38	46.67	59.71	55.59
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.50	1.44			0.52	1.49			0.63	1.75	
	Genotypes (G)		0.87	2.48			0.98	2.79			1.15	3.18	
	Interaction (G X C)		1.51	4.30			1.70	4.83			1.96	5.52	

chickpea genotypes. The early drought stress resulted in significantly reduction in SCMR value for both the year 2012-13 and 2013-14. The difference among the genotype over the year and the interaction with moisture stress condition were statistically significant.

In the year 2012-13, under normal condition the genotype Digvijay (65.18) recorded the highest SCMR value at 50 % flowering followed by ICC 4958 (64.12). The genotype Digvijay (58.66) demonstrated the highest SCMR value at 50 % flowering under early drought stress condition followed by Vijay (57.07). While the genotype Phule G 09103 (36.10) recorded the lowest SCMR value under early drought stress.

In the year 2013-14 the genotype Digvijay (64.00 and 58.05) recorded the highest SCMR value at 50 % flowering followed by the genotype ICCV 4958 (62.10 and 51.67) under normal and early drought stress condition, respectively.

On the basis of pooled analysis, the tolerant genotype Digvijay (58.35) recorded the highest SCMR value at 50 per cent flowering under early drought stress condition followed by ICC 4958 (53.85). However, the susceptible genotype Phule-G-09103 (37.23) recorded the lowest SCMR value at 50 per cent flowering under early drought stress condition.

4.3.3.8 SCMR value at grain filling stage

The data on SCMR value at grain filling stage as influenced by moisture stress in chickpea genotypes are presented in Table 23. It was evident from the table that, there was

considerable variation in SCMR value among all genotype studies. The differences amongst the genotypes over the year and their interaction with moisture stress condition were statistically significant.

In the year 2012-13 the tolerant genotype Digvijay (58.60) recorded the maximum SCMR value under normal condition at grain filling stage followed by the genotype Vijay (56.83). Under early drought stress, the significantly highest SCMR value was recorded by the genotype Digvijay (52.68) except ICC 4958 (49.56). At grain filling stage under late drought stress condition the genotype Digvijay (49.43) recorded the highest SCMR value. The susceptible genotype Phule-G-0305-3 (30.20) recorded significantly lowest SCMR value at grain filling stage under late drought stress.

In the year 2013-14 the genotype Digvijay recorded the highest SCMR value (56.15 and 51.01) under normal and early drought stress condition at grain filling stage. The genotype Vijay (47.33) recorded the highest SCMR value under late drought stress followed by Digvijay (46.70). The significantly lowest SCMR value was recorded by the susceptible genotype Phule -G-0305-3 (32.91) under late drought stress condition.

On the basis of pooled analysis, the genotype Digvijay (57.37, 52.15 and 48.07) recorded the maximum SCMR value under normal, early and late drought stress condition at grain filling stage. The genotypes Phule-G-0305-3 (31.56) recorded the significantly lowest SCMR value at grain filling stage under late drought stress condition.

Table 23 : Mean SCMR values at pod filling as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	52.28	41.85	30.20	41.44	51.49	36.13	32.91	40.18	51.88	38.99	31.56	40.81
2	Phule G 0405-44-2	55.38	47.09	42.41	48.29	52.67	45.00	43.00	46.89	54.03	46.05	42.70	47.59
3	Phule G 09103	55.57	46.27	43.00	48.28	51.52	40.33	37.14	43.00	53.54	43.30	40.07	45.64
4	ICCV 11112	56.23	45.61	40.81	47.55	52.72	45.67	39.33	45.91	54.47	45.64	40.07	46.73
5	ICCV 11117	55.60	48.00	41.91	48.50	56.29	50.33	42.41	49.68	55.95	49.17	42.16	49.09
	Susceptible Mean	55.01	45.76	39.66	46.81	52.94	43.49	38.96	45.13	53.97	44.63	39.31	45.97
6	Phule G 0511-43-2	55.37	45.93	41.52	47.61	53.77	50.52	41.58	48.62	54.57	48.23	41.55	48.12
7	Phule G 0752	51.06	44.35	43.23	46.21	51.28	49.29	42.21	47.59	51.17	46.82	42.72	46.90
8	ICC 4958	54.79	49.59	48.88	51.09	53.26	48.88	44.15	48.76	54.02	49.24	46.52	49.92
9	Vijay	56.83	46.08	43.53	48.81	55.44	50.33	47.33	51.04	56.14	48.21	45.43	49.93
10	Digvijay	58.60	52.68	49.43	53.57	56.15	51.61	46.70	51.48	57.37	52.15	48.07	52.53
	Tolerant mean	55.33	47.73	45.32	49.46	53.98	50.13	44.40	49.50	54.65	48.93	44.86	49.48
	Mean	55.17	46.75	42.49	48.14	53.46	46.81	41.68	47.32	54.31	46.78	42.08	47.73
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		0.18	0.51			0.44	1.26			0.41	1.16	
	Genotypes (G)		2.08	2.76			1.90	2.53			2.40	3.18	
	Interaction (G X C)		1.26	3.59			1.15	3.29			1.48	4.16	

4.3.3.9 APAR at 50 % flowering

The data on absorbed photosynthetically active radiation absorbance values at 50 per cent flowering as influenced by the moisture stress in chickpea genotypes are depicted in Table 24.

In the year 2012-13 under normal condition at 50 per cent flowering stage the genotype Digvijay ($994.4 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded maximum absorbed PAR followed by the genotype ICCV 4958 ($954.2 \mu \text{ mol m}^{-2}\text{s}^{-1}$). The genotype Digvijay ($894.1 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest absorbed PAR under early drought stress. Under late drought stress condition at 50 per cent flowering the genotype ICC 4958 ($976.1 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest absorbed PAR followed by Digvijay ($927.7 \mu \text{ mol m}^{-2}\text{s}^{-1}$).

In the year 2013-14, the genotype Vijay recorded the highest absorbed PAR (1009.9 , 890.3 and $999.8 \mu \text{ mol m}^{-2}\text{s}^{-1}$) under normal, early and late drought stress condition, respectively. The genotype, Phule G-0405-44-2 ($633.2 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the lowest absorbed PAR under early drought stress condition at 50 per cent flowering.

On the basis of average of two year the genotypes ICC 4958 ($975.30 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest absorbed PAR at 50 per cent flowering stage under normal condition. Under early drought stress condition at 50 per cent flowering stage the genotype ICC 4958 ($879.35 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest absorbed PAR followed by the genotype Digvijay ($862.45 \mu \text{ mol m}^{-2}\text{s}^{-1}$).

Table 24 : Mean absorbed photo synthetically active radiation absorbance values ($\ll \text{mol m}^{-2}\text{s}^{-1}$) at 50% flowering as influenced by the moisture stress in chickpea genotype

SN	Year / Genotype	2012-13				2013-14				Average			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	849.4	761.1	859.2	823.23	944.3	897	989.4	943.57	896.85	829.05	924.30	883.40
2	Phule G 0405-44-2	906.6	810.1	915.3	877.33	946.9	633.2	817.8	799.30	926.75	721.65	866.55	838.32
3	Phule G 09103	912.1	831.7	914.3	886.03	928.1	683.4	985.5	865.67	920.10	757.55	949.90	875.85
4	ICCV 11112	926.8	834.3	928.2	896.43	950.9	692.4	977.1	873.47	938.85	763.35	952.65	884.95
5	ICCV 11117	944.3	848.4	934.6	909.10	955.3	678	939	857.43	949.80	763.20	936.80	883.27
	Susceptible Mean	907.84	817.1	910.32	878.43	945.10	716.80	941.7	867.89	926.47	766.96	926.04	873.16
6	Phule G 0511-43-2	936.1	788.2	946	890.10	926.5	852.1	936.7	905.10	931.30	820.15	941.35	897.60
7	Phule G 0752	880.8	779.3	859.4	839.83	1013.7	686.3	986.1	895.37	947.25	732.80	922.75	867.60
8	ICC 4958	954.2	871.4	976.1	933.90	996.4	887.3	959.2	947.63	975.30	879.35	967.65	940.77
9	Vijay	872.9	821.7	871.3	855.30	1009.9	890.3	999.8	966.67	941.40	856.00	935.55	910.98
10	Digvijay	994.4	894.1	972.7	953.73	946.1	830.8	957.4	911.43	970.25	862.45	965.05	932.58
	Tolerant mean	927.68	830.9	925.10	894.57	978.52	829.36	967.8	925.24	953.10	830.15	946.47	909.91
	Mean	917.76	824.0	917.71	886.50	961.81	773.08	954.8	896.56	939.79	798.56	936.26	891.53

4.3.3.10 APAR at grain filling stage

The data on absorbed photosynthetically active radiation absorbance values at grain filling stage as influenced by the moisture stress in chickpea genotypes are depicted in the Table 25.

In the year 2012-13 under normal condition at grain filling stage the genotype Phule-G-0752 ($848.7 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded maximum absorbed PAR followed by the genotype ICCV 11112 ($835.1 \mu \text{ mol m}^{-2}\text{s}^{-1}$). The genotype Vijay ($788.4 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest absorbed PAR under early drought stress. Under late drought stress the genotype ICCV 11117 ($701.0 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest absorbed PAR followed by Vijay ($694.3 \mu \text{ mol m}^{-2}\text{s}^{-1}$) at grain filling stage.

In the year 2013-14, the genotype Phule G-0511-43-2 recorded the highest absorbed PAR (530.1 , 507.2 and $425.4 \mu \text{ mol m}^{-2} \text{ s}^{-1}$) under normal condition, early and late drought stress condition, respectively. The genotype, Phule G-09103 ($334.4 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the lowest absorbed PAR under late drought stress condition at grain filling stage.

On the basis of average of two year the genotypes Phule G-0752 ($667.8 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest absorbed PAR at grain filling stage under normal condition. The genotype Phule G 0511-43-2 ($611.3 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest absorbed PAR under early drought stress. Under late drought stress condition at grain filling stage the genotype Vijay ($544.8 \mu \text{ mol m}^{-2}\text{s}^{-1}$) recorded the highest absorbed PAR.

Table 25 : Mean absorbed photo synthetically active radiation absorbance values ($\ll \text{mol m}^{-2}\text{s}^{-1}$) at grain filling as influenced by the moisture stress in chickpea genotype

SN	Year / Genotype	2012-13				2013-14				Average			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	725.7	628.5	607.2	653.8	466.8	356.9	397.2	407.0	596.3	492.7	502.2	530.4
2	Phule G 0405-44-2	802.3	775.1	606.3	727.9	519.4	471.3	386.2	459.0	660.9	623.2	496.3	593.4
3	Phule G 09103	745.2	717.4	579.8	680.8	459.3	356.7	334.4	383.5	602.3	537.1	457.1	532.1
4	ICCV 11112	835.1	756.0	699.4	763.5	446.0	384.8	355.7	395.5	640.6	570.4	527.5	579.5
5	ICCV 11117	802.3	759.9	701.0	754.4	425.4	352.9	370.0	382.8	613.9	556.4	535.5	568.6
	Susceptible Mean	782.1	727.4	638.7	716.1	463.4	384.5	368.7	405.5	622.8	555.9	503.7	560.8
6	Phule G 0511-43-2	763.8	715.3	603.3	694.1	530.1	507.2	425.4	487.6	646.9	611.3	514.4	590.8
7	Phule G 0752	848.7	730.1	613.1	730.6	486.9	478.2	380.6	448.6	667.8	604.2	496.8	589.6
8	ICC 4958	779.3	722.3	676.7	726.1	459.8	482.9	361.2	434.6	619.6	602.6	519.0	580.4
9	Vijay	814.6	788.4	694.3	765.8	466.8	410.7	395.3	424.3	640.7	599.5	544.8	595.0
10	Digvijay	719.2	716.9	630.4	688.8	468.1	475.4	409.9	451.1	593.7	596.2	520.1	570.0
	Tolerant mean	785.1	734.6	643.6	721.1	482.3	470.9	394.5	449.2	633.7	602.7	519.0	585.2
	Mean	783.6	731.0	641.2	718.6	472.9	427.7	381.6	427.4	628.2	579.3	511.4	573.0

4.3.3.11 Relative water content (RLWC) at 50 per cent flowering

The data on relative water content (RLWC) in the chickpea genotypes as influenced by moisture stress are depicted in Table 26. The pre flowering stressed leaves showed corresponding decline in RLWC over unstressed control in the leaves of all the chickpea genotypes.

In the year 2012-13 the genotype Vijay recorded the highest (73.65 %) relative water content followed by the genotype Phule G-09103 (72.95) under normal condition at 50 per cent flowering. The tolerant genotype ICC 4958 (65.92 %) recorded the highest relative water content under early drought stress condition. Significantly lowest RLWC was recorded by susceptible genotype ICCV 11117 (50.29 %) under early drought stress.

In 2013-14 under normal condition the genotype Vijay (72.94 %) recorded the highest RLWC contact followed by ICC 4958 (71.90 %) while under early drought stress condition, the genotype Digvijay (67.59 %) maintained the highest RLWC followed by ICC 4958 (66.12 %).

On the basis of pooled analysis, the genotype ICC 4958 recorded the highest (72.77) RLWC at 50 per cent flowering under normal condition followed by Vijay (72.01). Under early drought stress, the tolerant genotype Digvijay maintained the highest (66.67 %) RLWC followed by ICC 4958 (66.02 %). The susceptible genotype ICCV 11117 recorded the significantly lowest (51.48 %) RLWC under early drought stress condition at 50 per cent flowering.

Table 26 : Mean per cent relative water content at 50 % flowering as influenced by moisture stress in Chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	66.96	58.68	66.76	64.14	66.92	56.14	65.99	63.02	66.94	57.41	66.38	63.58
2	Phule G 0405-44-2	61.94	54.07	65.01	60.34	65.55	56.97	65.58	62.70	63.74	55.52	65.30	61.52
3	Phule G 09103	72.95	59.16	67.98	66.70	68.29	57.09	66.99	64.12	70.62	58.13	67.49	65.41
4	ICCV 11112	66.14	55.42	63.69	61.75	64.45	55.33	63.27	61.02	65.30	55.37	63.48	61.38
5	ICCV 11117	63.07	50.29	61.94	58.43	61.34	52.68	60.64	58.22	62.21	51.48	61.29	58.33
	Susceptible Mean	66.21	55.53	65.08	62.27	65.31	55.64	64.49	61.82	65.76	55.58	64.79	62.04
6	Phule G 0511-43-2	69.85	64.20	71.10	68.38	66.14	61.80	68.13	65.36	67.99	63.00	69.62	66.87
7	Phule G 0752	70.87	65.67	70.99	69.18	67.20	62.38	70.89	66.82	69.04	64.02	70.94	68.00
8	ICC 4958	71.81	65.92	70.55	70.04	71.90	66.12	70.13	69.38	72.77	66.02	70.34	69.71
9	Vijay	73.65	65.28	71.81	69.39	72.94	65.80	70.62	69.79	72.01	65.54	71.21	69.59
10	Digvijay	70.68	65.74	69.86	68.76	70.37	67.59	69.99	69.32	70.52	66.67	69.92	69.04
	Tolerant mean	71.23	65.36	70.86	69.15	69.71	64.74	69.95	68.13	70.47	65.05	70.41	68.64
	Mean	68.72	60.44	67.97	65.71	67.51	60.19	67.22	64.97	68.12	60.32	67.60	65.34
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.25	0.69			0.32	0.90			0.35	0.97	
	Genotypes (G)		0.63	1.79			0.54	1.52			0.72	2.01	
	Interaction (G X C)		1.09	3.11			0.93	2.64			1.03	2.85	

4.3.3.12 Relative water content at grain filling stage

The data pertaining to relative water content at maturity as influenced by moisture stress in chickpea genotype are depicted in Table 27. At grain filling stage progressive trend of significant decline in RLWC was observed in both season in early and late drought stressed leaves of chickpea genotypes.

In the year 2012-13 the tolerant genotype Vijay recorded the highest RLWC (69.62%) at grain filling stage under normal condition followed by the genotype Digvijay (69.41%). Under late drought stress the genotype Vijay (66.64 %) recorded the highest RLWC at grain filling stage followed by the genotype Digvijay (65.26%).

In the year 2013-14 the genotype Digvijay recorded the highest (68.97 %) RLWC followed by the genotype Phule G-0511-43-2 (68.53 %) at grain filling stage. Under early drought stress the genotype Digvijay recorded the highest RLWC (66.26 %) at grain filling stage followed by Phule-G-0511-43-2 (65.98 %). At grain filling stage under late drought stress the highest RLWC was recorded by the genotype Phule-G-0511 43-2 (63.89%). While the susceptible genotype Phule G-0405-44-2 (53.40 %) recorded at lowest RLWC at grain filling stage under late drought stress.

On the basis of pooled analysis at grain filling stage the tolerant genotype Digvijay recorded the highest RLWC (69.19 %) under normal condition. While the genotype Vijay maintained the highest RLWC (66.70 and 64.96 %) at early and late drought

Table 27: Mean per cent relative water content at grain filing stage as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	65.19	62.15	55.79	61.04	63.95	61.80	54.41	60.05	64.57	61.97	55.10	60.55
2	Phule G 0405-44-2	65.13	60.97	57.96	61.35	62.75	57.75	53.40	57.97	63.94	59.36	55.68	59.66
3	Phule G 09103	60.81	57.37	54.67	57.61	64.53	60.16	57.04	60.58	62.67	58.76	55.85	59.10
4	ICCV 11112	63.41	60.11	55.52	59.68	62.06	59.47	54.06	58.53	62.74	59.79	54.79	59.10
5	ICCV 11117	66.12	63.56	59.39	63.02	60.82	59.00	56.23	58.68	63.47	61.28	57.81	60.85
	Susceptible Mean	64.13	60.83	56.67	60.54	62.82	59.64	55.03	59.16	63.48	60.23	55.85	59.85
6	Phule G 0511-43-2	68.06	65.76	63.34	65.72	68.53	65.98	63.89	66.13	68.29	65.87	63.62	65.93
7	Phule G 0752	67.76	64.27	62.66	64.89	67.84	65.28	63.57	65.56	67.80	64.78	63.11	65.23
8	ICC 4958	68.04	65.40	65.21	66.22	64.26	63.50	62.91	63.56	66.15	64.45	64.06	64.89
9	Vijay	69.62	68.08	66.64	68.11	66.63	65.31	63.28	65.07	68.12	66.70	64.96	66.59
10	Digvijay	69.41	66.65	65.26	67.11	68.97	66.26	63.81	66.35	69.19	66.46	64.54	66.73
	Tolerant mean	68.58	66.03	64.62	66.41	67.25	65.27	63.49	65.33	67.91	65.65	64.06	65.87
	Mean	66.35	63.40	60.65	63.48	65.04	62.45	59.26	62.25	65.69	62.94	59.95	62.86
			SE+	CD(5%)			SE+	CD(5%)			SE+	CD(5%)	
	Conditions (C)		0.27	0.78			0.30	0.85			0.35	0.99	
	Genotypes (G)		0.46	1.31			0.41	1.30			0.56	NS	
	Interaction (G X C)		0.76	2.27			0.79	2.27			0.97	2.44	

stress condition, respectively. The susceptible genotype ICCV 11112 (54.79 %) recorded the lowest RLWC at grain filling stage.

4.3.4 Effect of moisture stress on morphological parameters

4.3.4.1 Plant height (cm) at 50 per cent flowering

The data on plant height (cm) as influenced by moisture stress in chickpea genotypes are presented in Table 28. A considerable variation for plant height (cm) was observed among all genotypes studied. The differences among genotypes over the season and their interactions were statistically significant for both the year 2012-13 and 2013-14.

In the year, 2012-13 the genotype Digvijay (43.70 cm) recorded the significantly highest plant height except Phule G 0752 (43.40 cm) under normal condition at 50 per cent flowering. Under early drought stress condition the genotype Digvijay (40 cm) maintained highest plant height at 50 per cent flowering. The genotype Phule G -0305-3 recorded the lowest (32.70 and 29.27 cm) plant height under normal and early drought stress condition, respectively.

In the year 2013-14, at 50 per cent flowering the genotype Phule G 0405-44-2 recorded significantly highest (47.70 and 43.70 cm) plant height at 50 per cent flowering under normal and early drought stress condition, respectively. Significantly lowest plant height was recorded by the genotype Vijay (23.40 cm) under early drought stress condition except the genotype ICC 11112 (25.03 cm).

Table 28 : Mean plant height (cm) at 50 % flowering as influenced by moisture stress in Chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	32.70	29.27	33.77	31.91	40.33	30.63	39.50	36.82	36.52	29.95	36.63	34.37
2	Phule G 0405-44-2	41.07	37.70	41.73	40.17	47.70	43.70	45.07	45.49	44.38	40.70	43.40	42.83
3	Phule G 09103	36.27	32.77	35.33	34.79	36.00	30.97	34.37	33.78	36.13	31.87	34.85	34.28
4	ICCV 11112	33.97	29.07	35.37	32.80	33.37	25.03	34.30	30.90	33.67	27.05	34.83	31.85
5	ICCV 11117	37.37	34.70	39.73	37.27	39.30	29.03	33.00	33.78	38.33	31.87	36.37	35.52
	Susceptible Mean	36.27	32.70	37.19	35.39	39.34	31.87	37.25	36.15	37.81	32.29	37.22	35.77
6	Phule G 0511-43-2	38.37	35.60	36.67	36.88	40.67	30.73	37.73	36.38	39.52	33.17	37.20	36.63
7	Phule G 0752	43.40	37.97	40.37	40.58	43.07	38.70	41.53	41.10	43.23	38.33	40.95	40.84
8	ICC 4958	35.77	35.30	39.37	36.81	37.37	32.77	38.37	36.17	36.57	34.03	38.87	36.49
9	Vijay	30.03	28.37	29.33	29.24	30.70	23.40	29.03	27.71	30.37	25.88	29.18	28.48
10	Digvijay	43.70	40.00	39.27	40.99	36.67	32.70	34.93	34.77	40.18	36.35	37.10	37.88
	Tolerant mean	38.25	35.45	37.00	36.90	37.69	31.66	36.32	35.22	37.97	33.55	36.66	36.06
	Mean	37.26	34.07	37.09	36.14	38.52	31.77	36.78	35.69	37.89	32.92	36.94	35.92
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.25	0.72			0.26	0.76			0.32	0.90	
	Genotypes (G)		0.47	1.34			0.38	1.08			0.52	1.47	
	Interaction (G X C)		0.81	2.32			0.88	2.06			0.41	2.54	

On the basis of pooled mean the genotype Phule G 0405-44-2 recorded the highest (44.38 and 40.70 cm) plant height at 50 per cent flowering under normal and early drought stress condition, respectively. The genotype Vijay (25.88 cm) under early drought stress recorded the lowest plant height.

4.3.4.2 Plant height (cm) at maturity

The data on plant height (cm) at maturity influenced by moisture stress in chickpea genotypes are depicted in Table 29. The plant height was significantly reduced in all genotypes under early drought stress condition as compared to normal condition.

The genotypes Phule G-0405-44-2 recorded significantly highest plant height (57.23 cm) under normal condition at maturity in the year 2012-13. The same genotype, Phule-G-0405-44-2 maintained the highest plant height (47.73 and 50.03 cm) under early and late drought stress condition, respectively. The significantly lowest plant height was recorded by the genotype Vijay (37.03 and 38.03 cm) under early and late drought stress condition at maturity, respectively.

In the year 2013-14, Phule-G-0405-44-2 recorded the significantly highest plant height (58.57 cm) under unstressed normal condition, also maintained the highest plant height (43.70 and 54.70 cm) at pre and post flowering stress, respectively. At maturity, the genotype Vijay (36.43 cm) recorded significantly lowest plant height at normal condition. Under early drought stress the genotype Vijay (35.33 cm) recorded the lowest plant height followed by Phule G -0305-3 (37.58 cm).

Table 29 : Mean plant height (cm) at maturity as influenced by moisture stress in Chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	46.17	37.07	46.97	43.40	44.27	38.70	43.67	42.21	45.22	37.88	45.32	42.81
2	Phule G 0405-44-2	57.23	47.73	50.03	51.67	58.57	43.70	54.70	52.32	57.90	45.72	52.37	51.99
3	Phule G 09103	43.37	42.10	43.40	42.96	43.07	38.10	41.40	40.86	43.22	40.10	42.40	41.91
4	ICCV 11112	44.43	41.80	43.13	43.12	43.70	39.40	43.70	42.27	44.07	40.60	43.42	42.69
5	ICCV 11117	48.03	43.20	47.30	46.18	42.37	40.30	40.30	40.99	45.20	41.75	43.80	43.58
	Susceptible Mean	47.85	42.38	46.17	45.46	46.39	40.04	44.75	43.73	47.12	41.21	45.46	44.60
6	Phule G 0511-43-2	45.73	41.63	43.07	43.48	42.10	38.27	44.67	41.68	43.92	39.95	43.87	42.58
7	Phule G 0752	45.73	43.93	44.73	44.80	47.37	39.63	46.33	44.44	46.55	41.78	45.53	44.62
8	ICC 4958	50.37	43.37	47.70	47.14	45.70	38.27	44.03	42.67	48.03	40.82	45.87	44.91
9	Vijay	38.77	37.03	38.03	37.94	36.43	33.63	35.40	35.16	37.60	35.33	36.72	36.55
10	Digvijay	49.40	43.93	44.03	45.79	45.37	38.63	44.00	42.67	47.38	41.28	44.02	44.23
	Tolerant mean	46.00	41.98	43.51	43.83	43.39	37.69	42.89	41.32	44.70	39.83	43.20	42.58
	Mean	46.92	42.18	44.84	44.65	44.89	38.86	43.82	42.53	45.91	40.52	44.33	43.59
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		0.35	1.01			0.44	1.27			0.49	1.38	
	Genotypes (G)		0.62	1.76			0.50	1.39			0.69	1.93	
	Interaction (G X C)		1.07	3.08			0.84	2.41			1.18	3.31	

On the basis of pooled mean the genotype Phule G 0405-44-2 recorded significantly highest (57.90) plant height at maturity under normal condition. While the genotype Vijay (35.33 cm) under early drought stress condition recorded the lowest plant height at maturity.

4.3.4.3 Secondary branches per plant at 50per cent flowering

The data on secondary branches per plant 50 per cent flowering as influenced by moisture stress in chickpea are presented in Table 30. A considerable variation was observed for secondary branches per plant among all the genotypes studied. The differences among the genotypes over the season and interactions were statistically significant for both the year 2012-13 and 2013-14.

In the year, 2012-13 the genotype Vijay (16.33) recorded the highest number of secondary branches under normal condition. However, the susceptible genotype Phule G-09103 recorded the lowest (8.67) secondary branches under early drought stress at 50 per cent flowering. On an average the genotype Digvijay (15.56) exhibited superior to secondary branches per plant.

In the year 2013-14, at 50 per cent flowering the genotype Digvijay (18.33) recorded the highest number of secondary branches under normal condition. Under early drought stress condition, the genotype Phule-G-0305-3 (10.67) recorded the significantly lowest number of secondary branches per plant.

Table 30 : Number of secondary branches per plant at 50% flowering as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	14.00	11.00	15.67	13.56	15.33	10.67	18.00	14.67	14.67	10.83	16.83	14.11
2	Phule G 0405-44-2	13.33	9.67	11.67	11.56	15.67	12.33	15.00	14.33	14.50	11.00	13.33	12.94
3	Phule G 09103	12.00	8.67	11.00	10.56	14.67	13.00	16.00	14.56	13.33	10.83	13.50	12.56
4	ICCV 11112	15.00	10.33	13.33	12.89	17.33	12.67	17.67	15.89	16.17	11.50	15.50	14.39
5	ICCV 11117	14.00	10.00	11.67	11.89	16.00	13.00	13.67	14.22	15.00	11.50	12.67	13.06
	Susceptible Mean	13.67	9.93	12.67	12.09	15.80	12.33	16.07	14.73	14.73	11.13	14.37	13.41
6	Phule G 0511-43-2	15.00	13.67	14.00	14.22	16.67	15.00	17.33	16.33	15.83	14.33	15.67	15.28
7	Phule G 0752	15.33	12.33	15.00	14.22	16.67	16.33	17.67	16.89	16.00	14.33	16.33	15.56
8	ICC 4958	14.67	11.33	13.00	13.00	17.00	14.67	17.33	16.33	15.83	13.00	15.17	14.67
9	Vijay	16.33	13.33	16.33	15.33	17.67	16.33	16.67	16.89	17.00	14.83	16.50	16.11
10	Digvijay	15.67	15.00	16.00	15.56	18.33	17.67	17.67	17.89	17.00	16.33	16.83	16.72
	Tolerant mean	15.40	13.13	14.87	14.47	17.27	16.00	17.33	16.87	16.33	14.57	16.10	15.67
	Mean	14.53	11.53	13.77	13.28	16.53	14.17	16.70	15.80	15.53	12.85	15.23	14.54
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.12	0.35			0.21	0.61			0.21	0.60	
	Genotypes (G)		0.28	0.81			0.28	0.80			0.35	0.97	
	Interaction (G X C)		0.48	1.41			0.51	1.54			0.66	1.68	

On the basis of pooled analysis, the genotypes Vijay and Digvijay recorded the highest (17.00) number of secondary branches under normal condition, while the genotype Phule-G-0305-3 recorded the lowest (10.83) number of secondary branches under early drought stress condition. On an average the genotype Digvijay (16.72) recorded highest mean number of secondary branches per plant.

4.3.4.4 Secondary branches per plant at maturity

The data pertaining to number of secondary branches per plant at maturity as influenced by moisture stress in chickpea genotypes are depicted in Table 31. The number of secondary branches per plant was significantly reduced under early drought stress. The differences amongst the genotypes over the season and their interaction were also found significant for both the year.

In the year 2012-13 at maturity the genotypes Vijay (17.40) recorded significantly highest number of secondary branches per plant under normal condition. Whereas, the genotype Digvijay (15.33) recorded the highest number of secondary branches under early drought stress at maturity. The susceptible genotype Phule G-09103 (8.93) recorded significantly the lowest number of secondary branches per plant under early drought stress.

In the year 2013-14, the genotype Phule-G-0305-3 and Digvijay recorded the highest number of secondary branches (18.67) under normal condition at maturity. Under early drought

Table 31 : Number of secondary branches per plant at maturity as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	15.03	11.70	15.93	14.22	18.67	11.50	16.33	15.50	16.85	11.60	16.13	14.86
2	Phule G 0405-44-2	14.67	10.67	11.80	12.38	15.27	13.53	15.20	14.67	14.97	12.10	13.50	13.52
3	Phule G 09103	14.23	8.93	12.17	11.78	15.67	13.10	15.33	14.70	14.95	11.02	13.75	13.24
4	ICCV 11112	15.23	11.20	13.80	13.41	18.33	13.40	17.33	16.36	16.78	12.30	15.57	14.88
5	ICCV 11117	13.87	10.67	12.27	12.27	16.37	12.97	13.90	14.41	15.12	11.82	13.08	13.34
	Susceptible Mean	14.61	10.63	13.19	12.81	16.86	12.90	15.62	15.13	15.73	11.77	14.41	13.97
6	Phule G 0511-43-2	15.22	13.60	14.87	14.56	17.67	15.77	17.00	16.81	16.44	14.68	15.93	15.69
7	Phule G 0752	15.33	13.20	15.43	14.66	18.00	17.07	16.67	17.24	16.67	15.13	16.05	15.95
8	ICC 4958	15.50	12.33	13.73	13.86	17.67	14.83	17.33	16.61	16.58	13.58	15.53	15.23
9	Vijay	17.40	14.00	17.20	16.20	18.00	16.73	17.67	17.47	17.70	15.37	17.43	16.83
10	Digvijay	15.67	15.33	15.93	15.64	18.67	17.67	18.17	18.17	17.17	16.50	17.05	16.91
	Tolerant mean	15.82	13.69	15.43	14.98	18.00	16.41	17.37	17.26	16.91	15.05	16.40	16.12
	Mean	15.22	12.16	14.31	13.90	17.43	14.66	16.49	16.19	16.32	13.41	15.40	15.05
			SE+	CD(5%)			SE+	CD(5%)			SE+	CD(5%)	
	Conditions (C)		0.16	0.47			0.19	0.55			0.21	0.61	
	Genotypes (G)		0.26	0.75			0.30	0.86			0.34	0.98	
	Interaction (G X C)		0.45	1.30			0.52	1.50			0.60	1.70	

stress the genotype Digvijay (17.67) recorded the highest number of secondary branches per plant.

On the basis of pooled analysis, at maturity the genotype Vijay (17.70) recorded the highest number of secondary branches per plant under normal condition, while the Digvijay (16.50) recorded the highest number of secondary branches per plant under early drought stress condition followed by Vijay (15.37).

4.3.5 Effect of water stress on osmolytes accumulation

4.3.5.1 Proline at 50 per cent flowering

The proline content in the leaves at 50 % flowering as influenced by moisture stress in chickpea genotypes were determined and presented in Table 32. It was evident from the Table that at 50 per cent flowering there was significant increase in proline accumulation in all genotypes under early drought stress and interaction were significant for both season.

At 50% flowering, the highest proline content was observed in genotype Phule-G-0405-44-2 (6.44 $\mu\text{moles g}^{-1}\text{FW}$) under normal condition followed by the genotype ICC 4958 (6.38 $\mu\text{moles g}^{-1}\text{FW}$). The tolerant genotype ICC 4958 (17.09 $\mu\text{moles g}^{-1}\text{FW}$) accumulated significantly highest proline content at 50 per cent flowering under early drought stress, during the year 2012-13.

In the year 2013-14 the genotype Vijay (7.94 $\mu\text{moles g}^{-1}\text{FW}$) recorded the highest proline content followed by the genotype

Table 32 : Proline accumulation ($\mu\text{moles g}^{-1}$ FW) at 50 % flowering as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	5.49	10.31	5.56	7.12	5.07	9.07	5.12	6.42	5.28	9.69	5.34	6.77
2	Phule G 0405-44-2	6.44	10.59	6.49	7.84	6.85	11.52	6.56	8.31	6.65	11.06	6.53	8.08
3	Phule G 09103	6.02	9.26	5.86	7.05	7.51	12.88	7.32	9.24	6.77	11.07	6.59	8.14
4	ICCV 11112	4.59	8.7	4.53	5.94	6.44	12.76	6.3	8.50	5.52	10.73	5.42	7.22
5	ICCV 11117	4.97	8.56	4.8	6.11	6.50	10.05	6.41	7.65	5.74	9.31	5.61	6.88
	Susceptible Mean	5.50	9.48	5.45	6.81	6.47	11.26	6.34	8.02	5.99	10.37	5.90	7.42
6	Phule G 0511-43-2	5.15	13.13	4.98	7.75	7.36	16.15	7.31	10.27	6.26	14.64	6.15	9.01
7	Phule G 0752	5.76	14.35	5.62	8.58	7.19	16.1	7.06	10.12	6.48	15.23	6.34	9.35
8	ICC 4958	6.38	17.09	6.31	9.93	7.65	17.54	7.46	10.88	7.02	17.32	6.89	10.41
9	Vijay	5.79	16.55	5.82	9.39	7.94	17.59	7.86	11.13	6.87	17.07	6.84	10.26
10	Digvijay	5.62	16.49	5.76	9.29	7.64	17.73	7.69	11.02	6.63	17.11	6.73	10.16
	Tolerant mean	5.74	15.52	5.70	8.99	7.56	17.02	7.48	10.68	6.65	16.27	6.59	9.84
	Mean	5.62	12.50	5.57	7.90	7.02	14.14	6.91	9.35	6.32	13.32	6.24	8.63
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.02	0.04			0.01	0.04			0.02	0.05	
	Genotypes (G)		0.06	0.16			0.07	0.21			0.08	0.22	
	Interaction (G X C)		0.10	0.28			0.12	0.37			0.11	0.31	

ICC 4958 ($7.65 \mu\text{moles g}^{-1}$ FW) under normal condition at 50 per cent flowering. The tolerant genotype Digvijay ($17.73 \mu\text{moles g}^{-1}$ FW) recorded the highest accumulation of proline at 50 % flowering under early drought stress condition followed by Vijay ($17.59 \mu\text{moles g}^{-1}$ FW).

Overall on pooled basis at 50 per cent flowering the tolerant genotype ICC 4958 ($17.32 \mu\text{mole g}^{-1}\text{FW}$) recorded the highest proline content in leaves under early drought stress followed by the genotype Digvijay ($17.11 \mu\text{mole g}^{-1}\text{FW}$). The susceptible genotype ICCV11117 ($9.31 \mu\text{mole g}^{-1}\text{FW}$) accumulated the lowest proline under early drought stress.

4.3.5.2 Proline at grain filling stage

The data on proline accumulation at grain filling stage as influenced by moisture stress in chickpea genotypes are depicted in Table 33. It was observed that there was considerable variation in proline accumulation amongst all genotypes studied and the proline accumulation was observed significantly more in stress condition than non stress normal condition. The interactions were statistically significant for both the year 2012-13 and 2013-14.

In the year 2012-13 the genotype Phule G-0405-44-2 ($4.07 \mu\text{moles g}^{-1}$ FW) recorded the highest proline content at grain filling stage followed by Digvijay ($3.97 \mu\text{moles g}^{-1}$ FW) under normal condition. Under early drought stress the tolerant genotype ICC 4958 ($5.56 \mu\text{moles g}^{-1}$ FW) recorded significantly highest proline accumulation in leaves except the genotype Vijay

Table 33 : Proline accumulation ($\mu\text{moles g}^{-1}$ FW) at grain filling stage as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	3.39	3.69	5.35	4.14	3.97	4.08	6.41	4.82	3.68	3.88	5.88	4.48
2	Phule G 0405-44-2	4.07	4.57	5.43	4.69	4.21	4.45	6.72	5.13	4.14	4.51	6.07	4.91
3	Phule G 09103	3.90	4.00	5.74	4.55	4.65	5.03	7.23	5.63	4.27	4.51	6.49	5.09
4	ICCV 11112	3.77	4.08	6.09	4.65	4.00	4.57	7.60	5.39	3.89	4.33	6.84	5.02
5	ICCV 11117	3.38	3.43	5.64	4.15	4.46	4.97	6.72	5.38	3.92	4.20	6.18	4.77
	Susceptible Mean	3.70	3.95	5.65	4.43	4.26	4.62	6.94	5.27	3.98	4.29	6.29	4.85
6	Phule G 0511-43-2	3.36	3.99	8.08	5.14	5.03	5.66	9.66	6.78	4.19	4.82	8.87	5.96
7	Phule G 0752	3.93	4.87	9.59	6.13	6.00	6.38	11.47	7.95	4.97	5.63	10.53	7.04
8	ICC 4958	4.07	5.56	10.22	6.62	6.17	6.52	13.64	8.78	5.12	6.04	11.93	7.70
9	Vijay	3.71	5.21	10.14	6.35	6.59	6.81	13.72	9.04	5.15	6.01	11.93	7.70
10	Digvijay	3.97	4.80	10.05	6.27	5.78	7.12	12.31	8.40	4.87	5.96	11.18	7.34
	Tolerant mean	3.81	4.89	9.62	6.10	5.91	6.50	12.16	8.19	4.86	5.69	10.89	7.15
	Mean	3.76	4.42	7.63	5.27	5.08	5.56	9.55	6.73	4.42	4.99	8.59	6.00
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.03	0.08			0.05	0.13			0.05	0.13	
	Genotypes (G)		0.06	0.18			0.09	0.25			0.09	0.26	
	Interaction (G X C)		0.11	0.31			0.15	0.43			0.16	0.45	

(5.21 $\mu\text{moles g}^{-1}$ FW). There was a significant increase in proline accumulation in leaves of all genotypes under late drought stress. However, the tolerant genotype ICC 4958 (10.22 $\mu\text{moles g}^{-1}$ FW) recorded the highest proline accumulation in leaves under late drought stress condition at grain filling stage.

In year 2013-14, the tolerant genotype Vijay (13.72 $\mu\text{moles g}^{-1}$ FW) recorded significantly highest proline accumulation in leaves at grain filling stage except the genotype ICC 4958 (13.64 $\mu\text{moles g}^{-1}$ FW) under the late drought stress condition.

On an average, on the pooled analysis, the tolerant genotype ICC 4958 and Vijay (11.93 $\mu\text{moles g}^{-1}$ FW) accumulates significantly highest proline under late drought stress condition at grain filling stage.

4.3.5.3 Glycine betaine at 50 per cent flowering

The glycine betaine accumulation in the leaves of chickpea genotypes under moisture stress is depicted in Table 34. The moisture stress at pre-flowering stage reflected in significant increase in glycine betaine accumulation in all chickpea genotypes examined.

In the year 2012-13 the tolerant genotype Vijay (8.22 $\mu\text{moles g}^{-1}$ FW) recorded significantly highest glycine betaine contact except the genotypes ICC 4958 (8.09 $\mu\text{moles g}^{-1}$ FW) at 50 per cent flowering under normal condition. The significantly highest glycine betaine to the extent of 13.85 $\mu\text{mole g}^{-1}$ FW was recorded by the tolerant genotype ICC 4958 except the genotype Vijay (13.72 $\mu\text{moles g}^{-1}$ FW) under early drought stress.

Table 34 : Glycine betaine accumulation ($\mu\text{moles g}^{-1}$ FW) at 50 % flowering as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	7.20	8.87	7.06	7.71	7.17	10.59	7.12	8.30	7.19	9.73	7.09	8.00
2	Phule G 0405-44-2	6.68	9.40	6.72	7.60	7.57	11.09	7.38	8.68	7.12	10.24	7.05	8.14
3	Phule G 09103	7.36	9.71	7.31	8.12	7.36	10.17	7.39	8.31	7.36	9.94	7.35	8.22
4	ICCV 11112	7.64	9.70	7.59	8.31	7.83	10.96	7.92	8.90	7.74	10.33	7.76	8.61
5	ICCV 11117	7.57	10.20	7.48	8.42	8.61	10.96	8.53	9.37	8.09	10.58	8.01	8.89
	Susceptible Mean	7.29	9.58	7.23	8.03	7.71	10.75	7.67	8.71	7.50	10.16	7.45	8.37
6	Phule G 0511-43-2	7.25	11.92	7.29	8.82	8.87	15.39	8.80	11.02	8.06	13.66	8.05	9.92
7	Phule G 0752	6.99	10.49	6.61	8.03	8.14	14.61	8.06	10.27	7.57	12.55	7.34	9.15
8	ICC 4958	8.09	13.85	7.94	9.96	7.57	17.74	7.59	10.97	7.83	15.80	7.77	10.46
9	Vijay	8.22	13.72	8.12	10.02	8.35	16.96	8.31	11.21	8.28	15.34	8.22	10.61
10	Digvijay	7.30	12.21	7.13	8.88	8.61	15.65	8.54	10.93	7.96	13.93	7.84	9.91
	Tolerant mean	7.57	12.44	7.42	9.14	8.31	16.07	8.26	10.88	7.94	14.25	7.84	10.01
	Mean	7.43	11.01	7.33	8.59	8.01	13.41	7.96	9.79	7.72	12.21	7.64	9.19
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.02	0.05			0.03	0.08			0.03	0.08	
	Genotypes (G)		0.08	0.21			0.10	0.27			0.11	0.30	
	Interaction (G X C)		0.13	0.37			0.16	0.46			0.14	0.41	

In the year 2013-14 the genotype Phule-G-0511-43-2 (8.87 $\mu\text{moles g}^{-1}$ FW) recorded the highest glycine betaine content under normal condition. While the tolerant genotype ICC 4958 (17.74 $\mu\text{moles g}^{-1}$ FW) recorded significantly highest glycine betaine accumulation in leaves under early drought stress condition.

On the pooled basis, the genotype Vijay (8.28 $\mu\text{moles g}^{-1}$ FW) recorded the highest glycine betaine content at 50 per cent flowering under normal condition. The tolerant genotypes ICC 4958 (15.80 $\mu\text{moles g}^{-1}$ FW) accumulate significantly the highest glycine betaine in leaves under early drought stress at 50 per cent flowering except the genotype Vijay (15.34 $\mu\text{moles g}^{-1}$ FW). The susceptible genotypes Phule-G-0305-3 (9.73 $\mu\text{moles g}^{-1}$ FW) accumulated the lowest glycine betaine content at 50 per cent flowering under early drought stress condition.

4.3.5.4 Glycine betaine at grain filling

The glycine betaine at grain filling stage as influenced by moisture stress in chickpea genotypes is presented in Table 35. The glycine betaine content was significantly increased in all genotypes under stress condition as compared to control condition.

In the year 2012-13 the genotypes Vijay (7.60 $\mu\text{moles g}^{-1}$ FW) recorded the highest glycine betaine content followed by the genotypes Digvijay (7.46 $\mu\text{ mole g}^{-1}\text{FW}$) under normal condition. Similar trend was observed under early drought stress, the genotypes Vijay (7.96 $\mu\text{ mole g}^{-1}\text{FW}$) accumulate highest

Table 35 : Glycine betaine accumulation ($\mu\text{moles g}^{-1}$ FW) at grain filling as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	6.53	7.10	7.85	7.16	6.34	6.89	7.89	7.04	6.43	6.99	7.87	7.10
2	Phule G 0405-44-2	6.57	6.94	8.92	7.48	5.53	6.26	7.07	6.29	6.05	6.60	8.00	6.88
3	Phule G 09103	6.17	6.68	7.85	6.90	6.29	6.89	7.17	6.78	6.23	6.78	7.51	6.84
4	ICCV 11112	7.12	7.54	8.71	7.79	5.97	6.29	7.89	6.72	6.55	6.92	8.30	7.26
5	ICCV 11117	6.76	7.38	8.92	7.69	6.66	7.02	8.13	7.27	6.71	7.20	8.52	7.48
	Susceptible Mean	6.63	7.13	8.45	7.40	6.16	6.67	7.63	6.82	6.39	6.90	8.04	7.11
6	Phule G 0511-43-2	6.24	6.76	9.86	7.62	6.55	7.07	10.28	7.97	6.39	6.92	10.07	7.79
7	Phule G 0752	6.03	6.42	8.87	7.11	6.73	6.86	9.78	7.79	6.38	6.64	9.33	7.45
8	ICC 4958	6.88	7.46	11.37	8.57	7.83	8.19	12.03	9.35	7.36	7.83	11.70	8.96
9	Vijay	7.60	7.96	10.98	8.85	8.14	8.66	12.57	9.79	7.87	8.31	11.78	9.32
10	Digvijay	7.46	7.80	11.17	8.81	6.43	6.68	9.11	7.40	6.94	7.24	10.14	8.11
	Tolerant mean	6.84	7.28	10.45	8.19	7.13	7.49	10.75	8.46	6.99	7.39	10.60	8.33
	Mean	6.74	7.20	9.45	7.80	6.65	7.08	9.19	7.64	6.69	7.14	9.32	7.72
			SE+	CD(5%)			SE+	CD(5%)			SE+	CD(5%)	
	Conditions (C)		0.07	0.19			0.03	0.08			0.06	0.18	
	Genotypes (G)		0.27	0.37			0.28	0.37			0.33	0.44	
	Interaction (G X C)		0.17	0.48			0.16	0.47			0.17	0.48	

glycine betaine followed by Digvijay ($7.80 \mu\text{moles g}^{-1} \text{FW}$). However, under late drought stress condition the tolerant genotype ICC 4958 ($11.37 \mu\text{moles g}^{-1} \text{FW}$) recorded the significantly highest glycine betaine accumulation in leaves than other genotypes except the genotype Digvijay ($11.17 \mu\text{moles g}^{-1} \text{FW}$) at grain filling stage.

The genotypes Vijay ($8.14 \mu \text{ mole g}^{-1}\text{FW}$) recorded significantly highest glycine betaine content at grain filling stage under normal condition during the year 2013-14. The highest glycine betaine content of $8.66 \mu \text{ mole g}^{-1}\text{FW}$ under early drought stress condition was observed in the leaves of Vijay under early drought stress. The tolerant genotype Vijay ($12.57 \mu\text{moles g}^{-1} \text{FW}$) and ICC 4958 ($12.03 \mu\text{moles g}^{-1} \text{FW}$) accumulate significantly highest glycine betaine in leaves at grain filling stage under late drought stress condition.

On the basis of pooled analysis, the genotype Vijay ($11.78 \mu\text{moles g}^{-1} \text{FW}$) accumulated significantly highest glycine betaine in leaves at grain filling stage than rest of genotypes except ICC 4958 ($11.70 \mu\text{moles g}^{-1} \text{FW}$) under late drought stress condition.

4.3.6 Effect of water stress on antioxidative enzymes

4.3.6.1 Superoxide dismutase at 50 per cent flowering

The effect of moisture stress on superoxide dismutase activity at 50 per cent flowering stage in the leaves of chickpea genotypes has been shown in Table 36. It was observed that, there was a considerable variation in SOD activity in all genotypes

studied and the SOD activity was significantly increased in all genotypes at 50 per cent flowering under early drought stress condition than the control condition.

In the year 2012-13, the genotype ICCV 11112 (112.69 units mg^{-1} protein) recorded the highest SOD activity under normal condition. The highest SOD activity of 159.33 units mg^{-1} protein was observed in tolerant genotype Digvijay followed by ICC 4958 (157.33 units mg^{-1} protein) at 50 per cent flowering under early drought stress condition. However, the susceptible genotype Phule G-0305-3 (117.95 units mg^{-1} protein) recorded the significantly lowest SOD activity under early drought stress condition.

In the year 2013-14, at 50 per cent flowering the genotype Phule G-0752 (102.96 units mg^{-1} protein) exhibited the highest SOD activity under unstressed condition. Under early drought stress the genotypes Digvijay (149.53 units mg^{-1} protein) showed maximum SOD activity at 50 per cent flowering.

On the basis of pooled analysis, the tolerant genotype Digvijay exhibited the maximum SOD activity of 154.43 units mg^{-1} protein at 50 per cent flowering under early drought stress condition followed by the genotypes ICC 4958 (153.25 units mg^{-1} protein). However, the susceptible genotype Phule-G-0305-3 (120.95 units mg^{-1} protein) recorded the lowest SOD activity under early drought stress.

Table 36 . Effect of moisture stress on superoxide dismutase activity (units mg⁻¹ protein) at flowering stage in the leaves of chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	106.12	117.95	104.82	109.63	93.21	123.94	91.63	102.93	99.67	120.95	98.23	106.28
2	Phule G 0405-44-2	110.02	132.39	115.47	119.29	89.21	116.13	91.06	98.80	99.62	124.26	103.27	109.05
3	Phule G 09103	108.52	130.07	111.31	116.63	91.03	126.34	94.75	104.04	99.78	128.21	103.03	110.34
4	ICCV 11112	114.52	127.73	112.69	118.31	94.05	124.81	92.14	103.67	104.29	126.27	102.42	110.99
5	ICCV 11117	109.02	128.1	112.24	116.45	97.99	133.78	102.17	111.31	103.51	130.94	107.21	113.88
	Susceptible Mean	109.64	127.25	111.31	116.06	93.10	125.00	94.35	104.15	101.37	126.12	102.83	110.11
6	Phule G 0511-43-2	121.23	153.84	118.19	131.09	97.06	138.86	99.22	111.71	109.15	146.35	108.71	121.40
7	Phule G 0752	108.21	154.94	111.86	125.00	101.14	147.01	102.96	117.04	104.68	150.98	107.41	121.02
8	ICC 4958	111.12	157.33	108.98	125.81	102.02	149.16	99.26	116.81	106.57	153.25	104.12	121.31
9	Vijay	103.36	156.45	105.68	121.83	95.97	142.21	93.48	110.55	99.67	149.33	99.58	116.19
10	Digvijay	110.26	159.33	113.3	127.63	99.03	149.53	102.45	117.00	104.65	154.43	107.88	122.32
	Tolerant mean	110.84	156.38	111.60	126.27	99.04	145.35	99.47	114.62	104.94	150.87	105.54	120.45
	Mean	110.24	141.81	111.45	121.17	96.07	135.18	96.91	109.39	103.15	138.50	104.18	115.28
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		0.70	2.20			0.59	1.07			0.79	2.72	
	Genotypes (G)		1.46	4.18			1.28	3.65			1.68	4.74	
	Interaction (G X C)		2.54	7.22			2.21	6.32			2.92	8.21	

4.3.6.2 Superoxide dismutase at grain filling stage

The effect of imposed water stress on superoxide dismutase activity at grain filling stage in the leaves of chickpea genotypes has been shown in Table 37. The SOD activity was significantly increased in all genotypes under late drought stress as compared to normal condition in both year 2012-13 and 2013-14.

In the year 2012-13, at grain filling stage the SOD activity was highest in genotype Phule-G-0511-43-2 (111.26 units mg^{-1} protein) followed by the genotype Digvijay (107.49 units mg^{-1} protein) under normal condition. Under late drought stress condition, the genotype Digvijay (151.33 units mg^{-1} protein) and Phule G-0511-43-2 (145.46 units mg^{-1} protein) exhibited significantly maximum SOD activity at grain filling stage.

In the year 2013-14, the genotypes Phule-G-0511-43-2 (95.03 units mg^{-1} protein) recorded maximum SOD activity under normal condition at grain filling stage. The tolerant genotypes Vijay (130.50 units mg^{-1} protein) recorded statistically significant SOD activity under late drought stress condition except the genotype Digvijay (127.70 units mg^{-1} protein).

On the basis of pooled analysis it is revealed that the tolerant genotype Digvijay demonstrated the highest (139.52 units mg^{-1} protein) SOD activity under late drought stress condition followed by Phule-G-0511-43-2 (135.13 units mg^{-1} protein) and Vijay (135.09 units mg^{-1} protein) while the susceptible genotype Phule-G-0305-3 (95.59 units mg^{-1} protein) recorded significantly

Table 37 . Effect of moisture stress on superoxide dismutase activity (units mg⁻¹ protein) at grain filling stage in the leaves of chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	85.88	91.55	93.13	90.19	80.00	86.85	98.04	88.30	82.94	89.20	95.59	89.24
2	Phule G 0405-44-2	94.61	92.00	107.26	97.96	79.08	85.46	96.01	86.85	86.85	88.73	101.64	92.40
3	Phule G 09103	95.67	100.40	109.25	101.77	85.80	89.00	99.55	91.45	90.74	94.70	104.40	96.61
4	ICCV 11112	96.23	99.00	111.33	102.19	87.42	89.19	98.23	91.61	91.83	94.10	104.78	96.90
5	ICCV 11117	96.56	98.33	103.18	99.36	89.65	96.33	101.80	95.93	93.11	97.33	102.49	97.64
	Susceptible Mean	93.79	96.26	104.83	98.29	84.39	89.37	98.73	90.83	89.09	92.81	101.78	94.56
6	Phule G 0511-43-2	111.26	115.47	145.46	124.06	95.03	97.00	124.80	105.61	103.15	106.24	135.13	114.84
7	Phule G 0752	105.04	109.59	139.32	117.98	86.67	97.76	121.49	101.97	95.86	103.68	130.41	109.98
8	ICC 4958	100.67	106.48	138.33	115.16	87.69	91.61	123.67	100.99	94.18	99.05	131.00	108.08
9	Vijay	99.67	114.64	139.67	117.99	86.25	94.59	130.50	103.78	92.96	104.62	135.09	110.89
10	Digvijay	107.49	110.00	151.33	122.94	90.79	85.40	127.70	101.30	99.14	97.70	139.52	112.12
	Tolerant mean	104.83	111.24	142.82	119.63	89.29	93.27	125.63	102.73	97.06	102.25	134.23	111.18
	Mean	99.31	103.75	123.83	108.96	86.84	91.32	112.18	96.78	93.07	97.53	118.00	102.87
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		0.59	1.09			0.55	1.56			0.70	1.96	
	Genotypes (G)		1.15	3.26			1.17	3.55			1.42	3.99	
	Interaction (G X C)		1.99	5.65			2.04	5.80			1.99	5.67	

lowest SOD activity at grain filling stage under late drought stress condition.

4.3.6.3 Catalase activity at 50 per cent flowering

Imposing the water stress by withholding irrigation at 40 DAS in early drought stress condition increased the catalase activity than under unstressed control (Table 38). At 50 per cent flowering, the catalase activity was significantly increased in all genotypes under early drought stress and interactions were found to be significant in both the year 2012-13 and 2013-14.

At 50 per cent flowering, the catalase activity of $8.71 \mu \text{mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$ was comparatively higher in genotype Phule-G-0405-44-2 followed by ICC 4958 ($8.30 \mu \text{mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$) under normal condition, during the year 2012-13. However, the tolerant genotype ICC 4958 demonstrated significantly highest activity of $18.18 \mu \text{mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$ at 50 per cent flowering under early drought stress condition. The susceptible genotype ICCV 11112 ($8.78 \mu \text{mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$) showed significantly lowest catalase activity under early drought stress at 50 per cent flowering.

In the year 2013-14, the tolerant genotype Vijay ($19.16 \mu \text{mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$) exhibited significantly maximum catalase activity under early drought stress at 50 per cent flowering than other genotypes except ICC 4958 ($19.05 \mu \text{mol H}_2\text{O}_2 \text{ decomposed mg}^{-1} \text{ protein min}^{-1}$).

Table 38 . Effect of moisture stress on catalase activity ($\mu\text{mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1}) at 50% flowering stage in the leaves of chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	6.42	9.74	6.52	7.56	9.07	13.26	8.96	10.43	7.75	11.50	7.74	8.99
2	Phule G 0405-44-2	8.71	11.21	8.63	9.52	9.15	12.13	9.18	10.15	8.93	11.67	8.91	9.84
3	Phule G 09103	7.19	9.91	7.26	8.12	7.78	10.43	7.70	8.64	7.49	10.17	7.48	8.38
4	ICCV 11112	7.33	8.78	7.27	7.79	8.45	9.43	8.51	8.80	7.89	9.11	7.89	8.30
5	ICCV 11117	7.78	11.54	7.68	9.00	9.02	12.72	8.81	10.18	8.40	12.13	8.25	9.59
	Susceptible Mean	7.49	10.24	7.47	8.40	8.70	11.59	8.63	9.64	8.09	10.91	8.05	9.02
6	Phule G 0511-43-2	8.09	14.46	8.12	10.22	8.15	14.76	8.23	10.38	8.12	14.61	8.18	10.30
7	Phule G 0752	7.86	16.40	7.59	10.62	8.40	17.88	8.34	11.54	8.13	17.14	7.97	11.08
8	ICC 4958	8.30	18.18	8.34	11.61	8.51	19.05	8.56	12.04	8.41	18.62	8.45	11.83
9	Vijay	6.24	13.33	6.16	8.58	9.09	19.16	9.15	12.47	7.67	16.25	7.66	10.52
10	Digvijay	6.79	15.84	6.65	9.76	8.28	18.00	8.31	11.53	7.54	16.92	7.48	10.65
	Tolerant mean	7.46	15.64	7.37	10.16	8.49	17.77	8.52	11.59	7.97	16.71	7.95	10.88
	Mean	7.47	12.94	7.42	9.28	8.59	14.68	8.58	10.62	8.03	13.81	8.00	9.95
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.06	0.16			0.09	0.27			0.09	0.26	
	Genotypes (G)		0.08	0.23			0.13	0.36			0.13	0.37	
	Interaction (G X C)		0.14	0.39			0.22	0.64			0.23	0.64	

On the basis of pooled analysis, it is observed that the tolerant genotype ICC 4958 ($18.62 \mu \text{ mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1}) demonstrated significantly highest catalase activity in leaves under early drought stress at 50 per cent flowering. The susceptible genotype ICCV 11112 recorded significantly lowest catalase activity at 50 per cent flowering under early drought stress condition.

4.3.6.4 Catalase activity at grain filling stage

The effect of moisture stress on catalase activity at grain filling stage in the leaves of chickpea genotype has been shown in Table 39. Under stress condition the catalase activity was significantly increased in all the genotypes studied.

In the year 2012-13, the genotypes Phule-G-0511-43-2 ($7.49 \mu \text{ mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1}) recorded significantly highest catalase activity under normal condition while the genotype Vijay ($9.82 \mu \text{ mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1}) recorded the highest catalase activity under early drought stress condition at grain filling stage. Under late drought stress condition the tolerant genotype Digvijay ($13.36 \mu \text{ mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1}) demonstrated significantly highest activity at grain filling stage.

The genotypes Vijay ($7.84 \mu \text{ mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1}) recorded maximum catalase activity at grain filling stage, during the year 2013-14. The tolerant genotypes Digvijay and ICC 4958 (16.00 and $15.50 \mu \text{ mol H}_2\text{O}_2$ decomposed mg^{-1}

Table 39 .Effect of moisture stress on catalase activity ($\mu\text{mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1}) at grain filling stage in the leaves of chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	4.14	4.28	5.38	4.60	5.56	6.07	7.58	6.40	4.85	5.18	6.48	5.50
2	Phule G 0405-44-2	4.03	4.29	5.23	4.52	5.04	5.51	7.16	5.90	4.54	4.90	6.20	5.21
3	Phule G 09103	4.39	4.64	5.88	4.97	6.28	6.50	8.08	6.95	5.34	5.57	6.98	5.96
4	ICCV 11112	6.42	6.57	7.89	6.96	5.37	5.80	6.29	5.82	5.90	6.19	7.09	6.39
5	ICCV 11117	5.86	6.06	8.49	6.80	8.21	8.59	11.62	9.47	7.04	7.33	10.06	8.14
	Susceptible Mean	4.97	5.17	6.57	5.57	6.09	6.49	8.15	6.91	5.53	5.83	7.36	6.24
6	Phule G 0511-43-2	7.49	8.15	12.50	9.38	6.45	6.79	11.24	8.16	6.97	7.47	11.87	8.77
7	Phule G 0752	5.67	5.81	10.30	7.26	7.46	7.57	14.41	9.81	6.57	6.69	12.36	8.54
8	ICC 4958	6.44	7.44	12.36	8.75	7.57	7.75	15.50	10.27	7.01	7.60	13.93	9.51
9	Vijay	5.73	9.82	10.10	8.55	7.84	7.90	13.52	9.75	6.79	8.86	11.81	9.15
10	Digvijay	6.60	9.09	13.36	9.68	7.44	7.68	16.00	10.37	7.02	8.39	14.68	10.03
	Tolerant mean	6.39	8.06	11.72	8.72	7.35	7.54	14.13	9.67	6.87	7.80	12.93	9.20
	Mean	5.68	6.62	9.15	7.15	6.72	7.02	11.14	8.29	6.20	6.82	10.14	7.72
			SE+	CD(5%)			SE+	CD(5%)			SE+	CD(5%)	
	Conditions (C)		0.10	0.30			0.04	0.11			0.09	0.27	
	Genotypes (G)		0.09	0.27			0.11	0.30			0.12	0.35	
	Interaction (G X C)		0.18	0.59			0.18	0.59			0.21	0.60	

protein min⁻¹) demonstrated significantly highest catalase activity under late drought stress condition.

On the basis of pooled analysis the tolerant genotypes Digvijay (14.68 μ mol H₂O₂ decomposed mg⁻¹ protein min⁻¹) showed significantly maximum catalase activity under late drought stress condition. The susceptible genotypes Phule-G-0405-44-2 (6.20 μ mol H₂O₂ decomposed mg⁻¹ protein min⁻¹) recorded minimum catalase activity at grain filling stage under late drought stress condition.

4.3.6.5 Ascorbate peroxidase (APX) activity at 50 per cent flowering

The data on ascorbate peroxidase (APX) activity as influenced by moisture stress at 50 per cent flowering are depicted in Table 40. The differences amongst the genotype over the season and their interactions were statistically significant for both the years 2012-13 and 2013-14 under stress condition as well as non stress condition. The APX activity was significantly increased in all genotypes at 50 per cent flowering under early drought stress condition.

In the year 2012-13, the highest APX activity of 451.10 n moles ascorbate oxidized mg⁻¹ protein min⁻¹ was the highest in genotype ICCV 11112, at 50 per cent flowering under normal condition. However under early drought stress the tolerant genotype Vijay (881.34 nmoles ascorbate oxidized mg⁻¹ protein min⁻¹) recorded significantly the highest APX activity at 50 per cent flowering.

Table 40 .Effect moisture stress on APX activity (nmoles ascorbate oxidized mg⁻¹ protein min⁻¹) at 50 % flowering stage in the leaves of chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	310.21	518.23	312.21	380.22	345.07	565.85	349.25	420.05	327.64	542.04	330.73	400.14
2	Phule G 0405-44-2	481.31	699.21	483.35	554.63	441.47	650.47	421.23	504.39	461.39	674.84	452.29	529.51
3	Phule G 09103	384.55	638.31	379.53	467.46	377.11	632.98	381.25	463.78	380.83	635.65	380.39	465.62
4	ICCV 11112	451.10	652.61	453.65	519.12	434.55	655.88	436.52	508.99	442.82	654.25	445.09	514.05
5	ICCV 11117	444.34	757.59	442.25	548.06	414.07	703.46	418.56	512.03	429.20	730.53	430.41	530.05
	Susceptible Mean	414.30	653.19	414.20	493.90	402.45	641.73	401.36	481.85	408.38	647.46	407.78	487.87
6	Phule G 0511-43-2	338.02	670.56	339.52	449.37	319.94	676.97	321.21	439.38	328.98	673.77	330.37	444.37
7	Phule G 0752	320.01	769.57	315.56	468.38	360.84	784.74	367.85	504.48	340.43	777.16	341.71	486.43
8	ICC 4958	398.24	776.88	392.57	522.56	411.39	857.41	415.62	561.47	404.82	817.14	404.10	542.02
9	Vijay	333.67	881.34	335.02	516.68	318.13	771.52	324.20	471.28	325.90	826.43	329.61	493.98
10	Digvijay	306.64	743.36	315.25	455.08	336.50	778.01	334.36	482.96	321.57	760.69	324.81	469.02
	Tolerant mean	339.32	768.34	339.58	482.41	349.36	773.73	352.65	491.91	344.34	771.04	346.12	487.16
	Mean	376.81	710.77	376.89	488.16	375.91	707.73	377.01	486.88	376.36	709.25	376.95	487.52
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		1.86	5.31			1.33	3.80			1.99	5.58	
	Genotypes (G)		3.55	10.13			2.40	6.85			3.72	10.46	
	Interaction (G X C)		6.16	17.55			4.18	12.35			6.44	18.11	

In the year 2013-14, the genotype Phule-G-0405-44-2 (441.47 nmoles ascorbate oxidized mg^{-1} protein min^{-1}) recorded the highest APX activity at 50 per cent flowering under normal condition. The tolerant genotype, ICC 4958 demonstrated significantly highest APX activity of 857.41 nmoles ascorbate oxidized mg^{-1} protein min^{-1} than other genotypes.

On the basis of pooled analysis the tolerant genotypes Vijay (826.43 nmoles ascorbate oxidized mg^{-1} protein min^{-1}) exhibited significantly highest APX activity at 50 per cent flowering than all other genotypes except ICC 4958 (817.14 nmoles ascorbate oxidized mg^{-1} protein min^{-1}) under early drought stress condition. The susceptible genotype Phule-G-0305-3 (542.04 nmoles ascorbate oxidized mg^{-1} protein min^{-1}) recorded significantly lowest APX activity under early drought stress.

4.3.6.6 APX activity at grain filling stage

The effect of moisture stress on APX activity at grain filling stage in the leaves of chickpea genotypes is presented in Table 41. A considerable variation in APX activity amongst all genotypes was observed and APX activity was significantly increased in all genotypes under late drought stress than the non stressed normal condition. The difference amongst the genotypes over the season and their interactions were statistically significant for both the years 2012-13 and 2013-14 under stress condition as well as non-stress condition.

At grain filling stage, the genotype Vijay (308.78 nmoles ascorbate oxidized mg^{-1} protein min^{-1}) exhibited

Table 41 . Effect moisture stress on APX activity (nmoles ascorbate oxidized mg⁻¹ protein min⁻¹) at grain filling stage in the leaves of chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	237.77	242.01	273.31	251.03	227.19	240.31	287.79	251.77	232.48	241.16	280.55	251.40
2	Phule G 0405-44-2	248.87	265.85	356.62	290.45	258.21	290.21	391.30	313.24	253.54	278.03	373.96	301.84
3	Phule G 09103	248.40	274.78	371.29	298.15	262.25	274.42	376.70	304.45	255.32	274.60	373.99	301.30
4	ICCV 11112	282.33	296.80	358.84	312.65	251.99	283.89	361.90	299.26	267.16	290.34	360.37	305.96
5	ICCV 11117	275.45	306.76	397.33	326.51	296.48	285.78	357.70	313.32	285.97	296.27	377.51	319.92
	Susceptible Mean	258.57	277.24	351.48	295.76	259.22	274.92	355.08	296.41	258.89	276.08	353.28	296.08
6	Phule G 0511-43-2	282.54	306.84	493.51	360.96	262.69	291.01	479.58	344.43	272.62	298.92	486.55	352.70
7	Phule G 0752	307.60	318.22	462.28	362.70	311.34	339.14	499.62	383.36	309.47	328.68	480.95	373.03
8	ICC 4958	286.72	291.67	506.84	361.74	268.64	272.33	469.29	336.75	277.68	282.00	488.06	349.24
9	Vijay	308.78	317.87	541.47	389.37	282.33	307.22	500.78	363.44	295.55	312.55	521.12	376.41
10	Digvijay	277.67	288.32	482.78	349.59	285.34	303.90	488.53	359.26	281.51	296.11	485.65	354.42
	Tolerant mean	292.66	304.58	497.37	364.87	282.07	302.72	487.56	357.45	287.36	303.65	492.47	361.16
	Mean	275.61	290.91	424.43	330.32	270.65	288.82	421.32	326.93	273.13	289.87	422.87	328.62
			SE_±	CD(5%)			SE_±	CD(5%)			SE_±	CD(5%)	
	Conditions (C)		1.06	3.04			0.94	2.69			1.23	3.47	
	Genotypes (G)		3.22	9.17			3.33	9.50			4.01	11.2	
	Interaction (G X C)		5.57	15.88			5.78	16.46			6.95	19.59	

significantly the highest APX activity under normal condition than all genotypes except Phule-G-0752 (307.60 nmoles ascorbate oxidized mg^{-1} protein min^{-1}) during the year 2012-13. The tolerant genotype Vijay also demonstrated significantly highest APX activity of 541.47 nmoles ascorbate oxidized mg^{-1} protein min^{-1} than all other genotypes at grain filling stage under late drought stress condition.

In the year 2013-14 the tolerant genotypes Vijay (500.78 nmoles ascorbate oxidized mg^{-1} protein min^{-1}) recorded the highest APX activity at grain filling stage under late drought stress condition followed by the genotype Phule-G-0752 (499.62 nmoles ascorbate oxidized mg^{-1} protein min^{-1}).

On the basis of pooled analysis it is observed that, the tolerant genotype Vijay (521.12 nmoles ascorbate oxidized mg^{-1} protein min^{-1}) exhibited significantly the highest APX activity at grain filling stage under late drought stress condition.

4.3.7 Effect of moisture stress on yield contributing characters

4.3.7.1 Number of pods per plant

The data on number of pods per plant as influenced by moisture stress in chickpea genotypes are depicted in Table 42. The differences amongst the genotypes over the season and stress condition were statistically significant and their interactions were statistically significant for the year 2012-13 and 2013-14.

In the year, 2012-13 the genotype Vijay (77.70) recorded significantly highest number of pods under normal

Table 42 : Number of pods per plant as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	68.04	58.56	65.07	63.89	65.61	50.76	56.00	57.46	66.83	54.66	60.54	60.67
2	Phule G 0405-44-2	68.74	61.46	63.06	64.42	65.33	53.73	59.83	59.63	67.04	57.60	61.45	62.03
3	Phule G 09103	66.96	57.78	64.27	63.00	62.00	50.74	58.00	56.91	64.48	54.26	61.13	59.96
4	ICCV 11112	64.93	54.67	56.23	58.61	68.90	52.53	60.70	60.71	66.92	53.60	58.47	59.66
5	ICCV 11117	55.30	29.86	43.10	42.75	53.43	40.00	46.93	46.79	54.36	34.93	45.02	44.77
	Susceptible Mean	64.79	52.47	58.35	58.54	63.05	49.55	56.29	56.30	63.92	51.01	57.32	57.42
6	Phule G 0511-43-2	66.67	59.00	64.33	63.33	64.23	59.67	63.67	62.52	65.45	59.33	64.00	62.93
7	Phule G 0752	71.26	63.27	64.00	66.18	73.87	62.67	67.33	67.96	72.57	62.97	65.67	67.07
8	ICC 4958	52.67	46.00	50.33	49.67	55.33	51.67	52.60	53.20	54.00	48.83	51.47	51.43
9	Vijay	77.70	68.33	72.53	72.86	75.23	69.67	72.00	72.30	76.47	69.00	72.27	72.58
10	Digvijay	68.70	64.09	66.00	66.26	71.77	61.90	67.67	67.11	70.23	63.00	66.83	66.69
	Tolerant mean	67.40	60.14	63.44	63.66	68.09	61.11	64.65	64.62	67.74	60.63	64.05	64.14
	Mean	66.10	56.30	60.89	61.10	65.57	55.33	60.47	60.46	65.83	55.82	60.68	60.78
			SE₊	CD(5%)			SE₊	CD(5%)			SE₊	CD(5%)	
	Conditions (C)		0.32	0.91			0.24	0.68			0.35	0.97	
	Genotypes (G)		0.74	2.12			0.69	1.98			0.88	2.49	
	Interaction (G X C)		1.29	3.08			1.20	3.44			1.53	4.30	

condition. The genotype ICC 4958 (52.67) recorded the lowest number of pods per plant under normal condition. Under early drought stress condition, the susceptible genotype ICCV 11117 recorded significantly lowest (29.86) number of pods per plant, while genotypes Vijay maintained significantly highest (68.33) number of pods per plant. The genotype Vijay (72.53) also maintained significantly highest number of pods per plant under late drought stress condition while the susceptible genotypes ICCV 11117(43.10) recorded lowest number of pods per plant.

In the year 2013-14, similar trend was observed in the tolerant genotype Vijay, which recorded the highest (75.23, 69.67 and 72.00) number of pods per plant under normal, early drought and late drought stress condition.

On the basis of pooled analysis, the tolerant genotypes Vijay (76.47) recorded significantly highest number of pods per plant than all genotypes under normal condition except Phule-G-0752 (72.57). Under early and late drought stress, tolerant genotype Vijay maintained significantly highest (69.00 and 72.27) number of pods per plant, respectively.

4.3.7.2 Grain yield per plant (g)

The data on grain yield per plant (g) as influenced by moisture stress in chickpea genotypes are depicted in Table 43. The differences among the genotypes are the season under stress condition and their interactions were statistically significant.

In the year 2012-13, the genotype Digvijay recorded significantly highest grain yield per plant (19.97 g) under normal

Table 43 : Grain yield per plant (g) as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	16.38	10.87	13.29	13.51	15.37	10.71	12.62	12.90	15.87	10.79	12.96	13.21
2	Phule G 0405-44-2	15.90	11.40	13.66	13.65	15.22	10.88	12.83	12.98	15.56	11.14	13.24	13.31
3	Phule G 09103	13.28	9.31	11.29	11.29	12.36	8.39	10.79	10.51	12.82	8.85	11.04	10.90
4	ICCV 11112	17.97	12.08	14.15	14.73	16.07	10.12	13.18	13.12	17.02	11.10	13.66	13.93
5	ICCV 11117	16.56	9.98	14.63	13.72	16.66	9.45	14.31	13.47	16.61	9.71	14.47	13.60
	Susceptible Mean	16.02	10.73	13.40	13.38	15.14	9.91	12.75	12.60	15.58	10.32	13.07	12.99
6	Phule G 0511-43-2	16.29	13.76	14.61	14.89	14.82	12.34	13.34	13.50	15.56	13.05	13.98	14.19
7	Phule G 0752	15.81	13.44	14.44	14.56	15.98	14.01	15.01	15.00	15.90	13.72	14.73	14.78
8	ICC 4958	14.54	13.52	14.36	14.14	15.09	13.93	14.77	14.60	14.82	13.73	14.56	14.37
9	Vijay	16.13	14.17	15.39	15.23	16.23	14.28	15.11	15.20	16.18	14.23	15.25	15.22
10	Digvijay	19.97	17.33	17.80	18.37	19.84	16.96	18.05	18.28	19.90	17.15	17.92	18.32
	Tolerant mean	16.55	14.44	15.32	15.44	16.39	14.30	15.25	15.32	16.47	14.37	15.29	15.38
	Mean	16.28	12.59	14.36	14.41	15.76	12.11	14.00	13.96	16.02	12.35	14.18	14.18
			SE+	CD(5%)			SE+	CD(5%)			SE+	CD(5%)	
	Conditions (C)		0.11	0.32			0.15	0.42			0.16	0.46	
	Genotypes (G)		0.36	1.02			0.33	0.95			0.42	1.19	
	Interaction (G X C)		0.62	1.77			0.58	1.64			0.73	2.06	

condition followed by genotype ICCV 11112 (17.97 g). Under early drought stress the grain yield per plant was significantly reduced in all genotypes. However, the genotypes Digvijay (17.33 g) recorded the highest grain per plant followed by the genotype Vijay (14.77 g). Similar trend was observed under late drought stress, the genotypes Digvijay (17.80 g) recorded the highest grain yield per plant followed by Vijay (15.39 g).

In the year 2013-14 the genotypes Digvijay (19.84 g) recorded significantly highest grain yield per plant under normal condition. The tolerant genotype Digvijay (16.96 g) also maintained highest grain yield per plant under early drought stress condition, while susceptible genotypes ICC 11117 (9.45 g) recorded the lowest grain yield per plant. Significantly highest grain yield was recorded by Digvijay (18.05 g) under late drought stress condition.

On the basis of pooled analysis, the tolerant genotype Digvijay (19.90 and 17.15 and 17.92 g) recorded the highest grain yield per plant under normal, early drought stress and late drought condition. The susceptible genotypes Phule G-09103 (12.82, 8.85 and 11.04 g) recorded the lowest grain yield per plant under normal, early and late drought stress condition, respectively.

4.3.7.3 Test weight (g)

The test weight (g) as influenced by moisture stress in chickpea is depicted in Table 44. It was evident from the table that there was a considerable variation for 100 seed weight among

Table 44 : Test weight (g) as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	20.64	19.63	19.36	19.88	21.59	20.53	20.71	20.94	21.11	20.08	20.04	20.41
2	Phule G 0405-44-2	20.90	20.27	19.72	20.30	20.38	20.31	19.07	19.92	20.64	20.29	19.40	20.11
3	Phule G 09103	24.87	24.66	23.14	24.22	24.07	22.57	21.65	22.76	24.47	23.62	22.40	23.49
4	ICCV 11112	23.90	23.06	20.83	22.60	25.78	22.66	21.44	23.29	24.84	22.86	21.13	22.94
5	ICCV 11117	34.03	33.20	31.99	33.07	34.69	33.01	31.83	33.18	34.36	33.11	31.91	33.13
	Susceptible Mean	24.87	24.16	23.01	24.01	25.30	23.82	22.94	24.02	25.08	23.99	22.97	24.02
6	Phule G 0511-43-2	21.54	20.66	19.34	20.51	21.73	20.97	19.78	20.83	21.64	20.82	19.56	20.67
7	Phule G 0752	25.35	24.41	24.14	24.63	27.64	25.16	25.17	25.99	26.49	24.78	24.66	25.31
8	ICC 4958	34.61	32.60	32.26	33.16	33.90	32.68	30.55	32.38	34.26	32.64	31.40	32.77
9	Vijay	20.26	19.07	18.09	19.14	20.66	18.47	18.31	19.15	20.46	18.77	18.20	19.15
10	Digvijay	24.24	22.93	22.10	23.09	25.98	23.67	22.69	24.11	25.11	23.30	22.39	23.60
	Tolerant mean	25.67	24.44	23.76	24.62	26.51	24.74	24.02	25.09	26.09	24.59	23.89	24.86
	Mean	25.27	24.30	23.38	24.32	25.91	24.28	23.48	24.56	25.59	24.29	23.43	24.44
			SE+	CD(5%)			SE+	CD(5%)			SE+	CD(5%)	
	Conditions (C)		0.11	0.31			0.12	0.35			0.14	0.40	
	Genotypes (G)		0.30	0.85			0.29	0.82			0.36	1.01	
	Interaction (G X C)		0.52	NS			0.49	NS			0.62	NS	

all the genotypes studied. The difference among the genotype over the season was statistically significant and their interactions with moisture stress condition were statistically non-significant.

In the year 2012-13, the genotype ICC 4958 recorded the highest (34.61 g) test weight under normal condition followed by ICC 11117 (34.03 g). Under early drought stress genotype ICCV 11117 recorded the highest (33.20 g) test weight. The genotypes ICC 4958 (32.26 g) exhibited the highest test weight followed by the genotypes ICCV 11117 (31.99 g) under late drought stress condition.

In the year 2013-14, the genotype ICCV 11117 recorded the highest test weight (34.69, 33.01 and 31.83 g) under normal, early drought and late drought stress condition, followed by ICC 4958 (33.90, 32.68 and 30.55 g) respectively. While the genotype Vijay recorded the lowest test weight (20.66, 18.47 and 18.31 g) under normal, early drought and late drought stress condition, respectively.

On the basis of pooled analysis, the genotype ICCV 11117 exhibited the highest test weight (34.36, 33.11 and 31.91 g) under normal early and late drought stress condition, respectively.

4.3.7.4 Harvest index (%)

The data on harvest index as influenced by moisture stress in chickpea genotype are presented in Table 45. A considerable variation was not observed in harvest index amongst all genotypes studied.

Table 45 : Harvest index as influenced by moisture stress in chickpea genotypes

SN	Year / Genotype	2012-13				2013-14				Pooled			
		Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean	Normal	EDS	LDS	Mean
1	Phule G-0305-3	34.10	32.28	31.25	32.54	36.62	34.56	34.02	35.07	35.36	33.42	32.64	33.81
2	Phule G 0405-44-2	30.25	28.56	28.23	29.01	28.55	30.23	28.62	29.13	29.40	29.40	28.43	29.07
3	Phule G 09103	34.56	32.25	30.26	32.36	34.56	33.25	29.52	32.44	34.56	32.75	29.89	32.40
4	ICCV 11112	27.89	26.52	25.56	26.66	31.25	29.52	26.59	29.12	29.57	28.02	26.08	27.89
5	ICCV 11117	36.61	33.25	31.25	33.70	34.82	32.31	32.28	33.14	35.72	32.78	31.76	33.42
	Susceptible Mean	32.68	30.57	29.31	30.85	33.16	31.97	30.21	31.78	32.92	31.27	29.76	31.32
6	Phule G 0511-43-2	34.26	32.25	31.56	32.69	32.26	31.29	30.29	31.28	33.26	31.77	30.93	31.99
7	Phule G 0752	38.03	36.65	36.01	36.90	34.37	34.02	33.31	33.90	36.20	35.34	34.66	35.40
8	ICC 4958	34.12	32.92	32.06	33.03	34.50	33.15	31.90	33.18	34.31	33.04	31.98	33.11
9	Vijay	41.15	37.69	35.52	38.12	37.86	35.86	35.07	36.26	39.51	36.78	35.30	37.19
10	Digvijay	36.38	35.03	34.02	35.14	36.42	34.56	33.99	34.99	36.40	34.80	34.01	35.07
	Tolerant mean	36.79	34.91	33.83	35.18	35.08	33.78	32.91	33.92	35.94	34.34	33.37	34.55
	Mean	34.73	32.74	31.57	33.02	34.12	32.88	31.56	32.85	34.43	32.81	31.57	32.93
			SE+	CD(5%)			SE+	CD(5%)			SE+	CD(5%)	
	Conditions (C)		0.46	1.32			0.35	1.01			0.50	1.43	
	Genotypes (G)		0.81	2.31			1.04	2.96			1.14	3.21	
	Interaction (G X C)		1.53	NS			1.80	NS			1.98	NS	

The differences amongst the genotypes over the season were statistically significant for the year 2012-13 and 2013-14 and their interaction were non-significant under stress condition.

In the year 2012-13, the genotype Vijay (41.15 and 37.69%) recorded the highest harvest index followed by the genotype Phule-G-0752 (38.03 % and 36.85 %) under normal and early drought stress condition, respectively.

In the year 2013-14 the genotypes Vijay (37.80, 35.86 and 35.07 %) recorded the highest harvest index followed by the genotype Digvijay (36.42, 34.56 and 33.99 %) at normal, early and late drought stress condition, respectively.

On the basis of average of two year, similar trend was observed. The genotype Vijay (39.51, 36.78 and 35.30 %) recorded highest harvest index at normal, early and late drought stress condition, respectively. The susceptible genotype ICC 11112 recorded the lowest (28.02 and 26.08 %) harvest index at early and late drought stress condition, respectively.

4.3.8 Correlation study

The data on correlation between physio-biochemical parameter and yield and yield contributing character in chickpea under moisture stress at pre and post anthesis are depicted in Table 46.

During the year, 2012-13 at pre flowering stress the physiological parameters at 50 per cent flowering *viz.*, rate of photosynthesis (0.79**), SCMR value (0.74**) and relative water content (0.74**) showed significant and positive correlation with

Table 46 . Correlation between physio-biochemical parameters and yield and yield contributing characters in chickpea under moisture stress at pre and post anthesis.

SN	Characters	EDS		LDS	
		2012-13	2013-14	2012-13	2013-14
1.	Days to 50% Flowering	0.33	0.25	0.09	-0.20
2.	Days to maturity	0.19	0.09	0.32	0.05
3.	Rate of photosynthesis at 50 %flowering	0.79**	0.79**	0.71**	0.51**
4.	Rate of photosynthesis at maturity	0.55**	0.64**	0.53**	0.75**
5.	Rate of transpiration 50 % flowering	-0.40*	-0.66**	-0.32	-0.08
6.	Rate of transpiration at maturity	-0.37*	-0.72**	-0.27	-0.45*
7.	Stomatal conductance 50 % flowering	0.68**	0.71**	0.36*	0.07
8.	Stomatal conductance at maturity	0.43**	0.87**	0.54**	0.55**
9.	SCMR value 50 % flowering	0.74**	0.86**	0.50**	0.60**
10.	SCMR value at maturity	0.47**	0.55**	0.40	0.66**
11.	Pant height 50 % flowering	0.35	0.09	0.09	-0.18
12.	Pant height at maturity	0.01	0.26	0.10	0.11
13.	No. of secondary branches 50 %flowering	0.82**	0.76**	0.36*	0.21
14.	No. of secondary branches at maturity	0.80**	0.78**	0.50**	0.50**
15.	Relative water content 50 % flowering	0.69**	0.85**	0.25	0.32
16.	Relative water content at maturity	0.74**	0.74**	0.63**	0.59**
17.	Proline 50 % flowering	0.79**	0.79**	0.07	0.37
18.	Proline at maturity	0.57**	0.84**	0.58**	0.67**
19.	Glycine betaine 50 % flowering	0.65**	0.82**	0.11	0.55**
20.	Glycine betaine at maturity	0.31	0.36*	0.65**	0.50**
21.	SOD activity 50 % flowering	0.74**	0.77**	0.10	0.33
22.	SOD activity at maturity	0.61**	0.12	0.58**	0.66**
23.	CAT activity 50 % flowering	0.66**	0.86**	0.21	0.14
24.	CAT activity at maturity	0.74**	0.44*	0.67**	0.77**
25.	APX activity 50 % flowering	0.44*	0.71**	0.25	0.27
26.	APX activity at maturity	0.30	0.33	0.51**	0.60**
27.	No. of pods	0.42*	0.67**	0.26	0.33
28.	Test weight	0.16	0.01	0.01	0.26

grain yield per plant. Significantly positive correlation with grain yield per plant was also observed during the year 2013-14. Rate of transpiration at 50 per cent flowering showed negative correlation (-0.40** and -0.66**) with grain yield per plant, during the year 2012-13 and 2013-14.

In the present study, number of secondary branches at maturity under pre- (0.80** and 0.78**) and post- (0.50**) flowerings showed positive correlation with grain yield per plant during the year 2012-13 and 2013-14.

The osmolytes, like proline (0.79**) and glycine betaine (0.65** and 0.82**) at 50 per cent flowering showed positive correlation with grain yield per plant under pre flowering stress during the year 2012-13 and 2013-14. These parameters , proline (0.58** and 0.67**) and glycine betaine (0.65** and 0.50**) at grain filling stage under late drought stress also showed significant and positive correlation with grain yield per plant during the year 2012-13 and 2013-14.

The antioxidative enzymes viz., SOD (0.74** and 0.77**), CAT activity (0.66** and 0.86**) and APX activity (0.44** and 0.71**) at 50 per cent flowering were showed significant and positive correlation grain yield per plant under pre flowering stress during the year 2012-13 and 2013-14, respectively. At grain filling stage, under post flowering stress SOD (0.58** and 0.66**), CAT (0.67** and 0.77**) and APX (0.51** and 0.60**) showed significant and positive correlation with grain yield per plant for both the years. The number of pods showed significant and positive correlation with grain yield per plant for the year 2012-13 and 2013-14.

5. DISCUSSION

The investigation entitled, “Physio-biochemical analysis for growth and yield in Chickpea under moisture stress” was undertaken during *rabi* 2012-13 and 2013-14 at Pulses Improvement Project, Central Campus, Mahatma Phule Krishi Vidyapeeth, Rahuri under rainout shelter.

Total thirty chickpea genotypes were screened for moisture stress induced by PEG 6000 with five different osmotic potential levels of 0, -0.2, -0.4, -0.6 and -0.8 Mpa. On the basis of germination percentage, root and shoot length, vigour index and proline content five susceptible and five tolerant genotypes were selected for pot and field experiment. In pot study these genotypes were subjected to two progressive stresses i.e. moderate (-0.4 Mpa) and severe (-1.2 MPa) in pot study. The water stress condition was created by irrigating the pots with PEG solution and comparative physio-biochemical studies were performed in control and moisture stress treatments. The same set of chickpea genotypes were evaluated with three replications under three conditions *viz.*, normal, early and late drought stress with a view to study the relative sensitivity of chickpea genotypes under rain out shelter in respect of phenological, physiological, biochemical and yield and yield contributing characters. The results presented in previous chapter are discussed here under.

5.1 Screening of chickpea genotypes under polyethylene glycol (PEG 6000) induced water stress

Identifying drought tolerant mechanism is essential for measuring stress resistance in large breeding population. For drought stress, polyethylene glycol (PEG 6000) compound has been used to

induce the osmotic stress to maintain uniform water potential throughout experimental period. Using such methodology, selection from a large number of breeding lines can be done shortly and economically.

5.1.1 Germination percentage

The mean germination per cent of chickpea genotype as shown in Table 2a was 88 per cent in control and it was declined with decrease in osmotic potential of -0.2 MPa with (72.9%), -0.4 MPa (54.8 %), -0.6 MPa (11.4%) . At -0.6 MPa stress condition only fourteen genotypes showed resistance to stress and germination per cent was ranged from 10 to 47%. The germination in all genotypes stopped completely at -0.8 MPa moisture stress. The genotype Digvijay showed maximum germination percentage (47.5%) at -0.6 MPa, followed by ICC 4958 (45%).

The primary action of osmotic inhibition is retardation of water uptake which is crucial for germination. The results of this study are in agreement with the observations of Yucel *et al.* (2010) in chickpea. They observed that PEG adversely affects germination percentage.

The results of this study are also in agreement with the observation of Gurbuz *et al.* (2009) and Macar *et al.* (2009) in chickpea. Gupta *et al.* (1993), Gholami *et al.* (2010) and Homayoun *et al.* (2011) observed significant decrease in percentage of germination, germination rate and growth parameters. Sleimi *et al.* (2013) observed that at high PEG concentration (10 g L⁻¹), the inhibition of seed germination was highly significant for all the chickpea varieties studied.

5.1.2 Root length (cm)

The root length (cm) was significantly decreased from 20.07 to 13.99, 11.09 and 6.91 cm under 0 to -0.2 MPa, -0.4 MPa and -0.6 MPa osmotic potentials, respectively. The inhibition of growth under stress condition is the result of inhibition of cell division, cell elongation or both. These results are in agreement with Gholami *et al.* (2010) and Alam *et al.* (2002), who reported decreased root length with decreased osmotic potential. They also noted that decreasing the root length which was varied between cultivar as well as between osmotic potentials.

The genotype Phule G-511-43-2 was found superior in respect of root length under osmotic stress condition. Veer and Sharma (2010) reported that the length of stem, root and number of lateral roots were decreased with increase in PEG concentration in blackgram. Macar *et al.* (2009) reported that root lengths of all chickpea genotypes at -0.4 MPa potential of both PEG and NaCl were found to be less than the control. The results are also in agreement with Yucel *et al.* (2010).

5.1.3 Shoot length (cm)

It has been suggested that osmotic stress modifies the biochemical changes taking place in cell wall during growth thereby preventing the extension. Not only germination was inhibited but growth of seedlings was also affected as the osmotic potential was decreased. The genotype ICC 4958 (11.65 and 8.05 cm) recorded the highest shoot length at -0.2 and -0.4 MPa PEG induced osmotic stress. At -0.6 MPa the genotype Vijay recorded highest shoot length (2.50 cm). These results showed that radical and plumule growth of the seedling was adversely affected by water stress.

Gupta *et al.* (1993) found that the length and biomass of epicotyls and hypocotyls were decreased under PEG induced water stress in chickpea. Macar *et al.* (2009) reported that the length of epicotyls of all the chickpea cultivars and lines decreased significantly with increasing PEG potentials and there was no considerable epicotyls elongation in all genotypes at -0.8 MPa.

The root and shoot length are the most important parameter for drought stress and the responses of chickpea genotypes were varied to different osmotic potentials. In our study, shoot length was more adversely affected than the root length. The results are in agreement with Yucel *et al.* (2010) and Okcu *et al.* (2005) who observed that the water stress decreased the shoot growth rather than root growth in chickpea and pea.

5.1.4 Root: Shoot Ratio

The root/shoot length ratio was 1.17, 1.91, 2.33 and 3.00 under 0, -0.2, -0.4 and -0.8 MPa osmotic stress, respectively (Table 2 b). The genotype Phule G-0701 recorded the highest root /shoot length ratio of 4.58 and 11.44 at -0.4 and -0.6 MPa osmotic stress, respectively.

The shoot growth is more sensitive than root growth to water stress. The reason may be that root is the first organ emerged from the seed; therefore its growth is faster than shoot growth. Macar *et al.* (2009) found that drought stress brought by PEG 6000 inhibited epicotyls elongation more than the root growth and decreased the shoot/root ratio.

5.1.5 Vigour Index

The genotype Phule G-0752 recorded the highest vigour index (24.77 and 17.42) at -0.2 and -0.4 MPa osmotic stress,

while the highest vigour index (5.20) at -0.6 MPa was recorded by the genotype Phule G-0511-43-2 .

5.1.6 Proline

The proline accumulation was increased in all the genotypes in response to decreasing osmotic potentials of PEG 6000 from 0 to -0.6 MPa. The genotype Vijay (12.67 and 18.84 μ moles g^{-1}fw) and Phule G-0752 (14.09 and 17.21 μ moles g^{-1}fw) accumulated the highest proline at -0.4 MPa and -0.6 MPa , respectively. While the genotypes Phule G-0405-44-2 and ICCV 11117 were recorded lowest (7.34 and 7.98 μ moles g^{-1}fw) proline content in leaves at -0.4 MPa osmotic stress.

Increase of proline causes the mediation of osmotic adjustment and thus, the plant will keep growing under drought stress. In addition to this, proline has a good impact on maintaining the structure of the enzymes and removal of reactive oxygen species (Kumar *et al.*, 2006). Since proline has hydrophilic property it might replace water molecules around nucleic acid, protein and membrane during water shortage. It might also prevent interaction between destabilize ions and cellular components by replacing the water molecules around these compounds, thereby protecting against destabilizing during moisture stress (Bayomi *et al.*, 2008).

On the basis of high germination percentage, under higher osmotic potential more root and shoot length, vigour index and proline content, Phule G 0511-43-2, Phule G 0752, ICC 4958, Vijay and Digvijay were selected as drought tolerant genotypes. The genotypes *viz.*, Phule G-0305-3, Phule G-0405-44-2, Phule G 09103, ICCV 11 112 and ICCV 11 117 were found more sensitive to drought and used as drought susceptible for pot and field study.

5.2 Physiological and biochemical basis for moisture stress tolerance in chickpea (pot study).

Chickpea responses were studied to progressive induced different levels of osmotic stress in the pot by using PEG 6000. Several methods which range from withdrawal of water to plants to the use of chemicals such as polyethylene glycol, mannitol etc. are being employed to create water stress in plants. It has been reasonably well established that polyethylene glycol induced water stress mimics that caused by withdrawal of water from plants.

5.2.1 Photosynthetic rate

In the present study, the PEG induced water stress decreased the net photosynthesis as osmotic stress increased. The significant decrease in the rate of photosynthesis was observed in both progressive mild and severe stress. A decrease of 55.4 and 29 per cent was noted in susceptible and tolerant genotypes under moderate stress, while sharp decrease of 88 and 75 per cent was observed in susceptible and tolerant genotypes, respectively, as compared to control under severe stress.

Stomatal closure can serve as a rapid and effective drought avoidance response. However, prolonged stomatal closure is not suitable as stomatal CO₂ uptake is also reduced and will ultimately limit photosynthesis assimilation and growth. The genotype ICC 4958 recorded the highest mean rate of photosynthesis (10.73 and 3.59 $\mu\text{mol m}^{-2}\text{s}^{-1}$) under moderate and severe stress, respectively. Decreased CO₂ diffusion from the atmosphere to the site of carboxylation is generally considered the main cause of decreased

photosynthesis under mild to moderate water limitations (Chaves *et al.*, 2003; 2009 and Grassi and Magnani, 2005)

The observations recorded in present investigation are in agreement with Basu *et al.* (2007) who reported that, the photosynthesis (P_{max}) and transpiration decreased concurrently in two chickpea lines, M 129 and Tyson with increasing water stress. Similar results were reported by Kataria and Singh (2013). Chaves *et al.* (2009) observed decrease in photosynthetic rate under stressful condition (salinity, drought and temperature) was normally attributed to a suppression in the mesophyll conductance and the stomata closure at moderate and severe stress.

5.2.2 Transpiration rate

The mean rate of transpiration was higher in unstressed control condition than in moderate and severe stress. The reduction in rate of transpiration was with 11.97 per cent in susceptible genotypes and in tolerant genotypes with 16.60 per cent over unstressed control under moderate stress.

The higher per cent decrease in the rate of transpiration of 84.3 was observed in tolerant genotypes than the susceptible genotypes with 77.1 per cent reduction under severe stress. Limited rate of transpiration was observed by the tolerant genotype ICC 4958 (1.39 and 0.43 m mol m⁻²s⁻¹) followed by Vijay (1.45 and 0.53 m mol m⁻²s⁻¹) under moderate and severe stress, respectively.

Basu *et al.* (2007) reported that the transpiration decreased concurrently in two chickpea lines M 129 and Tyson with increasing water stress. Stomata closure is known to have a more inhibitory effect on transpiration of water than that of CO₂ diffusion into leaf tissue (Chaves *et al.*, 2009 and Sikuku *et al.*, 2010).

5.2.3 Stomatal Conductance

Stomatal conductance seems to be the main factor in photosynthesis and limited transpiration under drought conditions. The mean stomatal conductance of the leaves of chickpea genotypes was significantly decreased under stress condition. The tolerant genotypes Vijay showed minimum reduction in stomatal conductance which was decreased from 0.22 to 0.15 and 0.22 to 0.11 mol m⁻²s⁻¹ under moderate and severe stress, respectively.

Ritchi *et al.* (1990) observed in wheat that the most drought resistant genotypes had greater stomatal conductance under water stress condition than the susceptible genotypes. Krouma (2010) reported that the tolerant chickpea genotypes had highest stomatal conductance, which controls photosynthesis and transpiration and in turn, plant growth.

In the present study, significant decrease in net photosynthesis, stomatal conductance and transpiration were associated with the significant decrease in plant growth. The tolerant genotypes ICC 4958, Digvijay and Vijay had the higher photosynthetic activity and stomatal conductance under stress. Decrease in plant water status was associated with significant decline in net photosynthetic rates. Decreased relative water content under drought leading to reduced leaf turgour might be responsible for the observed reduction in photosynthesis during the present study. Reduction in stomatal conductance under stress was reported in linseed by Khan *et al.* (2010) and in chickpea by Hirich *et al.* (2014).

5.2.4 SCMR values

The chlorophyll meter (SPAD-502 Minolta, Tokyo, Japan) also known as SPAD (Soil Plant Analysis Development) meter, can

quickly and reliably assesses the N status of a crop based on leaf area. In addition, a SPAD chlorophyll meter reading (SCMR) is an indicator of the photosynthetically active light –transmittance characteristics of the leaf, which is dependent on the amount of chlorophyll per unit leaf area (chlorophyll density). The ability to maintain high chlorophyll density under water deficit condition has suggested as a drought tolerance in barley (This *et al.*, 2000).

Leaf photosynthesis is generally correlated with chlorophyll content per unit leaf area and hence the SPAD chlorophyll meter can provide a useful tool to screen the genotype variation in potential photosynthetic capacity under drought condition.

SCMR value was affected during present investigation which shows that progressive stress along with some other environmental factor may affect photosynthetic ability of the plant system. Overall on the mean basis, it is observed that susceptible genotypes Phule G-09103 (38.04) and Phule G-0305-3 (39.05) were more sensitive than the tolerant genotypes Vijay (48.44) and ICC 4958 (48.02). Minimum reduction in RLWC was observed in Vijay from 57.50 to 47.25 and 40.48 per cent (17.9 and 29.7 per cent decrease) over unstressed plant, respectively. A reason for decrease in SCMR value as affected by water stress deficit is that the moisture stress produces reactive oxygen species (ROS) such as O_2^- and H_2O_2 , which can lead to lipid peroxidation and consequently, chlorophyll destruction with decreasing chlorophyll content due to changing green colour of the leaf into yellow. Naderikharaji *et al.* (2008) reported similar decrease in SPAD value under stress in rapeseed.

5.2.5 Relative Water Content (RLWC)

Limited supply of water lead to dehydration of plants, decrease in RLWC and loss of turgour of leaves, which can results in stomata closes. Maximum decrease in RLWC from 68.32 to 56.87 and 30.58 per cent (16.7 and 55.2 per cent reduction) was observed in susceptible genotype Phule G-09 103 at moderate and severe stress. While minimum decrease in RLWC over control plants was observed in ICC 4958 with 14.2 and 18.18 per cent reduction in both progressive moderate and severe stress, respectively.

The ability of plant to maintain the turgour and related physiological process even under water stress condition has a great practical significance and it is related with drought resistance in terms of osmoregulatory activities. Decrease in RLWC in PEG induced water stress was reported in rice leaves (Zgalli, 2005 and Bayomi *et al.*, 2008).

5.2.6 Proline

A variety of organic solutes accumulates in osmotically stressed plants in which proline appears to be widely distributed osmolytes under stress condition. In the present investigation, it was observed (Table 8) that a severe progressive stress in chickpea leads to about 3.35 fold more accumulation of proline as compare to control while 1.57 fold increase was observed in progressive mild stress.

The tolerant genotypes Digvijay, Vijay and ICC 4958 accumulated higher proline content under stress condition. The highest per cent increase of 78.7 and 281.5 was observed in tolerant genotype than 32.3 and 182.2 in susceptible genotypes under moderate and severe stress, respectively over unstressed control.

Several investigators have reported the significant positive relationship between free proline content in leaves and PEG –induced osmotic stress in various crops as observed in the present study. Patil (2010) observed that PEG stress increased mean proline content upto 7-folds in tolerant as compared to 3-folds in susceptible sorghum genotypes. Kumar *et al.* (2011a) observed that a severe progressive stress in pigeon pea leads to about 25 fold more accumulation of proline as compared to control of same age group while 6 fold increase was observed in progressive mild stress. Zgali *et al.* (2005) reported 10 fold increase in proline accumulation under PEG induced water stress condition in tomato plants.

In the present study, an increase in proline accumulation on imposition of osmotic stress by PEG may be due to multifunctional role of proline 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 to recover from stress as proposed by Szabados and Savoure (2009).

5.2.7 Glycine Betaine

The effect of PEG 6000 induced osmotic stress of -0.4 MPa and -1.2 MPa clearly demonstrated significant variation in glycine betaine accumulation among susceptible and tolerant genotypes. The mean per cent increase in the level of glycine betaine in the tolerant genotypes was 72.2 and 189.9 per cent as against 41.7 and 93.3 per cent in susceptible genotypes under moderate and severe stress, respectively. The susceptible genotypes showed the lowest accumulation in glycine betaine under stress condition probably because of reduction in relative leaf water content.

The drought tolerant genotypes recorded more GB than susceptible ones. In the present study, an increase in GB accumulation on imposition of water stress by PEG may be due to choline monooxygenase activity, the rate-limiting step for GB synthesis and expression of gene induced under osmotic stress (Rathinasabapathi *et al.*, 1997). Patil (2010) observed that PEG-6000 -induced osmotic stress increased mean GB content up to 3.71 -folds in tolerant as compared to 1.57-folds in the leaves of susceptible *rabi* sorghum genotypes.

The tolerant genotype ICC 4958 accumulated significantly highest glycine betaine content of 25.16 and 12.65 $\mu\text{mol g}^{-1}\text{fw}$ followed by Digvijay with 22.96 and 14.23 $\mu\text{mol g}^{-1}\text{fw}$ under severe and moderate stress condition, respectively than all genotypes under study.

The results are in agreement with Khan *et al.* (2010) and Wu *et al.* (2014), who reported the significant increase in glycine betaine content in linseed and sorghum seedlings under NaCl stress compared to the control.

Antioxidative enzymes :

5.2.8 Super oxide dismutase

Superoxide radicals are toxic byproducts of oxidative metabolism. Thus, the dismutation of superoxide radicals into H_2O_2 and O_2 by SOD is necessary to protect the plant tissue from damage. It is found that SOD activity of tolerant genotypes was higher than the sensitive genotypes. The results (Table 10) revealed that SOD activity differ between susceptible and tolerant genotypes under PEG induced osmotic stress. The higher per cent increase in SOD activity of 88.8 and 184.7 per cent in tolerant genotypes than 69.1 and 143.6

per cent increase in susceptible genotype over unstressed control at -0.4 MPa and -1.2 MPa, respectively. The present study shows that tolerant genotypes probably developed efficient antioxidative defense system than susceptible ones to dismutase active oxygen species generated to cope with oxidative stress under extremely adverse condition.

Similar results were reported in various crops by several investigators. Kumar *et al.* (2011a) reported that the activities of SOD and POD in pigeon pea enhances by increasing in both progressive stress induced by PEG as compare to control.

Ahmadizadeh *et al.* (2011) have reported that SOD can be used as one of the biochemical markers in indirect selection of drought tolerant genotypes using 20 per cent PEG under laboratory condition in wheat

5.2.9 Catalase

The catalase activity of chickpea leaves was increased in both mild and severe stress condition as compare to control plant leaves (Table 11). Catalase is tetrameric heme containing enzymes with the potential to directly dismutase H_2O_2 into H_2O and O_2 and is indispensable for ROS detoxification during stress condition. The higher per cent increase in CAT activity of 83 and 331.2 in tolerant genotypes and a minimum of 46.2 and 217.9 per cent in susceptible types over unstressed control under moderate and severe stress, respectively.

The higher activity observed in tolerant genotypes *viz.*, ICC 4958, Vijay and Digvijay indicate their ability to scavenge harmful radicals under water stress conditions which could cause membrane

damage. The significantly highest catalase activity of 36.02 $\mu\text{mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1} was found in ICC 4958 than genotypes under severe stress (-1.2 MPa) condition while Vijay (15.36 $\mu\text{mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1}) showed significantly highest catalase activity under moderate stress (-0.4 MPa). The increase in catalase activity was reported in several crops under NaCl stressed condition : in linseed by Khan *et al.* (2010), in cucumber by Du *et al.* (2010) and in chickpea by Rasool *et al.* (2013).

5.2.10 Ascorbate Peroxidase Activity

The results revealed that the stressed leaves of susceptible chickpea genotypes showed lower mean APX activity than tolerant genotypes. As regards the mean per cent increase over control, the susceptible types exhibited a much lower per cent increase of 80.3 and 171.6 while tolerant types with higher mean of 195.4 and 313.2 per cent under moderate and severe stress, respectively.

APX is involved in scavenging of H_2O_2 in water-water and ASH-GSH cycle and utilizes ASH as the electron donor. Significantly highest activity of 1521.66 and 1015.51 n moles of ascorbate oxidized mg^{-1} protein min^{-1} was observed in a tolerant genotype ICC 4958 at severe and moderate stress, respectively.

Similar results were reported in various crops by several researchers. Vardhini and Rao (2003) reported that in sorghum, the ascorbate peroxidase activity was increased upto 20 per cent PEG-induced osmotic stress in drought tolerant as well as susceptible cultivars than the unstressed control.

The results were in agreement with the findings of Turkan *et al.* (2005) and Wang *et al.* (2009) who reported an increase in APX activity in *Phaseolus oculifolius* and alfalfa seedlings under PEG

induced osmotic stress. Raheleh *et al.* (2012) also reported that the chickpea leaf APX activity in tolerant genotypes was higher than the susceptible genotypes under drought condition.

Thus, it indicates that plants have developed an antioxidant defense system to cope with oxidative damage under extremely adverse conditions that include H₂O₂ sensitive antioxidative enzyme APX.

5.3 Physiological and biochemical basis for moisture Stress tolerance in chickpea under rain out shelter

Soil moisture stress is a major hazard to successful crop production throughout the world. It reduces the productivity by delay or prevention crop establishment, destruction of established crop, alteration of physiological and biochemical metabolism in plant. The field experiment was carried out during *rabi* 2012-13 and 2013-14 in rain out shelter at Pulses Improvement Project, MPKV, Rahuri.

Five susceptible and five tolerant genotypes were grouped in three sets *viz.*, irrigated, EDS and LDS. Water stress treatment was investigated at 40 DAS. Normal plans were given three irrigations (at 20, 40 and 60 DAS) from the date of sowing to maturity. Early drought stressed or pre-anthesis drought stressed plants (EDS) received two irrigations (20 and 60 DAS) whereas post-anthesis drought stressed (EDS) plant received two irrigations at (20 and 40 DAS). Stress cycle of seven days was given in both the condition.

5.3.1 50 % flowering

Days to 50 % flowering were significantly affected due to moisture stress and the plant under moisture stress enhanced

flowering (38 to 43 DAS) whereas well-watering treatments had delayed flowering (43 to 50 days).

The delay in days to 50 per cent flowering suggested that irrigation prolongs the vegetative period. Overall, the genotype Vijay (38.33 DAS) required lowest number of days to 50 per cent flowering, followed by Phule-G-09103 (38.50) under early drought stress, while under well watering treatment the genotype Phule-G-0305-3 (50.33 DAS) required highest number of days to 50 per cent flowering.

Kanouni (2001) have reported delay in flowering due to irrigation in chickpea. Similar results were reported by Yaqoob *et al.* (2012), Bakhsh *et al.* (2007) who observed that chickpea plants under moisture stress had produced early flowering.

5.3.2 Days to maturity

Overall days to maturity of tested chickpea genotypes ranged from 107 days in Phule-G-305-3 to 111.50 in Phule-G-09103 under normal condition (Table 15). Under late drought stress days to maturity ranged from 99.33 days in Phule-G-0305-3 to 105.83 in ICC 4958.

As a matter of fact, moisture stress always shortens the life cycle of crop plant which results in early maturity. The above results indicate that the genotypes which have faster vegetative growth with early start to vegetative phase may help in escaping the terminal drought.

The results are in agreement with the result of Khatri *et al.* (2004) which were reported that some varieties mature earlier than all the entries indicating that they can escape from high temperature stress prevailing at later stage of the crop season. Similar results were reported by Yaqoob *et al.* (2012) in chickpea.

5.3.3 Photosynthesis

All the plants cultivated under drought stress exhibited a significant decrease in photosynthesis activity as compared to the control. At 50 per cent flowering, the rate of photosynthesis was decreased by 30 and 13 per cent in susceptible and tolerant genotypes, respectively under early drought stress over normal condition.

One of the main reason why water stress inhibits growth and photosynthetic abilities of plants in the breakdown of balance between the production of reactive oxygen species (ROS) and the antioxidant defense. The water stress markedly inhibit rate of photosynthesis at grain filling stage. The tolerant genotypes showed highest pooled mean rate of photosynthesis ($5.80 \mu \text{mole m}^{-2}\text{s}^{-1}$) as compared to susceptible genotypes ($4.45 \mu \text{mole m}^{-2}\text{s}^{-1}$) under late drought stress condition due to withholding of irrigation at 60 DAS.

Reduction of photosynthesis is consider to be major yield limiting factor, as it influences leaf expansion, leaf senescence, portioning of assimilates and biomass production. So the yield component affected most by drought is dry matter accumulation largely as a consequence of poor canopy expansion.

The observation recorded in the present investigation are in agreement with the findings of Konwar *et al.* (2007) , Channappagondar *et al.* (2010), Kataria and Singh (2013) and Iqbal *et al.* (2014) they reported significant reduction in rate of photosynthesis, respiration, intercellular CO_2 and stomatal conductance under stress and the drought resistant promising entries exhibited higher values of photosynthetic rate, lowest transpiration rate and higher values of RWC.

A strong relationship between biomass production and net photosynthesis under water deficit was reported by Krouma (2010). Overall, the tolerant genotype Digvijay recorded the highest mean rate of photosynthesis under stress conditions at 50 per cent flowering as well as at grain filling stage.

The present results showed that the growth, photosynthetic activity and osmotic adjustment were interdependent. We suggest that the tolerance or sensitivity of chickpea at drought is related to its capability to maintain good leaf water status. This parameter is highly correlated with stomatal conductance, which controls photosynthesis and transpiration and in turn, plant growth. The tolerance observed in the genotypes Digvijay, ICC 4958 and Vijay seems to have been the result of the equilibrium between many physiological parameters (RLWC, stomatal conductance, photosynthesis and transpiration) ensuring the plant growth under drought stress.

5.3.4 Transpiration

The rate of transpiration was significantly reduced under moisture stress. However, the tolerant genotypes showed limited rate of transpiration under stress condition as compared to susceptible genotypes. At 50 per cent flowering, transpiration was decreased by 21 and 15.1 per cent in tolerant and susceptible genotypes, respectively under early drought stress. The transpiration rate was significantly decreased under late drought stress followed by early drought stress at grain filling stage.

Overall, the tolerant chickpea genotypes *viz.*, Vijay, ICC 4958 and Digvijay recorded limited rate of transpiration under stress condition. Ashraf and Bashir (2003) and Krouma (2010) reported

that the transpiration rate was significantly decreased by drought. Crop plant with limited water availability seems to use water more economically. Water stress conditions leads to change in leaf hydraulics, which leads to closure of stomata due to fall of water potential. Stomata closer inhibits the transpiration process.

5.3.5 Stomatal conductance

Stomatal conductance seems to be main factor in higher photosynthesis and limited transpiration under drought condition.

The mean stomatal conductance was decreased from 0.22 to 0.16 mol m⁻²s⁻¹ in susceptible genotypes from control to stressed plants while 0.25 to 0.21 mol m⁻²s⁻¹ in tolerant chickpea genotypes at 50 per cent flowering stage. At grain filling stage, the tolerant genotype Vijay recorded highest 0.16, 0.15 and 0.14 mol m⁻²s⁻¹ stomatal conductance under normal, early drought and late drought stress conditions, respectively. Stomatal closure is a drought avoidance mechanism and is one of the first step in a plants adaptation to water deficit, allowing the water status to be maintained in the plant. For most of the plants drought avoidance is achieved primarily through regulation of stomatal conductance in response to soil and atmospheric water deficit.

Appropriate stomatal behavior may be useful for improving drought response by reducing water loss and increasing transpiration efficiency and efficient measurement of stomatal conductance would increase the reliability of assessing cultivar performance under drought.

The lower canopy conductance in terminal drought tolerant near isogenic lines of pearl millet saves water under non-

stressed conditions, allowing plant to have water available to fill up grains (Kholova *et al.*, 2010).

Hinkossa *et al.* (2013) reported that superior performance under drought stress was associated with the maintenance of higher stomatal conductance as well as greater ability to remobilize biomass to reproductive sinks (pods and seed) in common bean.

Krouma (2010) reported a significant decrease in stomatal conductance under drought stress. The observations recorded in the present investigation are in agreement with the findings of Khan *et al.* (2010), Hirich *et al.* (2014) and Iqbal *et al.* (2014) who reported a significant decrease in stomatal conductance under stress.

The physiological changes observed could be the results of deleterious effect of water deficit on important metabolic processes as well as responses of various defense mechanisms by the plant under drought stress. Relatively higher stomatal conductance allows diffusion of more CO₂ into the leaf cells leading to higher rates of photosynthesis, which is positively correlated with yield of cultivars under drought situation.

5.3.6 SCMR values

Leaf chlorophyll content is a key indicator of the physiological status of a plant. Chlorophyll content per unit leaf area (chlorophyll density) have been used as an index of photosynthetic capacity and growth of many crop plants and it is most important to crop performance because chlorophyll is a photosynthetic pigment. The major role of these pigments is to absorb and reacts with visible light in the photosynthesis.

Total chlorophyll index recorded by using the instrument SPAD meter at 50 per cent flowering and pod development stage

showed that the SCMR value significantly decreased in all genotypes under drought stress, but the reduction were not as great as in tolerant genotypes.

The tolerant genotype Digvijay demonstrated the lowest decrease of 8.9 per cent in SCMR value under early drought stress over unstressed control at 50 per cent flowering. The susceptible genotypes however, recorded maximum 28.9 per cent reduction in SCMR value at 50 per cent flowering stage.

At grain filling stage, the mean per cent decrease in SCMR value was the lowest in tolerant genotypes than susceptible genotypes with 10.4 and 17.30 per cent under early drought stress while 17.9 and 27.1 per cent under late drought stress condition, respectively. The tolerant genotype Digvijay recorded the highest mean SCMR value of 48.07 under late drought stress condition.

Drought stress can also alter the tissue concentration of chlorophylls and carotenoids (Macer and Ekmekki, 2009). While increased chlorophyll and carotenoid content under drought stress may be related to the decrease in leaf area, it also can be a defensive response to reduce the harmful effects of drought stress.

Rajababu *et al.* (2010) observed that the groundnut cultivars recorded significantly higher SPAD values under moisture stress conditions. These characters are contributed the drought tolerance mechanism in groundnut. Ulemale *et al.* (2013) reported that the tolerant genotypes exhibited higher values of chlorophyll content and SPAD index under irrigated condition.

5.3.7 RLWC

High relative water content and low excised leaf water loss are associated with drought resistance and these parameter have also

been proposed as more valuable indicators of plant water status in comparison to other water potential parameters under drought. Water stress causes water loss within the plant and therefore causes reduction in its relative water content. The metabolic activity in the leaf tissue can be evaluated by measuring RLWC, it can be considered as an integral measure of plant water status. The tolerant genotypes maintained the higher value of relative leaf water content under stress and non stress condition. Therefore these genotypes seem to be drought tolerant.

Drought stress significantly reduced RLWC of all genotypes. Drought induced reduction in RWC occurred to a greater extent in the drought susceptible genotypes (15.4 %) and to a lesser degree in the tolerant genotypes (7 %) under early drought stress at 50 per cent flowering stage. Under late drought stress the tolerant genotypes Vijay recorded higher RLWC at grain filling stage followed by Digvijay. In general, relative water content (RLWC) was higher in drought tolerant genotype than susceptible genotypes. Overall the genotype Vijay, Digvijay and ICC 4958 showed less reduction in RLWC in drought stress compared to others. These results are in agreement with Gunes *et al.* (2008) and Ulemale *et al.* (2013).

Talebi *et al.* (2013) reported that drought stress significantly reduced RWC of all chickpea genotypes. Drought stress induced reduction in RLWC to a greater extent in the drought susceptible genotype ELIP05-74 C (38 %) and lesser degree in the tolerant genotype ELIP-153 C (7.8%).

Decreased RWC under moisture stress leading to reduced leaf turgor might be responsible for the reduction in photosynthesis observed in the present study. Garg *et al.* (2001) have also observed

significant differences in rate of net photosynthesis under increasing water stress in the moth bean genotypes. Also found that increasing water stress progressively decreased plant water potential, leaf area, net photosynthetic rate, starch and nitrate reductase activity in moth bean genotype.

According to results of Bayomi *et al.* (2008), RWC may be attributed to difference in the ability of the variation to absorb more water from the soil and/or the ability to control water loss through stomata. The RLWC parameter can be used to select high yielding genotypes because it maintains cell turgor under water stress environment to gave relatively higher yield.

5.3.8 Plant height

The vegetative phase governs the overall phenotypic expression of the plant and prepares the plant for next important reproductive phase. The plant height and branches constitute vegetative phase and perform specific function.

The average plant height ranged between 35.33 and 52.37 cm under moisture stress condition at maturity. Due to withholding of irrigation at 40 DAS (preflowering stress) the mean plant height was significantly reduced from 45.91 to 40.52 cm under early drought stress at maturity. Overall, the genotype Phule G 0405-42-2 recorded the highest plant height followed by ICC 4958 (44.91 cm).

The significant reduction in plant under stress condition was reported by Shinde *et al.* (2010). Manivannam *et al.* 2007 reported a reduction in plant height could be attributed to decrease in cell division, cell enlargement under water stress. Under the water deficiency, cell elongation of higher plants can be inhibited by

interruption of water flow from xylem to the surrounding elongation cells leading to reduced number of branches.

5.3.9 Number of secondary branches

Drought affects various morphological and physiological processes, resulting in reduced growth, development and economic yield of crop. Water stress has prominent effect on leaf number, total leaf area and secondary branches causing invariable reduction under rain fed condition (Basu *et al.* 2007).

Water stress impose at pre flowering (EDS) affected the mean number of secondary branches of susceptible chickpea genotypes and reduced from 14.73 to 11.33 at 50 per cent flowering. The trend remains same at grain filling stage also. Overall the genotype Vijay and Digvijay recorded the highest number of secondary branches per plant.

According to findings of Lakshmamma and Lakshmi (2010) under moisture stress condition the drought tolerant genotypes maintained their growth. The reduction in plant height, number of primary and secondary branches and leaf area due to moisture stress was recorded by Ulemale *et al.* (2013).

Mathur *et al.* (2005) and Kumar *et al.* (2004) reported that the seed yield per plant showed highly significant and positive correlation with number of secondary branches and emphasized that number of secondary branches can be considered as a selection criterion for improvement in chickpea yield.

5.3.10 Proline

Proline is one of the osmolytes, which increases faster than other amino acids in plant under water deficit stress and help the plant to maintain cell turgour. At 50 per cent flowering, the

highest pooled mean proline content in tolerant was 16.27 $\mu\text{moles g}^{-1}\text{FW}$ than the susceptible type with 10.37 $\mu\text{mole g}^{-1}\text{FW}$ under imposed early drought stress.

At the grain filling stage, further increase in proline content with similar trend was observed under stressed condition. The tolerant genotypes recorded highest per cent increase of 124 than the 58 per cent increase in susceptible genotypes over unstressed control under late drought stress condition. The tolerant genotypes ICC 4958, Vijay and Digvijay recorded highest proline accumulation under stress condition. Macar (2009) revealed that, proline provides a protection to cell by controlling turgor, make a cover on membrane and macromolecules which provides structural protection and play a role of free radicals scavenging. Decrease of proline content from the flowering to the podding stage in chickpea was reported by Raheleh *et al.* (2012).

Osmotic adjustment involves an active accumulation of cellular solutes such as proline and soluble proteins within the plant in response to lowering of the soil water potential and reducing the harmful effects of water deficit. As a consequence of solute accumulation, the osmotic potential of the cell is lowered which in turns, attracts water into the cell and leads to maintaining its turgour (Moinuddin and Khanna Chopra, 2004). Osmotic adjustment has been reported also in chickpea under water deficit conditions (Gunes *et al.* 2008).

Increase of proline causes the mediation of osmotic adjustment and thus the plant will keep growing under drought stress. The genotypes which have high proline content might increase the synthesis ability of osmotic regulators for protection from the

damage. It has been suggested that besides osmoregulation properties, antioxidative activity of proline could be involved in protection against injury caused by stress (Fusan and Oz, 2007). The result of this field study is in agreement with the observation of Rizvi *et al.* (2014) who found 3.23 times more proline accumulation in Pusa-1108 under moisture stress at flowering stage over control.

The observation recorded in the present investigation are in agreement with the findings of Manna *et al.* (2014), Rizvi *et al.* (2014), Hirich *et al.* (2014) who found high accumulation of proline in stress.

5.3.11 Glycine betaine

The glycine betaine accumulation in the leaves of chickpea genotypes at 50 per cent flowering and grain filling stage under stressed and unstressed condition is depicted in Table 34 and 35. The water stress under field condition reflected in an increase in glycine betaine accumulation in all genotypes examined. Further, the drought tolerant genotypes accumulated comparatively higher glycine betaine than susceptible genotypes over unstressed control.

At 50 per cent flowering, the pooled mean glycine betaine content under early drought stressed condition in tolerant type was highest with 14.25 $\mu\text{mole g}^{-1}$ FW than the susceptible type with 10.16 $\mu\text{mole g}^{-1}$ FW. The overall mean per cent increase in tolerant genotype with 79.4 per cent as against 35 per cent in susceptible genotypes over unstressed normal condition.

At grain filling stage comparative decline in glycine betaine content under both unstressed and stressed condition was observed in all the genotypes. However, higher glycine betaine content was observed in stressed than unstressed leaves. The pooled mean value

for glycine betaine accumulation in tolerant type with $10.60 \mu\text{moles g}^{-1}$ FW and susceptible types with $8.04 \mu\text{moles g}^{-1}$ FW under late drought stress condition. The per cent increase in glycine betaine content at late drought stress was highest in the tolerant types than susceptible with 51.6 and 25.8 per cent, respectively.

Comparatively lesser amount of glycine betaine was accumulated at grain filling than at 50 % flowering stage. The accumulation of GB is an adaptive response to abiotic environmental stress (Yang *et al.*, 2003), GB : total amino acid ratio or availability of substrate choline or increased/decreased transport of choline into chloroplast where GB is naturally synthesized (Ashraf and Foolad, 2007).

GB content showed a significant increase in linseed plant under NaCl stress compared to the control. The level of GB content was lower in unstressed plants supplied with CaCl_2 or GA_3 alone when compared with salt-stressed plants (Khan *et al.*, 2010). The glycine betaine in young soybean seedlings of ZH13 and BB52 was increased by 59.4% and 62.9% under 300mM NaCl treatment compared to control, respectively (Wu *et al.*, 2014).

5.3.12 Antioxidative enzymes

Antioxidant defense system plays a vital role in plant tolerance to stressful conditions. It is obvious from the results that drought has induced antioxidant enzyme activities more than the normal plants during the drought period. Results showed that increase in SOD, CAT and APX activities in chickpea genotypes may be related to induction of antioxidant responses that protect the plant from oxidative damage. Among the antioxidant enzymes, SOD

converts the toxic O_2^- to H_2O_2 which must be scavenged to the O_2 and H_2O by the antioxidant enzymes such as CAT and APX.

5.3.13 Superoxide dismutase (SOD)

Plants are well endowed with antioxidant molecules and scavenging systems which establish a link between tolerance to water stress and rise in antioxidant enzyme concentration in photosynthetic plants. SOD is thought to provide the first line of defense against toxic effect of ROS intermediates by converting H_2O_2 to H_2O and O_2 . Superoxide dismutase (SOD) activity increased in all chickpea genotypes under drought condition. The higher SOD activity was noticed at pre-anthesis stage than post-anthesis stage.

As far as genotype was concern overall maximum SOD activity in Digvijay (122.32 units mg^{-1} protein) and it was minimum in susceptible genotypes Phule G-0305-3 (106.28 units mg^{-1} protein) (Table 36) at 50 per cent flowering stage. The mean per cent increase in SOD activity was the highest in tolerant genotypes than susceptible with 43.7 and 24.4 per cent, respectively over control at 50 per cent flowering under early drought stress condition. At grain filling stage, SOD activity decreased than 50 per cent flowering stage. The overall mean per cent increase in SOD activity was highest in tolerant genotypes susceptible genotypes with 38.2 and 14.2 per cent, respectively over unstressed control under late drought stress condition. Superoxide dismutase constitutes the first line of defense via detoxification of superoxide radicals thereby maintaining membranes of plant tissue. The findings are in agreement with Rahelech *et al.* (2012) , Rasool *et al.* (2013) and Cevik *et al.* (2014)

who reported that SOD activity showed significant increase in all the chickpea genotypes at stress levels.

5.3.14 Catalase (CAT)

Catalase activity was measured during flowering and pod filling stage (Table 38 and 39) and observed that there was significantly higher CAT activity upon expose to drought stress in all the genotypes during flowering and grain filling stage. Catalase (CAT) eliminates H_2O_2 by breaking it down directly to water and oxygen.

Overall the tolerant genotypes ($16.71 \mu\text{mole } H_2O_2$ decomposed $\text{mg protein min}^{-1}$) had the highest mean CAT activity whereas susceptible genotypes should the lowest mean ($10.91 \mu\text{mole } H_2O_2$ decomposed $\text{mg protein min}^{-1}$) CAT activity (Table 38) at 50 per cent flowering under early drought stress.

Increase in catalase activity in response to drought stress suggests a prominent role for this enzyme in the protection of leaf tissue against oxidative damage. The higher per cent increase in CAT activity of 88.2 per cent in tolerant genotypes and minimum 33.1 per cent in susceptible type over unstressed control under late drought stress condition was observed at grain filling stage.

Catalase enzyme involved in regulation of intracellular level of H_2O_2 . Mafakheri *et al.* (2011) reported that an increase was observed in POX and CAT activity of chickpea cultivars under stress condition throughout the experiment. Rahelech *et al.* (2012), Kaur *et al.* (2013) and Rasool *et al.* (2013) reported that the content of catalase was increased as depending on drought. These findings are in accordance with the present results. Several investigations have reported an increase in activity of antioxidative enzyme CAT in various crops imposition of water stress.

5.3.15 Ascorbate Peroxidase Activity (APX)

APX convert H_2O_2 into H_2O along with the regeneration of NADH, therefore, the enzyme play important role in stress conditions.

At the grain filling stage, APX activity was found to decline than pre flowering stage. However, the activity was higher in stressed than the unstressed leaves. The susceptible genotypes exhibited the lowest APX activity. Among the tolerant genotypes Vijay demonstrated APX activity as 826.43 in n moles ascorbate oxidised mg^{-1} protein with the per cent increase of 153.6 over control at 50 per cent flowering under early drought stress. The overall activity of APX declined at post flowering than at pre flowering stage which may be due to isozymes of APX localized on different cell organelles among them Chl-APX isozymes are much more strongly inactivated by oxidative stress and found to be the primary target among the H_2O_2 sensitive enzymes (Mano *et al.*, 2001).

Omidi (2010) noticed that the enzyme activities of ascorbate peroxidase (APX) and catalase (CAT) were highly significant under drought stress.

APX which is primarily located both in chloroplasts and cytosol acts as a key enzyme of the glutathione-ascorbate pathway. It scavenges peroxides by converting ascorbic acid to dehydroascorbate and it is one of the most important enzymes playing a vital role in eliminating toxic H_2O_2 from plant cell in Asada-Halliwell pathway. In the present study drought stress increased total APX activity differed prominently in stressed leaves as compared to that of non-stressed leaves. At grain filling stage, under late drought stress higher activity of APX in tolerant genotypes was 492.47 n moles ascorbate oxidised mg^{-1} protein under stressed leaves, generally its activity was 1.71 fold

more over control, suggests a more effective H_2O_2 removal which might be produced by an enhanced activity of SOD in chickpea .

There is a fragile balance between ROS production and scavenging that defines the normal steady-state level of intracellular ROS. Under drought stress the balance suffers an upward shift, ROS production being enhanced due to stomatal closer and the concomitant limitation on CO_2 fixation. The avoidance of ROS production during drought stress is also an important strategy that enables plants to cope with water shortage with at extensive damage (Carvalho, 2008).

Raheleh *et al.* (2012) reported that APX activity in tolerant genotypes was significantly higher than the susceptible genotypes under drought condition and among the three investigated stages, the lowest level of APX activity belonged to the podding stage in all the studied genotypes. The early drought stress was affected more in reducing growth traits than late drought stress.

5.3.16 Number of pods

It was obvious from the present study that number of pods per plant were the most important character that responded positively to irrigation and hence contributed significantly to yield increase.

Chickpea varieties differed significantly for number of pods per plant under early, late drought stress and non stress condition (Table 42). Withholding irrigation at 40 DAS i.e. pre-flowering stage, resulted in 15.2 per cent reduction in mean number of pods over control under early drought stress. Overall the genotype, Vijay recorded maximum number of pods under irrigated as well as early drought stress condition. These findings are in accordance with

Ali *et al.* (1998). Shamsi *et al.* (2010) reported that the treatments with only one time irrigation at 50 per cent flowering and pod filling stages had 149.9 and 117.9 per cent increase in number of pods per plant, respectively as compared to treatment without irrigation. The differential behavior of genotypes to water stress may be attributed to their variable genetic makeup and impaired physiological mechanism of plants carried out in the presence of water. The major character affecting crop grain yield are number of pods and seeds per plant which reduce under drought stress (Davies *et al.*, 2000).

Bakhsh *et al.* (2007) emphasized that application of irrigation improved 48 per cent pods per plant on overall basis. Shinde *et al.* (1996) found the pods per plant to be the most affected trait by irrigation in chickpea. Fang *et al.* (2010) reported that the reduction of flower production due to water stress may reach up to 60 per cent in chickpea.

Pod abortion is known to be important in determining seed yield of chickpea when exposed to terminal drought (Leport *et al.*, 1999) but flower production and abortion are as well important factors reducing seed yield (Fang *et al.*, 2010). The quite low percentage of seed abortion in tolerant genotypes was in fact, related to the higher water uptake during the flowering period (Zaman-Allah *et al.*, 2011).

5.3.17 Grain yield per plant

A crop yield is an end product of various closely interlinked metabolic processes of the plant. Understanding of various physiological traits governing these metabolic processes in relation to ultimate yield is imperative for efficient development of crop plants. Water stress imposed at pre flowering and post flowering

affected grain yield per plant significantly. Several studies have shown that optimum yield can be obtained by irrigation at branching, flowering and pod formation stage. This investigation showed the negative effect of drought on yield of chickpea.

Moisture is more important at pre flowering stage, because it is the most damaging stage to yield and yield parameter and plant need abundant availability of moisture in the root zone.

After assessing the interaction in Table 43, it is clear that under stress and normal irrigated conditions tolerant genotypes maintained highest grain yield. Under stress condition, the susceptible genotypes showed the poorest performance in terms of economic yield. This might be because of lowest turgour potential of this genotype. Maintenance of turgour is crucial for the continuance of normal metabolism especially under drought conditions; the reduced turgour potential (RLWC) of these genotypes on account of poor osmotic adjustment might have lead to reduced photosynthesis and consequently poor yields. Rasales-Serna *et al.* (2004) reported similar results in common bean subjected to drought and observed that overall yield reductions due to drought treatments were greater in the drought susceptible cultivars than in the tolerant cultivars. Other researcher reported that drought resistant cultivar might be more efficient at photo assimilate production and translocation to the seeds, indicative of an obvious relationship between photosynthesis and yield.

Aslam *et al.* (2008) evaluated the performance of chickpea genotypes and reported significant variation in morphological, physiological phonological character in yield and yield components.

Overall the maximum grain yield per plant (19.90 g) under non stress condition was recorded by genotype Digvijay followed by Vijay (16.18 g). Digvijay also maintained highest grain yield per plant (17.15 and 17.92 g) under early and late drought stress, respectively. The superiority of Digvijay, Vijay and Phule G 752 observed under drought conditions was due to the production of more number of pods per plant, harvest index and better oxidative defense under stress.

Yield was found positively correlated with number of branches, 100 seed weight, number of pods per plant (Shamsi *et al.*, 2010) in chickpea. These traits can be used as reliable criterion in selection of water stress tolerant chickpea cultivars.

Our findings are in agreement with Yaqoob *et al.* (2012) who suggested that moisture stress at pre flowering stage being harmful and detrimental, is the most critical stage for screening chickpea germplasm under drought prone conditions. Patel and Hemantranjan (2012) concluded that early drought stress (pre-anthesis drought) was found to be more damaging than the late drought stress (post anthesis drought). It was also suggested that chickpea lines should be given moisture stress at pre flowering stage while screening against drought stress conditions. Among the irrigation levels two irrigations at pre-flowering and pod filling stages of chickpea recorded significantly higher seed yield as compared to all other treatment (Thomas *et al.*, 2010).

The higher yield of these genotypes was due to the production of higher number of pods per plant which was supported by the greater number of secondary branches per plant. The result of present study was in agreement with the result of Islam *et al.* (2008).

Yaqoob *et al.* (2013) revealed that plant recovery response of more than 50% studied lines could not properly recover after termination of moisture stress suggesting that moisture stress at pre flowering has usually lethal effect on chickpea plans.

5.3.18 Test weight

Hundred seed weight is important yield contributing character having direct effect on the final seed yield. The 100 seed weight was higher under non-stress condition than moisture stress condition.

On an average the genotype ICCV 11117 (34.36 g) recorded the highest test weight followed by ICC 4958 (34.26 g) under non stressed condition. Similar trend was observed under stress condition. Under late drought stress condition the genotype ICCV 11117 (31.91 g) recorded maximum 100 seed weight followed by the genotype ICC 4958 (31.40 g).

The decrease in 100 grain weight under stress condition might be due to lower photosynthetic translocation in the developing grain. It appears that moisture deficit at the time of pod formation and grain filling can decrease the rate of photosynthetic products transmission, resulting the grain shriveling. The irrigation at 60 DAS, moisture deficit has been resolved fairly; therefore, period of grain filling has lengthened as a result more photosynthetic products accumulated in the grains.

5.3.19 Harvest index

The harvest index is an important indicator of photosynthates translocation efficiency for conversion into economic yield. The harvest index is also an indirect measure of seed yield. In

general, the harvest index was higher under non-stress condition than moisture stress condition.

The average harvest index under stress and non stress conditions ranged between 26.08 to 39.51 per cent (Table 44). Overall the genotype Vijay recorded maximum 36.78 and 35.30 per cent harvest index followed by Phule-G-0752 (35.34 and 34.66 %) under early and late drought stress condition, respectively. The source sink relationship was observed to be better in the tolerant genotypes.

Overall the highest and lowest value of harvest index pertained to Vijay and ICC 11112, respectively. Having done an experiment on 25 chickpea genotypes under rainfed and irrigated condition in Syria. Singh and Singh (1991) concluded that grain size, grain yield, biological yield and harvest index were improved by irrigation, which is in agreement with the result of the present experiment.

Thomas *et al.* (2010) reported that one irrigation either at pre-flowering stage or at pod filling stage of chickpea being at par recorded significantly highest harvest index compared with no irrigation. Nagar *et al.* (2013) reported that the harvest index was significantly higher in tolerant genotypes. Under moisture stress conditions tolerant genotypes possessed better partitioning coefficient resulting in high seed weight and harvest index.

Correlation study :

The physiological characters *viz.*, rate of photosynthesis, stomatal conductance, morphological and yield contributing characters *viz.*, number of secondary branches and pods per plant, biochemical parameters like proline , glycine betaine, SOD ,

Cat and APX activity at 50% flowering under early drought stress and at maturity under late drought stress showed significantly positive correlation with grain yield per plant.

Kumar *et al.* (2014) reported that the seed yield per plant showed highly significant positive correlation with the number of pods per plant, number of secondary branches per plant.

6. SUMMARY AND CONCLUSIONS

Abiotic stress signaling is an important area with respect to increase in plant productivity. Drought is a worldwide problem, contrasting global crop production and quality seriously and recent global climate has made this situation more serious. Drought affects the growth, dry matter harvestable yield in plants. Timing, duration, severity and speed of development undoubtedly have pivotal role in determining how a plant responds to water stress deficit. Following drought, stomata close progressively with a parallel decline in net photosynthesis and water –use efficiency. In addition to other factors, changes in photosynthetic pigments one of paramount importance to drought tolerance. Stress induces production of reactive oxygen species (ROS) and these ROS cause oxidative damage to different cellular components including membrane lipid. Protective responses at the leaf level must be triggered quickly in response to the stress effects to prevent the photosynthetic machinery being irreversibly damaged. Scavenging of reactive oxygen species by enzymatic and non-enzymatic system, cell membrane stability, expression of aquaporins and stress proteins are vital mechanisms of drought tolerance.

Keeping the present state of research in view, a laboratory experiment along with a field experiment on “Physio-biochemical analysis for growth and yield in chickpea under moisture stress” was conducted during the *rabi* season of 2012-13 and 2013-14 at MPKV, Rahuri. Thirty genotypes were screened and five each of drought susceptible and drought tolerant were selected on the basis of high germination percentage, more root and shoot length, vigour index and proline content under higher osmotic potential. These ten genotypes

were further studied for physiological, biochemical and antioxidative enzymes activity in chickpea under progressive water stress induced by PEG 6000.

These genotypes were also evaluated under field capacity by imposing water stress at critical growth stage i.e. pre- and post-flowering stages. The results of the present investigations are briefly summarized as follows.

6.1 Chickpea genotypes screening under PEG 6000 induced osmotic stress

1. The genotype Phule G 0752, Digvijay , Vishal , Phule-G-511- 43-2, ICC 4958 and Vijay had maintained higher amount of germination percentage under PEG 6000 induced progressive stress.
2. Among the genotypes Phule G-511-43-2, Vishal, Digvijay, Vijay, ICC 4958 genotypes responded better as evident from shoot and root length under decreasing osmotic potential. While, the genotype Phule G-0305-3, Phule G-0405-44-2, Phule G-0752 and ICC 4958 under PEG 6000 induced osmotic stress recorded highest vigour index.
3. The genotypes Phule G-0511-43-2, Phule G-0752 and ICC 4958 under PEG 6000 induced osmotic stress accumulated higher proline content in their leaves.
4. In addition to germination, the longer root and shoot length, more vigour index with high proline accumulation, Phule G 511-43-2, Phule G 0752, ICC 4958, Vijay and Digvijay were found to more tolerant to

drought stress under PEG 6000 induced progressive stress.

6.2 Effect of induced water stress on physiological parameters

1. In pot study, the genotypes Digvijay, ICC 4958 and Vijay under PEG 6000 induced osmotic stress maintained higher rate of photosynthesis. These genotypes also recorded highest rate of photosynthesis under water stress created by withholding the irrigation under field condition at pre- and post- flowering stage.
2. In the pot study the genotypes ICC 4958, Vijay and Digvijay recorded the limited rate of transpiration under PEG 6000 induced osmotic stress. These genotypes also demonstrated lower rate of transpiration at 50 % flowering and grain filling stage under water stress created by withholding irrigation at pre- and post-flowering.
3. The genotypes Vijay, Phule G 0752, ICC 4958 and Digvijay recorded higher stomatal conductance under PEG 6000 induced osmotic stress. These genotypes also demonstrated similar stomatal behavior at 50 % flowering and grain filling stage under water stress created by withholding irrigation at pre- and post-flowering.
4. Higher SCMR seems to be an indicator of the genotypes ability for higher carbon assimilation and in turn seed yield even under moisture limited condition.
5. PEG induced water stress caused decrease in SCMR values in both progressive mild and severe stress. The genotypes *viz.*, Vijay, Digvijay and ICC 4958 recorded the

maximum SCMR values under stress and non-stress condition.

6. A significant decrease in the RLWC observed in both progressive mild and severe osmotic stress induced by PEG 6000 indicating that plants have the ability to sustain their water under mild stress, whereas this ability is lost under severe stress treatment. At severe stress significantly highest RLWC of 56.58 percent with the lowest decline of 18.8 per cent was observed in tolerant genotype ICC 4958.
7. The stressed leaves showed a considerable decline in RLWC over unstressed control in all the chickpea genotypes. The mean percent decline in RLWC was higher at pre-flowering than at post-flowering stage. The mean percent decline in RLWC was minimum in tolerant genotypes over unstressed control at pre- and post-flowering stage which indicate that tolerant genotypes retained more water under stressed condition.
8. Water stress induced reduction in RLWC occurred to a great extent in the drought susceptible genotypes ICCV 11117 and ICCV 11112 and to a lesser degree in the tolerant genotypes ICC 4958 and Digvijay.

6.3 Effect of induced water stress on accumulation of osmolytes

1. The genotypes Vijay, ICC 4958 and Digvijay accumulated highest leaf proline content under PEG 6000 induced osmotic stress. These genotypes also accumulated highest proline content in their leaves under water stress created

by withholding the irrigation under field condition at pre- and post- flowering stage.

2. The genotypes Vijay, ICC 4958 and Digvijay accumulated highest glycine betaine under PEG 6000 induced osmotic stress.. These genotypes also demonstrated highest proline content in their leaves under water stress created by withholding the irrigation under field condition at pre- and post- flowering stage.

6.4 Effect of induced water stress on antioxidative enzymes

1. The pot study exhibited significantly higher SOD activity in tolerant genotypes than the susceptible genotypes under severe stress followed by moderate stress.
2. The SOD activity was comparatively higher in tolerant genotypes than susceptible genotypes under early drought stress at grain filling stage.
3. The tolerant genotypes Digvijay and Vijay recorded the highest SOD activity than all other genotypes under field condition as well as under PEG 6000 induced osmotic stress in pot.
4. The significantly highest catalase activity in leaves was observed in tolerant genotypes, which increased during 50 % flowering over the grain filling stage. The significantly highest catalase activity of 18.62 $\mu\text{mol H}_2\text{O}_2$ decomposed mg^{-1} protein min^{-1} under early drought stress at 50 % flowering was observed in tolerant genotype ICC 4958. The susceptible genotype ICC 11112 and Phule G-0405-44-2 recorded the lowest catalase activity under stress condition at 50 % flowering and grain filling stage.

5. At pre-as well as post-flowering stage, the mean percent increase in catalase activity of stressed leaves over unstressed control was higher in tolerant genotypes than the susceptible genotypes.
6. A pot experiment of PEG-6000 induced osmotic stress depicted a similar trend in catalase activity in leaves as revealed field study.
7. Significantly increased APX activities were observed in moderate and severe stresses induced by PEG as compared to control in pot study.
8. The significantly higher APX activity was recorded in tolerant genotype ICC 4958 followed by Digvijay and Vijay in both progressive osmotic stresses induced by PEG 6000 in pot study.
9. The enzyme activity in the leaves declined considerably during post-flowering than pre-flowering stage. The significantly highest APX activity of 826.43 and 521.12 nmoles ascorbate oxidized mg^{-1} protein min^{-1} was observed under early and late drought stress at 50 percent flowering stage, respectively in the tolerant genotype Vijay in field studies

6.5 Effect of induced water stress on phenology

1. The genotypes Vijay, Phule G-09103, Phule G-0752 had produced early flowering where as well-watering treatments had delayed flowering in genotypes Phule G-0305-3 and Phule G-0405-44-2.
2. Mean days for physiological maturity were slightly reduced due to moisture stress as the genotypes tried to escape

from the stress condition. The genotypes Phule G-0305-3 Phule G-0752, ICCV 11112 recorded the minimum number of days to physiological maturity under moisture stress as well as non stress condition.

6.6 Effect of induced water stress on morphological character

1. Mean plant height was higher under non-stress than stress condition. The highest plant height recorded by genotype Phule G 0405-44-2 followed by ICC 4958.
2. Mean number of secondary branches per plant was higher under non stress condition. The genotypes Vijay, Digvijay and Phule G-0752 maintained highest number of secondary branches per plant under moisture stress.

6.7 Effect of induced water stress on yield contributing traits

1. The grain yield and yield contributing characters *viz.*, number of pods per plant, harvest index and test weight were higher under non stress condition than the moisture stress condition.
2. The highest numbers of pods per plant were recorded by Vijay, Digvijay and Phule G-0752 under stress condition. The genotypes Digvijay (17.15 and 17.92 g) recorded the highest seed yield per plant under early and late drought stress condition. The genotype ICC 4958 and Vijay maintained the minimum percent reduction in grain yield.
3. The genotype Vijay recorded the highest harvest index under stress conditions. The genotypes ICCV 111117 and ICC 4958 recorded the highest test weight under stress as well non stress conditions.

6.8 CONCLUSION

The laboratory screening of chickpea genotypes by exposing them to PEG-6000 induced osmotic stress differentiated their drought tolerance potential as evident from germination, root length, shoot length and seedling vigour index. The accumulation of proline under osmotic stress could also be considered while selecting the genotypes in breeding for drought tolerance. At an osmotic potential of -0.8 MPa the seeds of either tolerant or susceptible genotypes did not germinate.

Comparatively lower reduction in net photosynthetic rate, RLWC and higher accumulation of proline and glycine betaine was recorded in drought tolerant chickpea cultivars *viz.*, ICC 4958 and Vijay.

A perusal of the profile of antioxidative enzymes *viz.*, SOD, CAT and APX revealed better oxidative defense of drought tolerant genotypes *viz.*, ICC 4958 and Vijay. Based on the significant induction of antioxidative enzyme activities, we concluded that SOD and APX could be instrumental in predicting the drought tolerance of chickpea genotype.

The moisture stress at pre flowering stage of the crop growth is more deleterious to chickpea plants and it is suggested that chickpea lines should be evaluated at pre-flowering stage while screening against drought stress condition. The number of secondary branches per plant and number of pods per plant can be considered as selection criteria for the improvement of seed yield.

In the present study the screening under both laboratory and field conditions revealed that genotypes *viz.*, Vijay , Digvijay and ICC 4958 to be superior under moisture stress condition as evident from better yield over the years and therefore, these genotypes may be used as a source of drought tolerance in chickpea improvement programme.

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8. VITA

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of

DOCTOR OF PHILOSOPHY

in

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