Ionome and proteome assisted characterisation of drought tolerance in chilli (*Capsicum annuum* L.)

A Thesis Submitted

To

Sikkim University

In Partial Fulfilment of the Requirement for the

Degree of Doctor of Philosophy

By

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This is to certify that the thesis entitled **"Ionome and proteome assisted characterisation of drought tolerance in chilli (*Capsicum annuum* L.)"** submitted to **Sikkim University, Gangtok,** in partial fulfilment of the requirements of the award of the degree of **Doctor of Philosophy** in the **Department of Horticulture,** embodies the result of bonafide research work carried out by **Mr. Venkata Ramana Muddarsu** under my guidance and supervision. No part of the thesis has been submitted for any other degree, diploma, associateship or fellowship.

All the assistance and help received during the course of the investigation have been duly acknowledged by him.

I recommend this thesis to be placed before the examiners for evaluation.

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DECLARATION

I, Mr. Venkata Ramana Muddarsu, hereby declare that the thesis entitled "**Ionome and proteome assisted characterisation of drought tolerance in chilli (*Capsicum annuum* L.)**" was done by me and the contents of this thesis did not form basis of the award of any previous degree to me or, to the best of my knowledge, to anybody else and that the thesis has not been submitted by me for any other research degree in any other University/ institute.

The contents of this thesis have also been subjected to plagiarism check.

This is being submitted in partial fulfilment of the requirements of the degree of Doctor of philosophy in the Department of Horticulture, School of Life sciences, Sikkim University.

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Ionome and proteome assisted characterisation of drought tolerance in chilli (*Capsicum annuum* L.)

Submitted by Venkata Ramana Muddarsu under the supervision of Dr. S. Manivannan, Department of Horticulture, Sikkim University, Gangtok, 737102, India.

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(Venkata Ramana Muddarsu)

Affectionately dedicated

To my beloved Parents

Contents

List of Tables

List of Figures

List of Plates

Abbreviations

2-DE	Two-dimensional gel electrophoresis
ABA	Abscisic acid
Al	Aluminium
APX	Ascorbate peroxidase
ATP ase	Adenyl triphosphatase
B	Boron
Ba	Barium
Ca	Calcium
Ce	Cerium
Co	Cobalt
Cr	Chromium
Cs	Caesium
Cu	Copper
DAS	Days after sowing
DDW	Double distilled water
DNA	Deoxyribo Nucleic Acid
DTT	Dithiothreitol
Fe	Iron
Fe-EDTA	Ferric Ethylenediaminetetraacetic Acid
Ga	Gallium
$HgCl_2$	Mercuric chloride
I	Iodine
ICARDA	International Center for Agriculture Research in the Dry Areas
ICP-MS	Inductively coupled plasma mass spectrometry
IDA	Iminodiacetic acid
IEF	Isoelectric focusing
K	Potassium
LCMS	Liquid chromatography
Li	Lithium

Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
MoCo	Molybdenum cofactor
MPa	Mega Pascols
MSMS	Tandem Mass Spectrometry
Na	Sodium
NaCl	Sodium chloride
Ni	Nickel
P	Phosphorus
PEG	Polyethylene glycol
POD	Peroxidase
QTL	Quantitative trait loci
R.L	Root Length
R/S DW	Root to shoot dry weight
Rb	Rubidium
RDW	Root dry weight
ROS	Reactive oxygen species
S	Sulphur
S.L	Shoot length
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SDW	Shoot dry weight
Sn	Tin
Sr	Strontium
Ti	Titanium
UPLC	Ultra-Performance Liquid Chromatography
V	Vanadium
Zn	Zinc
Zr	Zirconium

CHAPTER-1

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the important members of the family Solanaceae. *Capsicum* probably evolved from an ancestral form in the Bolivian or Peru area (Heiser, 1976). It consists of about 22 wild species and five domesticated species (Bosland, 1994): Capsicum annuum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens and Capsicum pubescens. Chilli peppers have been shown to be domesticated more than 6000 years ago (Perry *et al.*, 2007) and the most economically important species in the world is *C. annuum* (Greenleaf, 1986 and Bosland, 1988).

In India, chilli production was 1519090 MT from 817390 ha. area (spice board, 2016). Cultivated chilli species has its unique place in the diet as a vegetable cum spice crop (Gadaginmath, 1992). India being largest exporter of chilli in the world, mainly exporting to USA, Canada, UK, Saudi Arabia, Malaysia, Germany etc. During 2015-16, 347,500 tonnes of chillies worth of Rs. 399,743.97 lakhs was exported, registering the highest share of export earnings from various spices and spice products (Spice board, 2016) by India. In global chilli market India has the highest share of 25% followed by china with 24%. Though, India has substantial share in the world hectarage under chilli crop, the productivity (1.76 t/ha) is low compared to the other hot pepper growing countries like Korea and Indonesia where it ranges at 2-3 t/ha (FAO, 2016). The main reason for low productivity is majority of chilli crop cultivation area (~ 50%) is under rainfed conditions (Dorji *et al.*, 2005). In the rainfed areas water scarcity and drought are the major concern.

Drought is one of the major abiotic stresses which results in significant reduction in morphological traits such as plant height, plant spread and dry matter accumulation (Shaozhong *et al*., 2001; Delfine *et al.*, 2002; Dorji *et al*., 2005 and Kumar and Phalke, 2009) affecting the physiological process thereby causing considerable economic yield loss in peppers (Delfine *et al*., 2000; Antony and Singandhupe, 2004; Kirada *et al*., 2007; Cafer *et al*., 2006; Sezen *et al*., 2006 and Showemimo and Olarewaju, 2007). Pepper is considered as one of the most sensitive crops to soil water deficit (González-Dugo *et al*. 2007). For high yields an adequate water supply is required during the total growing period. Reduction in water supply during the growing period in general has an adverse effect on yield. Low water availability prior and during flowering reduces the number of flowers and fruits (Jaimez *et al.*, 2000). Thus, water deficit is one of the major factors limiting chilli pepper productivity due to flower and fruit drop.

Drought is one of the most wide spread environmental stresses reducing yields by as much as 50% (Bray *et al*., 2000). Currently, approximately 1/3 of the world's arable land faces yield reduction due to cyclical or unpredictable drought, a great threat to agricultural production (Chaves and Oliveira, 2005). To meet the needs of the growing world population, it is essential to effectively utilize dehydrated soil in drought prone areas. Development and planting of drought tolerant cultivars is a cost-effective and practically acceptable approach for full utilization of water-limiting soil (Ashraf *et al.*, 2001).

Consequently, the development of drought-tolerant varieties will become increasingly important and efforts are directed towards a better understanding of plant responses to water deficit. Drought or water deficit affects directly the cellular metabolism of plant leading to a significant reduction in growth index and ultimately

crop yield. Hence, it is required to find out drought tolerant germplasm. Existence of genetic variability among genotypes is the most important component for the success of a breeding programme for increased drought resistance. The basic approach for development of drought tolerant genotypes is to select germplasm containing genetic variability for high yield potential and drought adaptive traits. Therefore, estimation of type and amount of total genetic variability associated with the target traits is equally important. Screening of cultivars based on physiological traits, morphological traits and biochemical traits is very crucial in selection of genotype to grow successfully in stress conditions. Field experiments related to water stress have been difficult to handle due to significant environmental or drought interactions with other abiotic stresses (Rauf, 2008). An alternative approach is to induce water stress through polyethylene glycol (PEG) solutions for the screening of the germplasm (Nepomuceno, 1988; Kulakarni and Deshpande, 2007; Khodarahmpour, 2011 and Rajendran *et al*., 2011). Polyethylene glycol with the molecular mass of 6000 and above is non-ionic, water soluble polymer which is not expected to penetrate intact plant tissues. This solution interferes with the roots to absorb water due to the reduction of osmotic potential (Dodd and Donovan, 1999 and sidari *et al.*, 2008). It has been reported that exposing plant roots to a PEG 6000 solution had no toxic symptoms at the plant level (Emmerich and Hardegree, 1990; Zgallai *et al.*, 2005). Since, PEG is a neutral polymer and highly soluble in water, it has been widely used to induce drought stress in plants (Zgallai *et al.*, 2005). Germinating seeds under PEG-simulated drought stress have several advantages compared with field or pot screenings, including easy to score root and shoot traits, controlled environmental conditions, thus increasing the repeatability, screening large numbers of genotypes in a small space within a short time, precise control of the concentration of mineral

nutrients (Kumar *et al*., 2014). It has been established that PEG induced drought stress mimics withdrawal of water from plants (Lawlor, 1970). This synthetically created water stress environment is used to provide the opportunity in selecting superior genotypes. On the basis of these facts, the present attempt was made to categorize chilli germplasm against drought stress to select suitable cultivars for drought tolerance.

Identifying and understanding mechanisms of drought tolerance is crucial for the development of tolerant crop varieties. For understanding the mechanism of drought tolerance, it is imperative to find out drought responsive proteins and ions for which novel approach would be proteomics and ionomics.

Plant adaptation to environmental stresses is controlled by cascades of molecular networks resulting in a combination of metabolic, physiological and morphological changes. Stress perception by osmosensors leads to signal transduction via primary and secondary messengers. Drought tolerance is controlled by regulatory proteins such as transcription factors, mitogen activated and calcium-dependent protein kinases and phospholipases, stress associated genes encoding functional proteins (Shinozaki and Dennis, 2003 and Beck *et al*., 2007). Thus enzymes involved in stress avoidance by maintaining the osmotic pressure and stabilisation of the quaternary structure of proteins as well as proteins involved in damage repair are synthesised. To understand these metabolic changes "Proteomics" is the important study area.

Ionome is the study of entire mineral nutrient and trace elements found in an organism (Salt *et al*., 2008). Elements are an essential component of every living cell. Elements are used to regulate the electrochemical balance of cellular compartments, as cofactors in biochemical reactions, and as structural components in biological

molecules and complexes. It is the dynamic network of elements, controlled by physiology and biochemistry of the plant, which is ultimately controlled by the genetic and environmental factors (Baxter, 2010).

Ionomics has the ability to capture information about the functional state of plant under different conditions, driven by genetic and developmental differences and by biotic and abiotic factors. Ionomics is a functional genomics approach for the identification of the gene(s) and gene networks that regulate the elemental composition, or ionome, of an organism. Comparison of the ionome of different genotypes across the multiple experiments will enhance the ability to identify drought stress and natural variants, as well as allow the identification of classes of ionomic profiles with common underlying physiological foundations (Baxter, 2009). Keeping above mentioned facts in view the present study entitled "Ionome and proteome assisted characterisation of drought tolerance in chilli (*Capsicum annuum* L.)" was formulated with the following objectives

OBJECTIVES

1. To collect and screen chilli germplasm for drought tolerance.
2. To elucidate profile of ions in the susceptible and tolerate germplasm lines.
3. To bring out profile of proteins in the susceptible and tolerate germplasm lines.
4. To correlate the ion and protein profiles, their significance towards the tolerance to drought.

CHAPTER-2

REVIEW OF LITERATURE

The present investigation entitled **"Ionome and proteome assisted characterization of drought tolerance in chili (*Capsicum annuum* L.)"** was carried out during 2015-16 and 2016-17 in plant growth and development laboratory of Department of Horticulture, Sikkim University, Gangtok, Sikkim. Keeping in view of the objectives of the present investigation, relevant literature on various aspects of ionome and proteome assisted characterization of stress tolerance, especially drought tolerance in Chili and other related crops has been reviewed here

2.1. Drought

As plants have a sedentary mode of life, they resort to many adaptive strategies in response to different abiotic stresses, such as salt, high light, low light, drought, cold, heat and flooding. These factors tend to alter plant's natural equilibrium and represent a driving force away from cellular homeostasis (Epstein *el. al.*, 1980). Among all the osmotic stresses to which plants may be exposed, drought-stress is probably the most limiting on plant productivity, both in natural and agricultural systems (Hanson and Hitz, 1982). In the changing climatic condition areas under drought have already expanded and this is expected to increase further. Exposure of plants to a water-limiting environment during various developmental stages appears to activate various physiological and developmental changes. Understanding of the basic mechanisms (biochemical and molecular) for drought stress, its perception, transduction and tolerance is still a major challenge in biology. When a plant is subjected to abiotic stress, a number of genes are turned on and off,

resulting in altered levels of several metabolites and proteins, some of which may be responsible for conferring a certain degree of protection to these stresses. Physiological and biochemical changes at the cellular level that are associated with drought stress include turgor loss, changes in membrane fluidity and composition, changes in solute concentration and protein-protein and protein-lipid interactions (Chaves *et al.*, 2003). Plant tissues can maintain turgor during drought by avoiding dehydration, tolerating dehydration or both (Kramer and Foyer, 1995). These types of stress resistance are controlled by developmental and morphological traits such as root thickness, the ability of roots to penetrate compacted soil layers and root depth and mass (Pathan *et. al*., 2004). Constitutive phenotypic traits (e.g. root thickness) are present even in the absence of stress conditions. By contrast, adaptive traits, such as osmotic adjustment and dehydration tolerance, arise in response to water deficit (Sirraj and Sinclair, 2002).

Drought stress is either moderate or extensive loss of water. Moderate loss of water leads stomata closure and limitations in gas exchange and extensive loss water causes disruption of metabolism and cell structure eventually cessation of enzyme catalyzed reactions (Smirnoff, 1993; Jaleel et al., 2007). Reduction of water content in plants leads to reduced water potential, turgor pressure, stomata closure and decreased cell enlargement. Drought stress affect the plant growth by physiological and biochemical process, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Jaleel *et al.*, 2008; Farooq *et al.*, 2008). The response of plants to drought stress differs significantly with intensity and duration as well as plant species and developmental stage (Chaves *et al.*, 2002; Jaleel *et al.*, 2008). The drought stress factors negatively affect growth and productivity and plants have evolved different mechanisms to respond to such

challenges. In plants, a better understanding of the morpho-anatomical and physiological basis of changes in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions.

2.2 Hydroponics

One of the important components of achieving sustainable and secure agriculture is to find out plants which are tolerant to abiotic stresses such as drought, salinity, heavy metal, cold stress, heat stress and other nutrient deficiencies. To achieve that hydroponics is one of the important scientific modelling tools, which facilitate accurate management of required environment. Drought is a particularly complex stress phenomenon that is difficult to model in any growth system. Water deficit may be imposed in hydroponics using osmotica such as mixed salts NaCl, mannitol, sorbitol and polyethylene glycol. The applied water stress in hydroponics is more controlled and homogeneous than in soil-based systems (Shavrukov *et al*., 2012). Exposure to polyethylene glycol (PEG-6000) solution, has been effectively used to mimic drought stress with limited metabolic interferences as those associated to the use of low molecular weight osmolytes that cannot be taken up by the plant (Hohl and Peter, 1991). It has been confirmed that PEG-induced drought screening is an effective drought screening method (Kulkarni and Deshpande, 2007). PEG has ability to keep the same water potential all over the experimental period (Hohl and Peter, 1991). Water potential decreased with increased PEG concentration and it caused decrease of seedling growth in tomato (Manoj and Uday, 2007). Similar type of results were found with decreasing water potential in Pearl millet (Radhouane. 2007), wheat (Dhanda *et al*., 2004), Barely (Kocheva and Georgive, 2003), rye grass

(Stacey *et al*., 2004), Black gram (Geetha *et al*., 1997) and potato (Gopal and Iwam, 2007).

2.3 Effect of drought stress on morpho-physiological and biochemical characters

The effects of drought stress on morphological, physiological and biochemical characters have been studied by several investigators. It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. Most of the drought effects appear on aerial or shoot parts which will bear mostly economic parts. Hence, shoot parameters are the one of the important characters to be considered while selecting the drought tolerant or resistant genotype or cultivar. Radhouane (2007) reported in pearl millet that shoot length was reduced along with increase osmotic stress in all ecotypes i.e. at -1.0 MPa 44% of shoot length was reduced whereas at -2.0 MPa 84.5% of reduction was found. In tomato, Kulakarni and Deshpande (2007) found that along with increasing PEG concentration shoot length was reduced. Reduction of shoot length was substantially less in mutant derivatives and hybrids which were found to be resistant to drought. Similar type of results were observed by Govindaraju *et al*. (2010) in peal millet where the reduction rate of shoot length was more in susceptible cultivars compared to tolerant cultivars. Bibi *et al*. (2012) in sorghum observed shoot length reduction to be 48.5% at -1.03MPa water stress. In lentil drought screening Singh *et al*., (2013a) found that ICARDA drought tolerant lines (ILL-10700, ILL-10823 and FLIP-96-51) were exhibiting maximum seedling survivability and least reduction of shoot and root length. Early and rapid elongation of root was important indication of drought tolerance. A root system with longer root length at deeper layer is useful in extracting water in upland conditions (Narayan, 1991 and Kim *et al.*, 2001). Leila Radhouse., (2007) in pearl millet reported that root length increased 15.8% at moderate drought

stress (-1.0MPa) whereas -2.0MPa drought stress condition root length reduction was 88%. Similar type of result was observed in chilli (Lakshmi Sahitya and Krishna, 2015). Kulakarni and Deshpande (2007) observed that root length was decreasing along with increasing drought stress and reduction of root length differed with genotypes in tomato. Kosturkova *et al.,* (2008) observed in soya bean, with increasing PEG concentration root length reduced by 2 to 3 times for the different genotypes compared to control. Reduction was less in Bulgarian lines particularly line-3 and line-5 which were showing drought tolerance. Govindaraju *et al.,* (2010) also observed similar type of results in pearl millet in which increased the water stress decreased root growth compared to control.

On set of water deficient reduces the plant-cell's water potential and turgor, which elevate the solutes' concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases, leading to reduction of leaf development and growth inhibition, which was reflected in shoot length, leaf area, number of leaves and number of internodes and other growth parameters (Lisar *et al.*,2012). Drought stress caused reduction of leaf area, number of leaves, number of internodes this was observed in many crops like chilli (Khan *et al.*, 2012), cowpea (Abayomi and Abidoye, 2009), soybean (Zhang *et al.*, 2004), bitter gourd (Shahbaz *et al.*, 2015), populus (Wullschleger *et al.*, 2005). Reduction of leaf area limits photosynthesis, and further decreases biomass production, this is the reason for the reduction of shoot dry weight and root dry weight along with increasing drought stress. Bibi *et al*., (2012) in sorghum observed that shoot and root dry weight decreased with increased drought stress. Similar results were obtained by Kulkarni and Deshpande (2007) and Nahar and Gretzmacher (2011) in tomato, Saddam *et al.*, (2014) in sorghum, Soni *et al.*, (2014) in *Vigna acountifolia* and Shahbaz *et al.,* (2015) in bitter gourd. The root-to-

shoot ratio increased under water-stress conditions to facilitate water absorption and to maintain osmotic pressure, although the root dry weight and length decreased as reported in sugar beet and populus (Lisar *et al.,* 2012).

Root to shoot dry weight estimates the distribution of dry matter between the root and shoot systems and it is a good indicator for effect on roots and shoot dry weight. Plants in dry condition often decrease the biomass production and contribute more biomass to roots, maintaining a higher root to shoot ratio (Yin *et al.,* 2005; Martin *et al.,* 2006; Villagara *et al.,* 2006 and Eric *et al.,* 2007) as an adaptation to drought resistance. Amino acid proline gets accumulated when plants exposed to drought stress. Proline plays major role as osmolyte besides that it acts as metal chelator, signalling molecule and antioxidative defence molecule. It has been discovered that proline improves stress tolerance by maintaining cell turgor or osmotic balance, stabilizing membranes and bringing the reactive oxygen species in to normal ranges by this way preventing oxidative burst in plants. Genotypes which accumulate high proline concentration under stress environment are generally considered to be tolerant (Yamada *et al.,* 2005; Vendruscolo *et al.,* 2007 and Abbas *et al.,* 2014). Kaur *et al.,* (2013) in chickpea observed that tolerant genotypes accumulated more proline content than sensitive ones. Lakshmi Sahitya and Krishna, (2015) reported in chilli that proline concentration was increased in all genotypes compared to control during drought stress and they also observed that drought tolerant genotypes accumulated higher concentration of proline content.

2.4 Ionomics

Elements, along with nucleic acids, proteins, and metabolites, are an essential building block of the living cell and are involved in almost every process in an organism. Understanding the functions and dynamics of elements is therefore critical

for understanding how life works (Baxter, 2010). Ionome is defined as the mineral nutrient and trace element composition of an organism, representing the inorganic component of cellular and organismal systems (Salt *et al*., 2008). It is a dynamic network of elements that are controlled by the physiology and biochemistry of the plant, which are ultimately controlled by the genome, in response to the environment. Lahner *et al.,* (2003) measured 18 elements of 6000 mutagenized M2 *Arabidopsis thaliana* plants (shoots) by using ICP-MS and separated 51 mutants with had altered elemental profiles. Based on results, it was found that 2-4% of *Arabidopsis thaliana* genome was involved in controlling the plant nutrients. From this experiment it was concluded that ionome profiling can be used as functional genomics tool for identification of genes involved in the accumulation of mineral nutrients and trace elements in plants. Rus *et al*., (2007) also reported that ionomics is one of the functional genomics strategies intended to rapidly identify the genes and gene networks involved in regulating how plants acquire and accumulate these mineral nutrients from the soil. Among 12 accessions of *Arabidopsis* 2 coastal accessions Ts-1(Spain) and Tsu-1(Japan) accumulated higher shoot levels of Na+ than do Col-0 and other accessions. They have identified AtHKT1known to encode transporter of Na+ in both Ts-1and Tsu-1. It was found with help of coupling of ionome profiling and DNA micro array based bulk segregant analysis and reverse genetics. Baxter *et al.,* (2008) established multivariable ionomic signatures for different physiologic state. This was achieved by studying *Arabidopsis thaliana* ionome profiling which were growing in different iron and phosphorus homeostasis condition. These multivariable ionomic signatures are potent enough to find specific physiological environmental or genetic perturbations. Buescher *et al*., (2010) found that in the plants, association of different elements and QTL of elements strongly affected by which environment they

are growing. This was observed by studying ionome profiling of 12 *Arabidopsis thaliana* accessions and 3 recombinant lines in different environments. Sánchez-Rodríguez *et al.,* (2010) reported that drought stress caused reduction of uptake macro-micro nutrients and in tolerant accessions or cultivars uptake nutrients was carried out in a better way than susceptible cultivars or accessions.

2.5 Drought Stress and Mineral Nutrients

2.5.1. Nutrient Uptake

Drought stress affects the balance in mineral nutrition of plants that leads to secondary effects. Drought stress reduces the mineral nutrients movement from root to shoot by decreasing transportation rates and changing membrane transporters role. Photosynthesis reduction, leaf senescence, nutrient uptake reduction, reduced cell growth and enlargement, leaf expansion, assimilation, translocation and transpiration are symptoms of drought stress and adversely affect the crop growth. The nutrient uptake of plants markedly influenced by the different factors *viz.* over use of land in agriculture activities, climate change, precipitation pattern, root morphology, soil properties, quantity and quality fertilizers, amount of irrigation (Patel., 1993; Barber., 1995 and Alam., 1999). Quantity of nutrient uptake by the plant depends on root structures such as root extension rate, root length root, radius and root hair density. Many nutrient elements are actively taken up by the plants, however, the capacity of plant roots to absorb water and nutrients generally decrease in drought stressed plants. Essential plant nutrients are known to regulate plant metabolism even when the plants exposed to drought by means of cofactor or enzymes activators (Nicholas., 1975). Mineral uptake can decrease when drought stress intensity increases (Rahman *et al.*,1971; Viets,1972; Nambiar, 1977; Tanguilig *et al.*, 1987; Kirnak *et al.*, 2003 and Singh and Singh, 2004;). For example, nutrients uptake concentration reduced along

with increased of water stress especially phosphorus, K+, Mg2+, Ca2+ in some crops (Foy,1983; Bie and Shinohara 2004 and Abdalla and El-Khoshiban, 2007), Fe3+, Zn2+ and Cu2+ in sweet corn (Oktem., 2008), Fe3+, K+ and Cu2+ in *Dalbergia sissoo* leaves (Singh and Singh, 2004)). This reduction of nutrient uptake was primarily due to soil water deficiency which in turn shortened the movement rate of elements in soil. Further, water stressed roots did not have the capability to uptake and translocate the nutrients.

On the contrary there were other reports stating that along with increase in the intensity of drought stress some elements uptake also increased. Tanguilig et al., (1987) reported potassium and calcium concentration increased in maize during water stress.

Potassium in drought tolerant wheat varieties (Sinha, 1978) and nitrogen, phosphorus, calcium, magnesium, zinc, and manganese in *Dalbergia* leaves (Singh and Singh, 2004) increased along with increasing drought stress. Nambiar (1977) reported that relative amount of potassium, calcium and magnesium increased in barley than in rye when drought stress was imposed. Although, reports stated calcium content increased during water stress (Tanguilig *et al.,* 1987 and Dogan and Akinci, 2011), Kırnak *et al.,* (2003) stated that drought stress reduced the calcium content in bell pepper and found that there was antagonistic affects of zinc and manganese on calcium uptake. Dogan and Akinci, (2011) reported that manganese content increased along with increasing water stress in *phaselous vulagaris*. Kidambi *et al.,* (1990) reported that phosphorus content in *Medicago sativa* (Alfa Alfa) and *Onobrychis viciifolia* (Sainfoin) increased when soil moisture supply decreased. On the other hand, Gomez-Beltranno (1982) observed no effect of moisture stress on nitrogen, phosphorus and potassium in alfa alfa. Based on the research carried out before the

mid 1950s, 12 of the 21 studies reported that P concentration decreased, and 9 studies stated that P status was not changed in plants due to drought stress (Gerakis *et al.,* 1975). Magnesium absorption increased by drought stress in many crops (Rahman *et al.,* 1971; Gerakis *et al.,* 1975). Contrary to this, in *Phaseolus vaulgaris* magenesium content decreased with increased drought stress (Dogan and Akinci, 2011).

Most of the earlier studies reported that drought stress restricted uptake of nutrient elements by crops. It also hampered the active transport of nutrients. Further, various nutrient ions responded differently during growth conditions under drought stress. Hence, studying the behaviour of different elements in various crops during water stress may give insight into understanding of drought response by plants.

2.5.2. Effect of drought induced differential uptake of nutrients

Among mineral nutrients, macro nutrients has important role to form structural components of plants and their insufficiency leads to sensitivity of plants. Micronutrient deficiency makes the plant susceptible to stress by effecting the enzyme activity and modulating the signal transduction pathways (Hajiboland, 2012).

2.5.2.1 Phosphorus

Water stress reduced the movement of phosphorus from soil to root and to the stem (Goicoechea *et al.,* 1997; Cramer *et al.,* 2009) this was due to phosphorus move only through diffusion. Hence, unavailability of sufficient moisture in soil causes phosphorus deficiency. In maize Radersma *et al.,* (2005) reported that lower soil moisture level caused reduction of phosphorus uptake and decreased plant biomass. Singh *et al.,* (2006) observed in cotton that drought stressed phosphorus deficiency caused reduction in plant height and leaf area. Hence, to avoid the P deficiency, Kang *et al.* (2014) placed phosphorus fertilizer deep into soil and increased wheat yield in semiarid areas. Under drought stress, phosphorus application had a positive effect on

plant growth such as increased root growth (Singh and Sale, 1998), leaf area and photosynthesis (Singh *et al.,* 2013), higher cell membrane stability and water relations (Singh *et al.,* 2006 and Kang *et al.,* 2014).

2.5.2.2 Potassium

Plant water status strongly influences the accumulation of potassium in leaves. Its role in the opening and closing of stomata has been well documented and potassium channels in stomatal guard cells are sensitive to plant water status (Taiz and Zeiger, 2006). Drought stress environment reduced the availability of soil potassium and limited the uptake of potassium by plants (Wang *et al.,* 2013). Premchandra *et al.,* 1991 reported, maize plants were better adapting to water deficient condition by the supplementation of potassium. Potassium nutrition increasesd cell membrane stability, leaf water potential, turgor potential and reduced stomatal resistance. An improved potassium nutritional status increased plant productivity by maintaining osmotic balance and influencing directly leaf characteristics like thickness and water content. Potassium nutrition increased plant total biomass and leaf area and also increased the water with holding capacity in plant tissues (Lindhauer, 1985). In water deficient condition potassium supplementation facilitated plant tolerance through osmotic adjustment, maintaining activity of aquaporins induced root growth, stabilized the cell membrane activity and detoxification of ROS (Wang *et al.*, 2013).

2.5.2.3 Magnesium

Magnesium is an important component of chlorophyll molecule. It is also play major role as cofactor for several enzymes associated with dephosphorylation, hydrolysis and stabilizing the structure of nucleotides and sugar accumulation (Xiao *et al.,* 2014 and Blasco *et al.,* 2015). Thalooth *et al.,* (2006) reported that magnesium

foliar application under water stress increased seed yield, net assimilation rate and crude protein content in mungbean. Magnesium alleviated the water stress by increasing root growth and root surface area that lead to improved uptake of water and nutrients (Waraich *et al.,* 2011).

2.5.2.4 Calcium

Finding of role of calmodulin in plant metabolism made calcium as not only macro nutrient but also multi functional element (Poovaiah and Reddy, 2000). Palta, (2000) reported that calcium had a major role during healing of stress caused injury by activating plasma membrane ATPase. These plasma membranes ATPase pump back the nutrients which were lost during drought stress damage. According to Marschener, (1995) calcium deficiency caused stunted growth of plants, premature shedding of blossoms and buds. Knight *et al.*, (1998) reported that during drought stress there was rapid changes in cytosolic free calcium in *Arabidopsis thaliana*. These changes in calcium levels mediated increased expression of drought responsive genes encoding proteins that protected plants. Nahar and Gretzmacher, (2002) reported in tomato that calcium uptake was reduced with reduction of soil water potential. Nayyar and Kaushal, (2002) found that combination of calcium and ABA application effectively alleviated the drought stress in wheat. Upadhaya et al. (2011) found that foliar spray of calcium chloride reduced water stress induced changes in tea.

2.5.2.5 Sulphur

Sulphur is one of the essential elements. It has the important role in formation of chlorophyll, proteins, enzymes and vitamins. In plants generally sulphur present in the form of cysteine, methionine, thiols and sulfolipids. Water stress restricts the accessibility of sulphur to shoot and it causes down regulation of sulphur assimilating

pathways in leaves. Insufficient supply of sulphur affected the nitrogen use efficiency that lead to the reduction of crop productivity and quality (Haneklaus *et al.,* 2007). Heidari *et al.,* (2011) reported that sulphur application reduced the damaging effects of drought stress in sesamum. Ahmad (2013) reported in maize that during drought stress glutathione reduced the activity of ROS. This glutathione synthesis depends on the sulphur assimilation pathway.

2.5.2.6 Zinc

Zinc is one of the important micronutrient essential for plant growth and development. In water stressed soils its mobility was restricted. Hence, its uptake by roots was also low (Marschner, 1995). Zinc deficiency during drought stress lead to increased stomata closure and produced reactive oxygen species (Hajiboland, 2012). In sunflower Khurana and Chatterjee, (2001) reported that zinc was very critical element in reproductive organs and its deficiency lead to the reduction of productivity. Blasco *et al.,* (2015) reported in *Brassica* species that anti-oxidative enzymes activity was low in Zn deficient plants compared to normal plants. It has been reported that zinc supplementation reduced the drought stress effects on plant growth by reducing the oxidative enzymes damagae, synthesis reactive oxygen species and increasing antioxidative enzymes like superoxide dismutase, peroxidise, catalase for detoxifying ROS (Waraich *et al.,* 2011 and Hajiboland, 2012).

2.5.2.7 Manganese

Manganese is also one of the important micronutrient. It is cofactor for several enzymes especially which involved in TCA cycle and shikimic acid pathway. Manganese also involved in PS-II, ATP synthesis and Ribulose-1,5-bisphosphate carboxylase reactions. In tea, Upadhyaya *et al.,* (2012) reported that manganese had the crucial role in maintaining SOD activity and chlorophyll

concentration. Manganese acted as scavenger for reactive oxygen species (Millaleo *et al.,* 2010). Gholamin and Khayatnezhad (2012) reported that application of sufficient amount of Mn increased grain yield and stress tolerance in wheat.

2.5.2.8 Iron

Iron involves in synthesis of chlorophyll and it is a part of enzymes which involved in nitrogen reduction and fixation, lignin formation and energy transfer. Soil water content affects both content and availability of iron to plants. If soil moisture content is sufficient, amount of Fe2+/Fe3+ ratio will be high and easily available to the plant. But in drought conditions, decreased amount of Fe2+/ Fe3+ ratio occurs possibly due to the increased O_2 levels in soil.

Higher O_2 reduced available iron for plant uptake because Fe3+ is less soluble than Fe2+ (Sardans et al., 2008). Water stress induced deficiency of Fe caused chlorosis in leaves. Drought induced oxidative stress can alleviated by iron nutrition because iron nutrition increased the antioxidant enzymes such as Cu/Zn super oxide dismutase, APX and POD. Lombardi et al., (2003) reported in onion that iron supplementation improves antioxidant enzymes.

2.5.2.9 Boron

Boron has important role in cell wall formation, lignin synthesis and xylem differentiation. Moreover, it enhances photosynthetic rate and integrity of membranes. Generally dry soils are boron deficient because of immobility. Even after applying sufficient amount of boron, roots cannot take up boron in drought stressed soils because mostly of the boron uptake takes place through passive process. Hajiboland and Farhanghi, (2011) reported in turnip that drought reduced the availability of boron

in soils. In spruce plants Motten *et al.*, (2015) observed that plant height, root dry weight and nutrient uptake was low under boron deficient condition.

2.5.2.10 Copper

Copper is important micro nutrient needed for nitrogen and carbohydrate metabolism. Copper availability in dry soils was low (Tom-Petersen *et al.,* 2004). Faize *et al.,* (2011) reported that over expression of cytosloic CuSOD/ZnSOD improved tolerance against water stress. Cu/ZnSOD reduced the water stress through balancing water use efficiency and reduction of hydrogen peroxide generation and electrolyte leakage.

2.5.2.11 Molybdenum

Molybdenum has important role in plant metabolism as a part of four enzymes *viz.* nitrate reductase, aldehyde oxidase, xanthin dehydronase and sulphite oxidase (Mendel and Hansch, 2002). Drought induced the molybdenum deficiency and thus made plants susceptible for drought stress (Hu and Schmidhalter, 2005). Mo was found to be an important component of MoCo (molybdenum cofactor) which improved the nitrogen metabolism (Mendel and Bittner, 2006). Molybdenum reduced the drought stress effects and increased water use efficiency due to its participation in enzymes of N metabolism, S metabolism and protein synthesis (Waraich *et al.,* 2011).

2.6 Proteomics

Proteins are essential to living organisms because they play central role in metabolic process. Stress induced alteration of gene expression to modulate metabolic process leads to alteration of cellular protein abundance. The proteome of each living cell is dynamic, altering in response to the individual cell's metabolic state and reception of intracellular and extra cellular signal molecules and many of proteins which expressed will be post translationally altered (Nanjo *et al.,* 2011). Proteomics

provides more direct assessment of biochemical process of monitoring the actual proteins performing the signalling, enzymatic, regulatory and structural functions encoded by the genome and transcriptome (Qureshi *et al*., 2007).

Hajheidari *et al.*, (2005) compared protein profiling of well watered and water stressed genotypes of sugar beet. At 157 DAS from the comparative protein profiling of leaf samples of the genotypes they have found that 79 protein spots significantly changed under drought which were mainly of Rubisco and 11 other proteins involved in redox regulation, oxidative stress, signal transduction, and chaperone activities. Rao *et al*., (2009) studied comparative protein profiling of two contrasting peanut genotypes for drought stress. 48 proteins were identified implicating a variety of stress response mechanisms in peanut. It was found that inter cellular and intra cellular signalling assisted proteins lipoxynase and 1L-myo-inositol-1-phosphate synthase were ample in tolerant genotype under stress condition and acetyl-CoA carboxylase a key enzyme of lipid biosynthesis was also increased along with epicuticular wax content (water conservation mechanism). Also there was considerable reduction of photosynthetic proteins in tolerant genotype in water stress condition. During drought stress cell wall strengthening, signal transduction, cellular detoxification and gene regulation was affected. Nelson and Peter (2012) identified proteins associated with plant drought tolerance by comparing two barley varieties Golden Promise (Sucueptible) and Basrah (Tolerant). From the physiological adaptations point of view variety Barsha exhibited higher relative water content in shoots and roots than golden promise. They have identified 24 leaf and 45 root proteins by comparative protein profiling in control and drought conditions of both variteis. This experiment concludes that drought tolerance of variety Barsha was driven by the enhanced regulation of ROS under drought. Kausar *et al*., (2013) in

barely found that under drought stress, metabolism and photosynthetic related proteins were increased in drought tolerant genotype whereas in susceptible genotype both types of proteins were decreased. By comparative proteomics Wang *et al*. (2015) in barley found that 38 drought tolerance associated proteins. These proteins characterized mainly in to Photosynthesis, stress responsive, metabolic process, energy and amino acid biosynthesis. Some proteins like melanoma associated antigen p97, type 1chlorophyll a/b binding protein b, glutathione S-tranferase 1, ribulosebisphosphate carboxylase large chain were found only in drought tolerant genotype and not in susceptible one. Cheng *et al.,* (2016) studied comparative proteomics to analyze proteome change of drought tolerant wheat cultivar and drought sensitive wheat cultivar subjected to range of dehydration treatments (18h, 24h and 48h) and rehydration treatment (24h) by using 2-DE. From the quantitative image analysis, they found 172 protein spots in drought tolerant cultivar and 215 proteins spots from drought sensitive cultivar with more than 2.5 fold increse. Most of the identified proteins were involved in metabolism, photosynthesis, defence and protein translation or processing or degradation, redox homeostasis, energy, transcription, cellular structure, signalling and transport in both cultivars. Bunding *et al.*, (2016) studied comparative proteomics between two contrasting genotypes of potato in drought tolerance. *In vitro* osmotic stress was created by using 0.2M sorbitiol. Shoot tips were collected from control and treatment for the proteome analysis. Protein profiles were analyzed using 2D-IEF/SDS-PAGE. The differentially abundant spots of the tolerant genotype comprised one chaperone and one hydrogen peroxide detoxifying protein.

Hence, it is evident that the molecular and cellular mechanisms of drought tolerance can be greatly understood through comparative the proteomics studies.

CHAPTER-3

MATERIALS AND METHODS

The present investigation entitled **"Ionome and proteome assisted characterisation of drought tolerance in chilli (*Capsicum annuum* L.)"** was carried out during 2015-16 and 2016-17 in plant growth and development laboratory of Department of Horticulture, Sikkim University, Gangtok, Sikkim. The details of materials used and methods employed during the present investigation are described below.

3.1 Experimental Materials

The experimental material for the present study was comprised of eight cultivars of chilli (***Capsicum annuum* L.**) and the list of cultivars and genotype along with their sources is given in Table 3.1.

3.2 Experiment Details

3.2.1 Seedlings rising

The seeds of all cultivars of chilli were sterilized with 70% ethanol for 1 min., followed by soaking in 0.1% $HgCl_2$ for 3 min. and then thoroughly washed with sterile distilled water for three times. Protrays were used for raising chilli seedlings. Protray holes are filled with mixture of coco peat and perlite. Single seed was sown in each holes and irrigation was given at regular intervals. The Protrays were kept in plant growth and development laboratory and optimum temperature (25℃) was maintained which resulted healthy seedlings production.

Table 3.1 List of cultivars and genotype along with their sources

Sl. No.	Cultivar	Source
1	Arka Lohit	Indian Institute of Horticulture Research Station, Hessaraghatta, Bengaluru, Karnataka
2	Arka Mohini	Indian Institute of Horticulture Research Station, Hessaraghatta, Bengaluru, Karnataka
3	LCA-334	Regional Agricultural research station, Lam, Guntur, Andhra Pradesh
4	LCA-353	Regional Agricultural research station, Lam, Guntur, Andhra Pradesh
5	G4	Regional Agricultural research station, Lam, Guntur, Andhra Pradesh
6	CA-960	Regional Agricultural research station, Lam, Guntur, Andhra Pradesh
7	LCA-625	Regional Agricultural research station, Lam, Guntur, Andhra Pradesh
8	Dallae Khursani	Sikkim Local Variety

3.2.2 Hydroponics set up required materials

A simple hydroponic set up was constructed by using utility trays (40cmx26cmx8cm) and protrays. Protrays were sized according to utility tray and made holes for holding seedlings. Aquarium air pumps (Sobo Company, India, SB-548A) were used for continued aeration. Each tray was filled with 4 litres of hydroponic solution supllimented with trace amounts of multi elements to provide nutrient to seedlings and solution was changed once in seven days.

3.2.3 Preparation of Hydroponics Stock solution (Hoagland's nutrient)

Stock solution was prepared according to Hoagland and Arnon (1950) with a minor modification as Fe-EDTA alone was prepared seperately. Stock solution was prepared carefully to avoid nutrient deficiencies and mineral toxicities not attributed to drought stress. For each macro nutrient stock solution preparation, crucial amount of reagent was dissolved in a beaker containing 500 ml distilled water and the contents were transferred to 1 liter volumetric flask. Finally volume was made up to 1liter with distilled water and stirred for 15 minutes in a magnetic stirrer for proper mixing. The solutions were maintained as stock solutions. The micro nutrients except the Fe-EDTA was also prepared as the procedure mentioned above (Table 3.2).

3.2.4. Fe EDTA preparation

Fe EDTA was prepared according Abram *et al* (1970). Solution A was prepared by dissolving 33.3 g of disodium EDTA in warm water (about 30^0C), containing 100.4 ml of 1N NaOH. Solution B was prepared by dissolving 24.9 g of $FeSO_4$-$7H_2O$ in 300 ml of hot water (about 70^0 C), containing 4 ml of 1 N HCl. Solution A and B were mixed and and added with 950 ml of distilled water, aerated vigorously for 12 hrs., then made to 1000 ml with distilled water and stored in amber colour bottle.

Table 3.2 List of chemicals required for Hoagland's solution preparation.

Component	Stock solution	ml of stock solution/1lit nutrient
Macro nutrients		
1 M NH_4H_2PO4	115.0257 g L^{-1}	1ml
1M KNO3	101.1032 g L^{-1}	6ml
1 M Ca(NO3)2.4H_2O	164.0878 g L^{-1}	4ml
1 M MgSO4.7H2O	120.3676 g L^{-1}	2ml
Micro nutrients		
H3BO3	2.86 g L^{-1}	1ml
$MnCl_2$ .4H_2O	1.81 g L^{-1}	
ZnSO4 .7H_2 O	0.22 g L^{-1}	
CuSO4. 5H_2O	0.08 g L^{-1}	
H_2MoO4. H_2O	0.02 g L^{-1}	
Fe EDTA		0.25ml

Nutrient solution prepared every time freshly by drawing the recommended quantity of stock solution each time. pH of solution was maintained between 5.5-6.0 after adding the solution containing 10 ng L^{-1} each of Ag, Al, B, Ba, Be, Bi, Ca, Cd, Cr, Cs, Cu, Fe, Ga, Ge, In, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Si, Ta, Ti, V, Zn, Zr and Hg analytes. For adjusting pH 1N NaOH and 1N HCl was used. In addition 1µg L^{-1} of all trace elements were added to the solution.

3.2.5 PEG treatment

Seedlings were transferred at the age of 14 days into a hydroponic system where, trays were filled with modified Hoagland's nutrient solution. Each tray was designated for different levels of drought stress. Different levels of drought stress was given 3 days after transplanting by adding different concentrations of PEG-6000 *viz.* 0%, 5%, 10% 15% and 20% into the designated trays

1. T1 - 0% control
2. T2 - 5% (50 g L^{-1})
3. T3 - 10% (100 g L^{-1})
4. T4 - 15% (150 g L^{-1})
5. T5 - 20% (200 g L^{-1})

The roots of seedlings of all the treatments were directly submerged in aerated growth solution and the shoots were supported to grow above the solution. Solution was changed once in every 7 days. Whole hydroponic culture system was maintained under optimum culture conditions at 16 hours photoperiod (70 µ mol M-2 s-1) at 28^0C temperature. After 30 days of treatment, measurements were recorded in the all treatments.

3.3 Observations on growth parameter

3.3.1 Shoot length

Shoot length was measured with the help of a scale. Height from the collar region to the base of the last fully opened leaf was recorded and expressed in centimetre. Three randomly selected plants were taken from each treatment for recording data and average was calculated.

3.3.2 Root length

Root length was measured with the help of a scale. From the collar region to growing tip of root was measured. Three randomly selected plants were taken from each treatment for recording data and average was calculated.

3.3.3. No. of leaves

No. of leaves per plant was counted in three randomly selected plants and average was calculated.

3.3.4 No. of internodes

No. of internodes per plant was counted in three randomly selected plants and average was calculated.

3.3.5 Leaf area per plant

Leaf area per plant was measured by using leaf area meter (model: 211, Systronics, India). Three randomly selected plants were taken from each treatment for recording data and average was calculated.

3.3.6 Fresh shoot weight

The Fresh shoot weight of plant was measured with the help of electronic balance immediately after taking from hydroponic system. Three randomly selected plants were taken from each treatment for recording data and average was calculated.

3.3.7 Fresh root weight

Roots were separated from collar region of the plant and weight was measured with the help of electronic balance immediately after taking from hydroponic system. Three randomly selected plants were taken from each treatment for recording data and average was calculated.

3.3.8 Shoot dry weight

Shoot dry weight was measured after drying in hot air oven at 60℃ for 72 hours and expressed in milligrams. Three randomly selected plants were taken from each treatment for recording data and average was calculated.

3.3.9 Root dry weight

Root dry weight was measured after drying in hot air oven at 60℃ for 72 hours and expressed in milligrams. Three randomly selected plants were taken from each treatment for recording data and average was calculated.

3.3.10 Root to shoot dry weight

Root to shoot dry weight ratio was calculated by dividing root dry weight by shoot dry weight.

3.4 Biochemical parameter

3.4.1 Proline

Proline was estimated spectrophotometrically following the method of Bates et al., (1973). The leaves weighing 250 mg were homogenized with 3% sulphosalicyclic acid. The homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant was collected. 2 ml of supernatant was made to react with 2 ml of freshly prepared ninhydrin (1.25 g of ninhydrin dissolved in a mixture of 30 ml of glacial acetic acid and 20 ml of 6 molar orthophosphoric acid with warming and stirring) and 2 ml of glacial acetic acid in a test tube and then was kept in a boiling

water bath at 100^0C for 1 hour. The reaction was terminated in an ice bath and then shifted to room temperature. Thereafter, the reaction mixture was extracted with 4 ml of toluene, mixed vigorously with test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from aqueous phase and absorbance was read at 520 nm using toluene as a blank. The concentration of proline in the sample was calculated from the slope of the proline standard curve using the undermentioned formula and the result was expressed as 'microgram 'of proline g^{-1} fresh weight of leaf.

□moles per gram tissue = [(□g proline/ml) x ml toluene)/115.5 □g/□mole] / [(g sample)/5]

3.5 Ionomics

3.5.1 Sample preparation

Leaves were washed first with tap water to remove the adhered dust particles followed by teepol solution. They were then washed by 0.1 N HCL solution and finally washed with double distilled water. This was followed by drying the samples in hot air oven at 60^o C. The dried samples were powdered with the help of Wiley's mill.

3.5.2 Sample Digestion

The microwave digestion of the sample was performed with the multiwave digestion system (Anton Par, Multiwave 3000). Digested samples were cooled and made up to volume of 50 ml with DDW (Double distilled water) in volumetric flask and then transferred to narrow mouth bottle with details of the sample for further analysis.

3.5.3 Ionome profiling

Ionome profiling was carried out with the help of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin Elemer, Nex ION 300X) system with cross flow nebulizer. Before analysing the sample the instrument was calibrated using standard reference material (peach level-NIST, 1547). Digested samples were analysed for ionomic constitution using multi elemental standard solution no.1 , 3 and 5 supplied by Perkin Elmer containing Ag, Al, B, Ba, Be, Bi, Ca, Cd, Cr, Cs, Cu, Fe, Ga, Ge, In, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Si, Ta, Ti, V, Zn, Zr and Hg analytes. Then digested sample analysed for the ionomic profiling and results were obtained directly in the units of ppm**.**

3.6 Proteomics

After finding out tolerant and susceptible cultivars based on in vitro screening. Comparative proteomic analysis was carried out for both tolerant and susceptible cultivars in control (C) and stress (T) condition. Drought tolerant cultivar in control condition (C1), Drought sucueptible cultivar in control condition (C2), Drought tolerant cultivar in stress condtion (T1), Drought susceptible cultivar in stress condition (T2). Proteomics analysis analysis procedure was as follows below.

3.6.1 Protein extraction

Protein was extracted following the method of Wang et al., (2003) in the following steps

- 1gm of plant tissue was weighed and liquid nitrogen grinding was done
- Metabolite Extraction Buffer was added to the ground sample and homogenized for 5 min.

- The homogenised material was centrifuged at 10,000 rpm for 10 min. at 4˚C. After the centrifugation the pellets alone were collected.
- To the pellets, 5 volumes of SDS buffer with protease inhibitors was added and incubated for 1hr. at room temperature on a shaker.
- Then centrifuged at 10,000 rpm for 15 min at 4˚C.
- To the supernatant, equal volumes of Tris buffered Phenol was added and shaking was done at room temperature for 30 min.
- Then again centrifuged at 10,000 rpm for 30 min. at 4˚C
- To the lower phenol layer, 6 volumes of 100mM ammonium acetate solution was added
- The contents were incubated overnight at -20˚C
- Then centrifuged at 10,000 rpm for 15 min. at 4˚C and pellets were collected
- The pellets were washed with pre-chilled acetone and centrifuged at 10,000 rpm for 15 min. at 4˚C.Again the pellets were collected and air dired.
- The air dried pellets were dissolved in 50mM ammonium bicarbonate which constituted the extracted protein.

3.6.2 SDS PAGE

The concentration of the sample and the protein profile was checked on a SDS PAGE. SDS was carried out as per procedure described by Laemmli (1970).

3.6.3 In solution Digestion Protocol

- 100µl of extracted protein was taken for digestion
- The sample was treated with 100 mM DTT (Dithiothreitol) at 95^{o}C for 1hr. followed by 250 mM IDA (Iminodiacetic acid) at room temperature in dark for 45min.
- The sample was then digested with trypsin and incubated overnight at 37˚C.

- From the incubated sample the peptides were extracted in 0.1% formic acid and incubated at 37˚C for 45 minutes.
- The solution was centrifuged at 10000 g and the supernatant was collected into a separate tube.
- The resulting sample was vacuum dried and dissolved in 20µl of 0.1% formic acid in water
- 10µL injection volume sample was used on C18 UPLC column for separation of peptides
- The peptides separated on the column were directed to Waters Synapt G2 Q-TOF instrument for MS and MSMS analysis.
- The raw data was processed by MassLynx 4.1 WATERS. The MSMS spectra of individual peptides were matched to the database sequence for protein identification on PLGS software, WATERS.

3.6.4 LCMS analysis

Liquid chromatography was performed on a ACQUITY UPLC system (Waters, UK). The separation of all samples was performed on ACQUITY UPLC BEH C18 column of 150mm X 2.1mm X 1.7µm dimension (Waters,UK). A gradient elution program was run for the chromatographic separation with mobile phase A (0.1% Formic Acid in WATER), and mobile phase B (0.1% formic Acid in ACETONITRILE) as per following conditions.

S.No	Time	Flow	%A	%B	Curve
1	Initial	0.300	98.0	2.0	Initial
2	1.00	0.300	98.0	2.0	6
3	30.00	0.300	50.0	50.0	6
4	32.00	0.300	50.0	50.0	6
5	40.00	0.300	20.0	80.0	6
6	45.00	0.300	20.0	80.0	6
7	50.00	0.300	98.0	2.0	6
8	55.00	0.300	98.0	2.0	6
9	60.00	0.300	98.0	2.0	6

A SYNAPT G2 QTOF (Waters, UK) equipped with an electrospray ionization (ESI) source was used for mass spectrometric detection. Samples analysis were performed in positive mode. The operation parameters were as follows

- Polarity ES+
- Analyser Resolution Mode
- Capillary (kV) 3.5000
- Source Temperature (℃) 150
- Sampling Cone 45
- Extraction Cone 4.5
- Source Gas Flow (mL/min) 30
- Desolvation Temperature (°C) 350
- Cone Gas Flow (L/Hr) 30
- Desolvation Gas Flow (L/Hr) 800

Acquisition

Acquisition Time

Start time : 0 min

End Time : 60 min

Source : ES

Acquisition Mode

Polarity : Positive

Analyzer Mode : Resolution

TOF MS

Da Range

Start : 50Da

End : 1500Da

Scanning Conditions

Scan Time : 0.5 Sec

Data Format : Continuum

Collision Energy

Function-1 Low Energy

Trap Collision Energy : On – 6V

Transfer Collision Energy : On – 6V

Function-2 High Energy

Ramp Trap Collision Energy : On – 20V to 45V

Ramp Transfer Collision Energy : Off

Cone Voltage

Cone Voltage: 40V

3.6.5 Mass spectrometric raw data analysis

The raw data acquired from the instrument was processed using PLGS software 3.0.2 within which data processing and database search was performed. The source of the sample being plant proteins for samples: C1, C2, T1, T2 , The protein database was downloaded from swissprot database (Rattus Proteins) and used for searching the proteins present in the samples. 3 runs of each sample were processed using the following search parameters in the software:

Search Parameters:

Peptide tolerance (ppm)	: 50
Fragment Tolerance (ppm)	: 100
Minimum no. of Fragment matches for Peptides	: 2
Minimum no. of Fragment matches for Proteins	: 5
Minimum no. of Peptide matches for Proteins	: 2
Missed Cleavages	: 1
Modification	:Carbamidomethyl_c,Oxidation_m
Database	: Uniprot: Capsicum annum
Search engine	: PLGS

3.7 Statistics

The experiment was laid out in factorial completely randomized design.

Factors

A. Cultivars- 8 numbers

B. Different concentration of PEG- 5 concentrations

Under each factor 3 replications were maintained and each replication had three biological replicates

3.7.2 Statistical analysis

The data on different parameters were analyzed by using analysis of variance (ANOVA) as suggested by Gomez and Gomez (1984). Valid conclusions were drawn only on significant differences between the treatment means at 0.05% level of significance. In order to compare treatment means, critical difference were calculated.

3.7.3 Principle component analysis (PCA)

PCA was performed by using JMP13.2.1 from SAS Software products.

CHAPTER-4

EXPERIMENTAL RESULTS

The present investigation entitled "Ionome and proteome assisted characterization of drought tolerance in chilli (*Capsicum annuum* L.)" was undertaken to evaluate and understand the mechanism of drought tolerance in chilli. The experimental results are presented under the following subheads

4.1 Screening Capsicum *annuum* germplasm for drought tolerance

Present study had revealed that all the observed growth parameters had shown highly significant variation between treatments as well as among cultivars (Table 4.1). It has been observed that at highest drought stress of 20% PEG condition (T5) LCA-353 could survive only seven days.

4.1.1 Shoot length

Shoot length was significantly reduced with increased drought stress in all cultivars compared to control (Figure 4.1 and Table 4.2). In the control condtion shoot length was ranging from 8.7 (LCA-353) to 13.47cm (LCA-334) with a mean of 10.57cm where as at 5% PEG (T2) it ranged from 4.72 (LCA-353) to 11.30cm (Arka Lohit) with a mean of 8.19. In 10% PEG (T3) condition shoot length ranged from 3.83 (LCA-353) to 8.80 (Arka Lohit) with a mean of 5.59cm. Shoot length was further reduced in 15% PEG (T4). Among the cultivars it ranged from 3.17 (LCA-353) to 6.52cm (Arka Lohit) with a mean of 4.54cm. In highest drought stress of 20% PEG (T5) condition, among the survived cultivars, the shoot length ranged from 3.5 (Arka Mohini) to 5.5cm (Arka Lohit) with a mean of 3.53. The mean performance of shoot length varied from 4.084 cm (LCA-353) to 8.94 cm (Arka Lohit). At the highest

Plate 1: Drought stress screening chilli under hydroponics system model

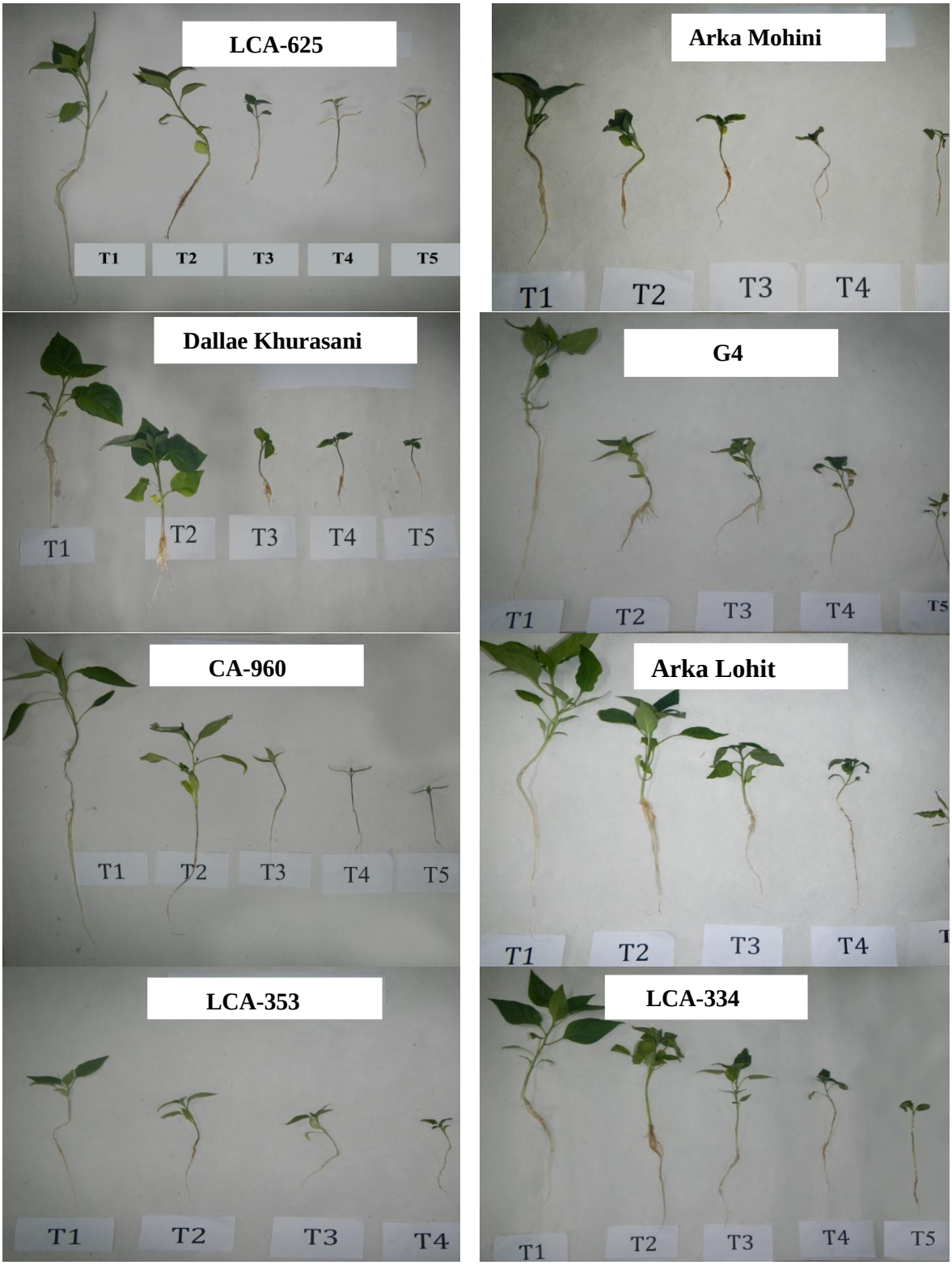

Plate No.2 Morpho-Physiological characters of *Capsicum annuum* L. cultivars exhibited during different level of Drought stress conditions

Table 4.1 Mean squares of 8 chilli cultivars for various plant traits under control and PEG stress conditions

Characters	Cultivars (G)	Treatment(T)	Interaction(GxT)	Error
D.F	7	4	28	78
Shoot length	40.311**	197.15**	2.2037**	0.6914
Root length	62.0109**	1112.8**	3.68002**	1.20352
No. of leaves	41.729**	129.28**	2.9548**	1.5536
No. of Internodes	8.2655**	49.304**	0.8994**	0.4085
Leaf area	964.51**	23383**	160.62**	60.978
Shoot dry weight	17956**	275995**	2144.7**	432.31
Root dry weight	7329.2**	59106**	634.65**	118.22
Root to shoot dry weight	0.094645**	0.139446**	0.010313**	0.00202
Proline	120162.9**	995877.1**	44490.21**	806.433

(*Significant at 5% df, ** Significant at 1% df)

Figure 4.1 Shoot Length (cm) of different genotypes at different concentration of PEG

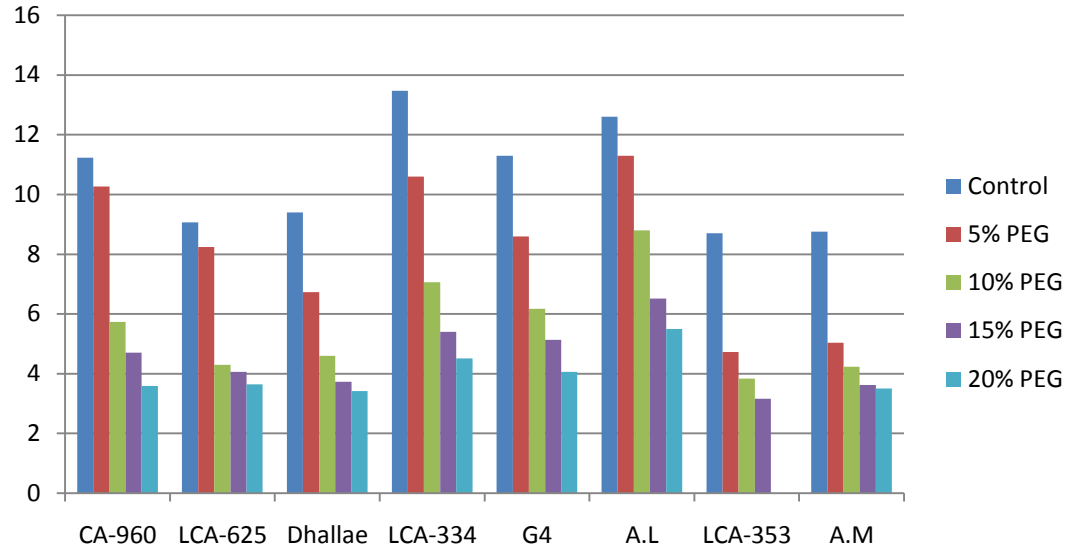

Table 4.2 Shoot Length (cm) of different genotypes at different concentration of PEG

Cultivars	Control (T1)	5% PEG (T2)	10% PEG (T3)	15% PEG (T4)	20% PEG (T5)	Mean
CA-960	11.23	10.27	5.73	4.70	3.59	7.10
LCA-625	9.07	8.24	4.30	4.07	3.65	5.86
Dallae	9.40	6.73	4.60	3.73	3.42	5.58
LCA-334	13.47	10.60	7.07	5.40	4.51	8.21
G4	11.30	8.60	6.17	5.13	4.07	7.05
Arka Lohit	12.60	11.30	8.80	6.52	5.50	8.94
LCA-353	8.70	4.72	3.83	3.17	0.00	4.08
Arka Mohini	8.76	5.03	4.23	3.62	3.50	5.03
Mean	10.57	8.19	5.59	4.54	3.53	

concentration of PEG (20%), cultivars Arka Lohit, LCA-334, G4 recorded 6.5cm, 5.4cm, and 5.1cm of shoot length respectively, which were higher than other cultivars.

4.1.2 Root length

In the present experiment, compared to control, root length was significantly reduced with increased drought stress in all cultivars (Figure 4.2 and Table 4.3). In the control condtion root length was ranging from 18.16 (Arka Mohini) to 23.33cm (LCA-334) with a mean of 20.84 cm where as at 5% PEG (T2) it was ranging from 9.9 (LCA-353) to 18.87cm (Arka Lohit) with a mean of 13.79cm. In 10% PEG (T3) condition root length ranged from 6.3 (LCA-353) to 13.03 cm (Arka Lohit) with a mean of 8.84cm. Root length was further reduced in 15% PEG (T4) and it ranged from 4 (LCA-353) to 10.95cm (Arka Lohit) with mean of 6.13cm. In highest drought stress of 20% PEG (T5) condition among the survived cultivars, it ranged from 3.53 (Arka Mohini) to 5.6cm (Arka Lohit) with mean of 3.72cm.

4.1.3 Number of Leaves

Increase in drought stress reduced the number of leaves (Figure 4.3 and Table 4.4). In the control condtion number of leaves ranged from 8 (Arka Mohini) to 12.33 (LCA-334) with a mean of 9.79 whereas at 5% PEG (T2) it ranged from 4 (Arka Mohini) to 9.67 (Arka Lohit) with a mean of 6.29. In 10% PEG (T3) condition number of leaves were showing more or less similar results as that of 5% PEG condition. Number of leaves got further reduced in 15% PEG (T4) induced drought stress and it ranged from 4 (LCA-353 and Arka Mohini) to 7.7 (Arka Lohit) with a mean of 5.04. In highest drought stress of 20% PEG (T5) condition, among the survived cultivars, it was ranging from 3.3 (Arka Mohini) to 4.33cm (Arka Lohit) with a mean of 3.5. The mean performance of the number of leaves varied from 4.13

Figure 4.2 Root Length (cm) of different genotypes at different concentration of PEG

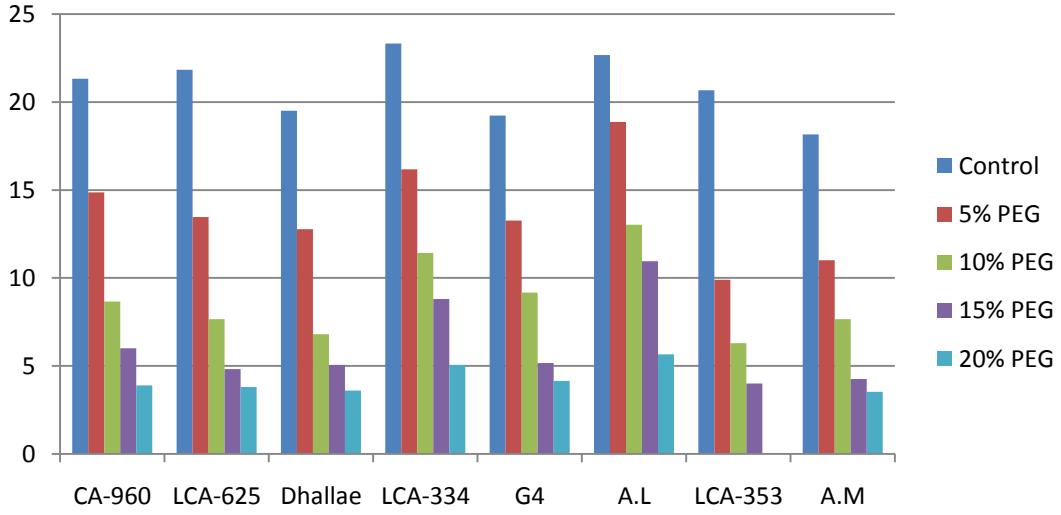

Table 4.3 Root Length (cm) of different genotypes at different concentration of PEG

Cultivars	Control (T1)	5% PEG (T2)	10% PEG (T3)	15% PEG (T4)	20% PEG (T5)	Mean
CA-960	21.3	14.9	8.7	6.0	3.9	11.0
LCA-625	21.8	13.5	7.7	4.8	3.8	10.3
Dallae	19.5	12.8	6.8	5.1	3.6	9.5
LCA-334	23.3	16.2	11.4	8.8	5.1	13.0
G4	19.2	13.3	9.2	5.2	4.2	10.2
Arka Lohit	22.7	18.9	13.0	11.0	5.7	14.2
LCA-353	20.7	9.9	6.3	4.0	0.0	8.2
Arka Mohini	18.2	11.0	7.7	4.3	3.5	8.9
Mean	20.8	13.8	8.8	6.1	3.7	

Figure 4.3 No. of Leaves of different genotypes at different concentration of PEG

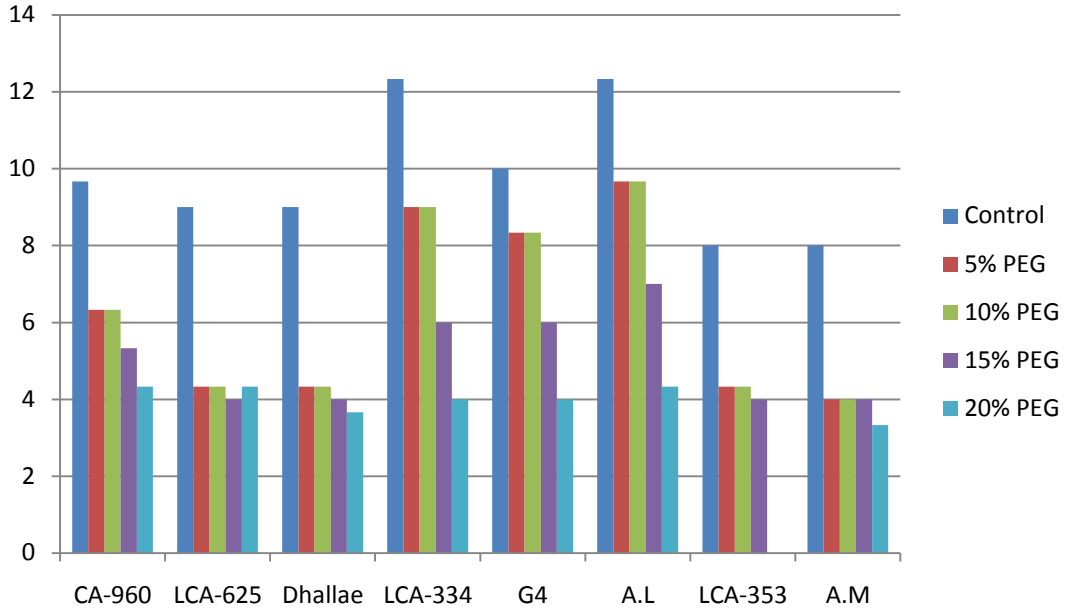

Table 4.4 No. of Leaves of different genotypes at different concentration of PEG

Cultivars	Control (T1)	5% PEG (T2)	10% PEG (T3)	15% PEG (T4)	20% PEG (T5)	Mean
CA-960	9.67	6.33	6.33	5.33	4.33	6.40
LCA-625	9.00	4.33	4.33	4.00	4.33	5.20
Dallae	9.00	4.33	4.33	4.00	3.67	5.07
LCA-334	12.33	9.00	9.00	6.00	4.00	8.07
G4	10.00	8.33	8.33	6.00	4.00	7.33
Arka Lohit	12.33	9.67	9.67	7.00	4.33	8.60
LCA-353	8.00	4.33	4.33	4.00	0.00	4.13
Arka Mohini	8.00	4.00	4.00	4.00	3.33	4.67
Mean	9.79	6.29	6.29	5.04	3.50	

(LCA-353) to 8.60 (Arka Lohit). Arka Lohit (8.60), LCA 334 (8.07), G4 (7.33) were statistically at par with each other.

4.1.4 Number of Internodes

Numbers of internodes were also reduced by increasing drought stress. In the control condition number of internodes were ranging from 4.33 (Arka Mohini) to 6.33 (LCA-334 and Arka Lohit) with a mean of 5.46 whereas at 5% PEG (T2) it was ranging from 3.3 (LCA-353) to 5.67 (Arka Lohit) with mean of 4.75. In 10% PEG (T3) condition number of internodes ranged from 2.33 (LCA-353) to 5.0 (Arka Lohit) with a mean of 3.3. Number of internodes in 15% PEG (T4) ranged from 2 (LCA-353) to 3.67 (Arka Lohit) with a mean of 2.63. In highest drought stress of 20% PEG (T5) condition, the reduction of no. of internodes also was high. Among survive cultivars, it ranged from 2 (Arka Mohini) to 3.0 (Arka Lohit) with a mean of 2.04. Mean performance of no.of internodes had revealed that highest number of internodes were recorded in Arka Lohit (4.67) whereas lowest no. of internodes were observed in LCA-353 (2.53) (Figure 4.4 and Table 4.5).

4.1.5 Leaf area

In the control condition leaf area ranged from 67.83 (Arka Mohini) to 102 cm^2 (Dallae) with a mean of 79.81 cm^2 whereas at 5% PEG (T2) was ranging from 33.33 (LCA-353) to 74.78 cm2 (Dallae) with a mean of 57.75 cm^2. In 10% PEG (T3) condition leaf area was ranging from 12.27 (LCA-353) to 43.96 cm^2 (Arka Lohit) with a mean of 23.04 cm^2. Leaf area in 15% PEG (T4) ranged from 6.07 (LCA-353) to 24.09 cm^2 (Arka Lohit) with a mean of 6.13 cm^2. In 20% PEG (T5) condition among the survived cultivars, it ranged from 5.20 (Arka Mohini) to 12.02 cm^2 (Arka Lohit) with a mean of 7.47 cm^2. From mean analysis, the highest leaf area was observed in Arka Lohit (47.38 cm^2) and lowest was in LCA-353 (24.15 cm^2) (Figure 4.5 and Table 4.6).

Figure 4.4 No. of Internodes of different genotypes at different concentration of PEG

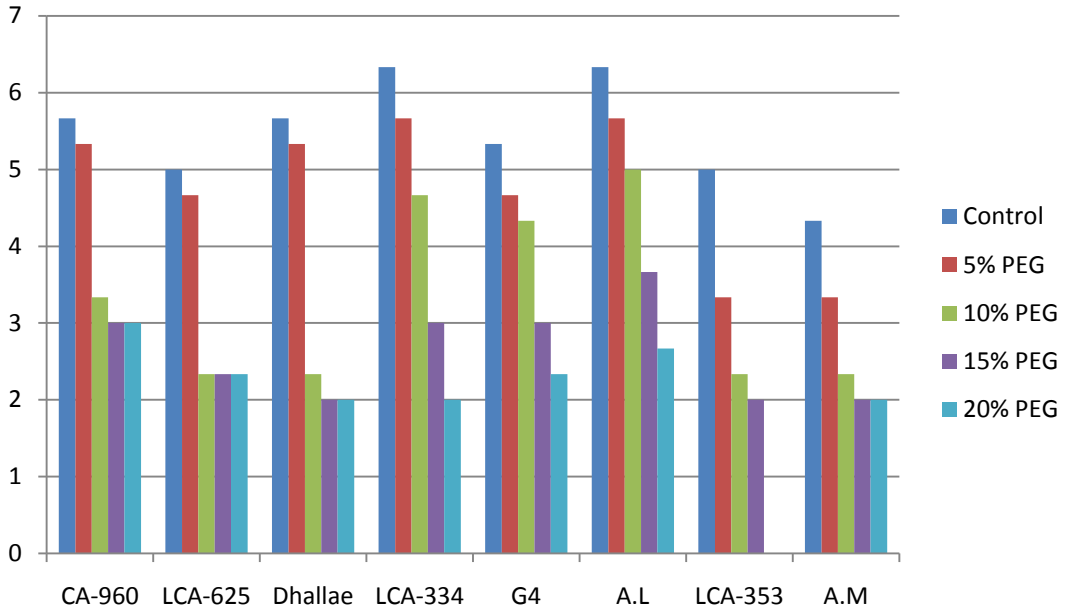

Table 4.5 No. of Internodes of different genotypes at different concentration of PEG

Cultivars	Control (T1)	5% PEG (T2)	10% PEG (T3)	15% PEG (T4)	20% PEG (T5)	Mean
CA-960	5.67	5.33	3.33	3.00	3.00	4.07
LCA-625	5.00	4.67	2.33	2.33	2.33	3.33
Dallae	5.67	5.33	2.33	2.00	2.00	3.47
LCA-334	6.33	5.67	4.67	3.00	2.00	4.33
G4	5.33	4.67	4.33	3.00	2.33	3.93
Arka Lohit	6.33	5.67	5.00	3.67	2.67	4.67
LCA-353	5.00	3.33	2.33	2.00	0.00	2.53
Arka Mohini	4.33	3.33	2.33	2.00	2.00	2.80
Mean	5.46	4.75	3.33	2.63	2.04	

Figure 4.5 Leaf area (cm^2) of different genotypes at different concentration of PEG

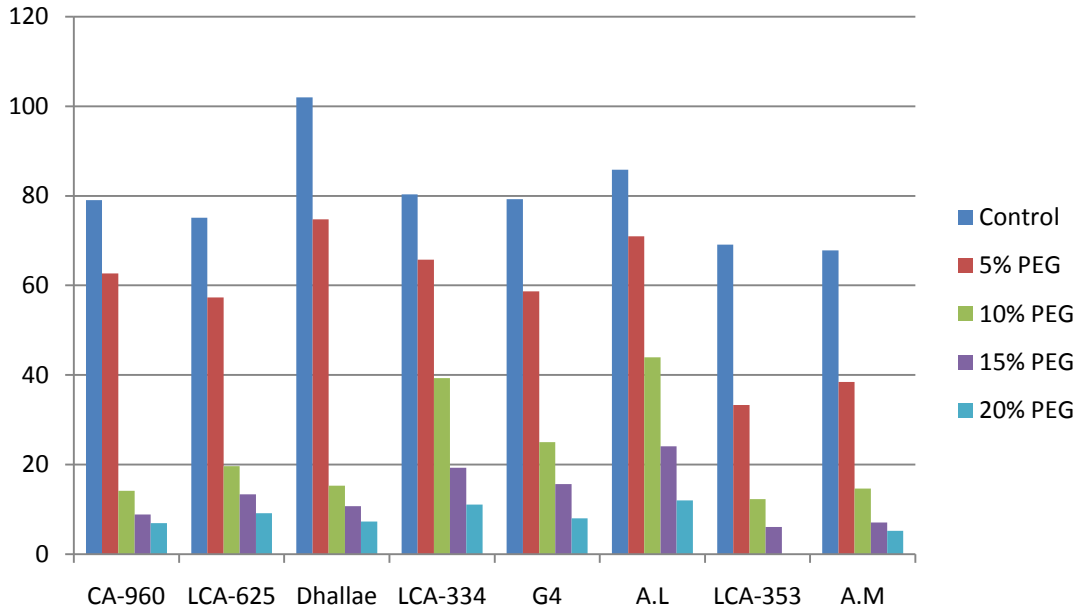

Table 4.6 Leaf area (cm^2) of different genotypes at different concentration of PEG

Cultivars	Control (T1)	5% PEG (T2)	10% PEG (T3)	15% PEG (T4)	20% PEG (T5)	Mean
CA-960	79.06	62.67	14.15	8.83	6.97	34.33
LCA-625	75.08	57.33	19.62	13.40	9.15	34.92
Dallae	102.00	74.78	15.32	10.74	7.30	42.03
LCA-334	80.33	65.75	39.33	19.27	11.09	43.16
G4	79.29	58.67	25.00	15.68	8.00	37.33
Arka Lohit	85.83	71.00	43.96	24.09	12.02	47.38
LCA-353	69.09	33.33	12.27	6.07	0.00	24.15
Arka Mohini	67.83	38.48	14.63	7.11	5.20	26.65
Mean	79.81	57.75	23.04	13.15	7.47	

4.1.6 Shoot dry weight

Shoot dry weight (Figure 4.6 and Table 4.7) in the control condition was ranging from 210 (Arka Mohini) to 385 mg (Arka Lohit) with a mean of 287.1 mg whereas at 5% PEG (T2) it was ranging from 143.6 (LCA-353) to 309.1 mg (Arka Lohit) with mean of 210.09 mg. In 10% PEG (T3) condition shoot dry weight was ranging from 66 (LCA-353) to 145.7 mg (Arka Lohit) with a mean of 103.8 mg. Shoot dry weight was further reduced in 15% PEG (T4) and it ranged from 41.3 (LCA-353) to 91.3 mg (Arka Lohit) with a mean of 64.3 mg. In highest drought stress of 20% PEG (T5) condition, among the survived cultivars, shoot dry weight ranged from 23.7 (Arka Mohini) to 48 mg (Arka Lohit) with a mean of 29.2 mg. Highest mean shoot dry weight was recorded in Arka Lohit (195.83 mg) followed by LCA 334 (174.68 mg) whereas lowest dry weight was recorded in LCA-353 (95.98 mg).

4.1.7 Root dry weight

Root dry weight (Figure 4.7 and Table 4.8) in the control condition ranged from 95.33 (Arka Mohini) to 180.33 mg (Arka Lohit) with a mean of 127.38 mg. At 5% PEG (T2) it ranged from 45 (LCA-353) to 171 mg (Arka Lohit) with a mean of 91.58 mg. In 10% PEG (T3) condition root dry weight was ranging from 20 (LCA-353) to 86.67 mg (Arka Lohit) with a mean of 43.92 mg. In 15% PEG (T4) of drought stress the root dry weight ranged from 8.47 (LCA-353) to 42 mg (Arka Lohit) with mean of 20.72 mg. At the highest drought stress condition of 20% PEG (T5), among the survived cultivars, root dry weight was ranging from 5.93 (Arka Mohini) to 23.33 mg (Arka Lohit) with a mean of 9.79 mg. The mean performance depicted that

Figure 4.6 Shoot dry weight (mg) of different genotypes at different concentration of PEG

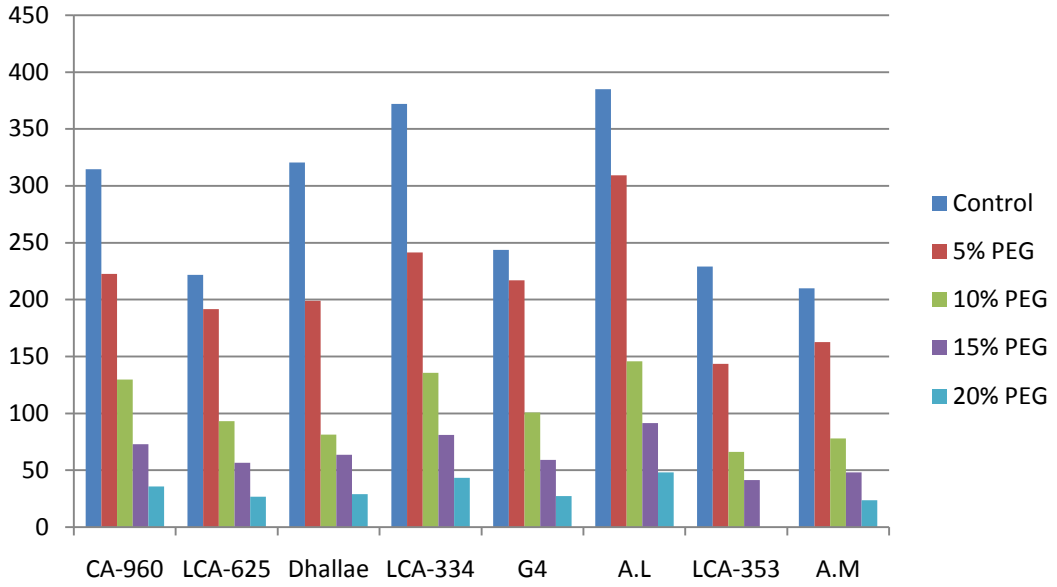

Table 4.7 Shoot dry weight (mg) of different genotypes at different concentration of PEG

Cultivars	Control (T1)	5% PEG (T2)	10% PEG (T3)	15% PEG (T4)	20% PEG (T5)	Mean
CA-960	314.8	222.5	129.7	73.0	35.6	155.1
LCA-625	221.7	191.7	93.1	56.7	26.7	118.0
Dallae	320.7	199.0	81.3	63.7	28.9	138.7
LCA-334	372.0	241.6	135.5	81.0	43.3	174.7
G4	243.7	217.1	100.7	59.0	27.2	129.5
Arka Lohit	385.0	309.1	145.7	91.3	48.0	195.8
LCA-353	229.0	143.6	66.0	41.3	0.0	96.0
Arka Mohini	210.0	162.7	78.0	48.0	23.7	104.5
Mean	287.1	210.9	103.8	64.3	29.2	

Figure 4.7 Root dry weight (mg) of different genotypes at different concentration of PEG

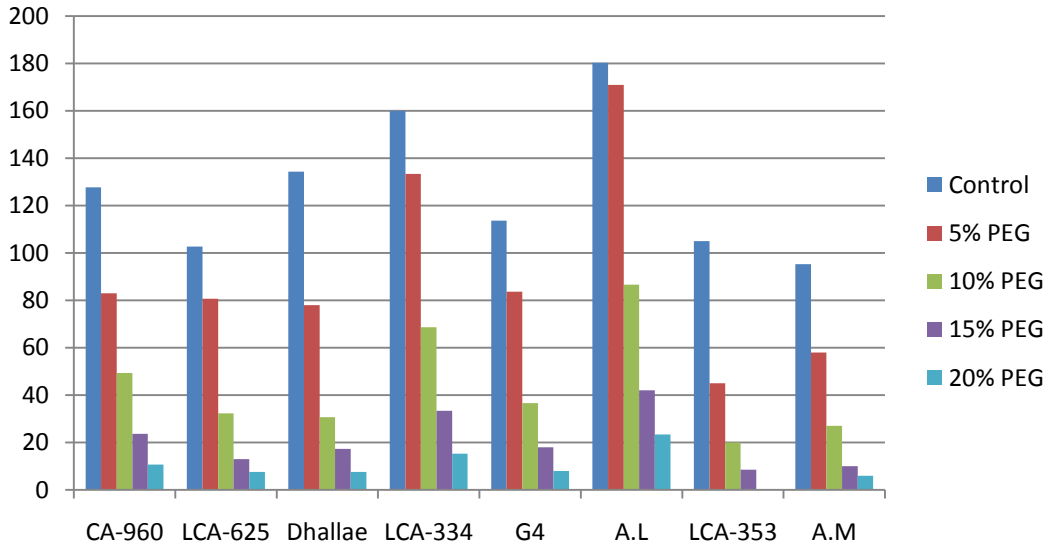

Table 4.8 Root dry weight (mg) different genotypes at different concentration of PEG

Cultivars	Control (T1)	5% PEG (T2)	10% PEG (T3)	15% PEG (T4)	20% PEG (T5)	Mean
CA-960	127.67	83.00	49.33	23.67	10.67	58.87
LCA-625	102.67	80.67	32.33	13.00	7.53	47.24
Dallae	134.33	78.00	30.67	17.33	7.57	53.58
LCA-334	160.00	133.33	68.67	33.33	15.28	82.12
G4	113.67	83.67	36.67	18.00	8.00	52.00
Arka Lohit	180.33	171.00	86.67	42.00	23.33	100.67
LCA-353	105.00	45.00	20.00	8.47	0.00	35.69
Arka Mohini	95.33	58.00	27.00	9.93	5.93	39.24
Mean	127.38	91.58	43.92	20.72	9.79	

highest root dry weight was recorded in Arka Lohit (100.67 mg) and lowest was recorded in LCA-353 (35.69 mg).

4.1.8 Root to shoot dry weight

Root to shoot dry weight estimates the distribution of dry matter between the root and shoot systems and it is a good indicator for effect on roots and shoot dry weight. In the control condition root to shoot dry weight was ranging from 0.41 (CA-960) to 0.49 cm (Arka Lohit) with a mean of 0.45 whereas at 5% PEG (T2) was ranging from 0.31 (LCA-353) to 0.55 (Arka Lohit) with a mean of 0.42. In 10% PEG (T3) condition root to shoot dry weight was ranging from 0.3 (LCA-353) to 0.59 (Arka Lohit) with mean of 0.40. Root to shoot dry weight was further reduced in 15% PEG (T4) and it ranged from 0.29 (LCA-353) to 0.49 (Arka Lohit) with mean of 0.31. At the highest drought stress of 20% PEG (T5) condition, among the survived cultivars, it was ranging from 0.21 (Arka Mohini) to 0.46 (Arka Lohit) with a mean of 0.205. The results (Figure 4.8 and Table 4.9) showed that root to shoot dry weight was decreased in all cultivars except LCA-334 and Arka Lohit. In Arka lohit root to shoot dry weight ratio was measured at 0.49, 0.55, 0.59, 0.49 and 0.46 at 0% PEG, 5% PEG, 10% PEG 15% PEG and 20% PEG drought stress conditions respectively whereas in LCA-334 root to shoot dry weight ratio was measured as 0.43, 0.56, 0.51, 0.41 and 0.35 at 0% PEG, 5% PEG, 10% PEG 15% PEG and 20% PEG drought stress conditions respectively.

4.1.9 Proline

As proline accumulation is a common response of plants to drought it was estimated in the present study. In the control condition proline was ranging from 156 (LCA-353) to 215 μg g^{-1} (LCA-625) with a mean of 191.1 μg g^{-1} whereas at 5% PEG (T2) it was ranging from 201 (LCA-353) to 262 μg g^{-1} (LCA-334) with a mean of

236.2 μg g^{-1}. In 10% PEG (T3) condition proline ranged from 316.7 (LCA-353) to 476.3 μg g^{-1} (Arka Lohit) with a mean of 393.8 μg g^{-1}. In 15% PEG (T4) proline content ranged from 438 (Dallae) to 728 μg g^{-1} (Arka Lohit) with mean of 580.01 μg g^{-1}. At the highest drought stress condition of 20% PEG (T5), among the survived cultivars, it was ranging from 533.7 (Dallae) to 942.0 μg g^{-1} (Arka Lohit) with a mean of 651.6 μg g^{-1}. The present experiment revealed that increased accumulation of proline has been observed in all the cultivars with increased PEG concentration. The mean proline accumulation was varied from 229 μg g^{-1} (LCA-353) to 519 μg g^{-1} (Arka Lohit) (Figure 4.9 and Table 4.10).

Figure 4.8 Root to shoot dry weight of different genotypes at different concentration of PEG

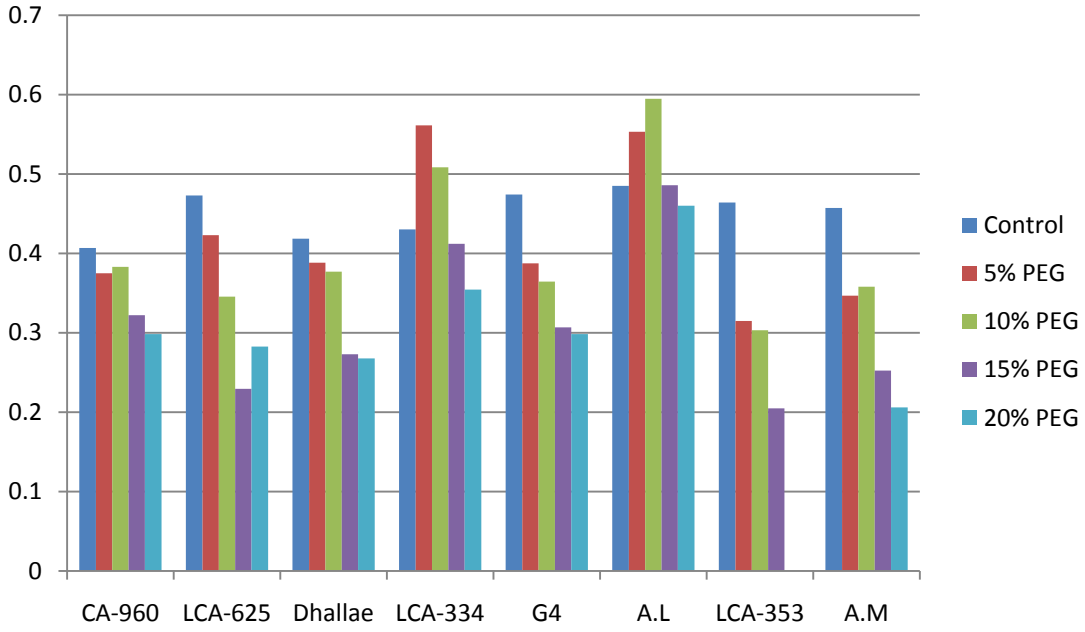

Table 4.9 Root to shoot dry weight of different genotypes at different concentration of PEG

Cultivars	Control (T1)	5% PEG (T2)	10% PEG (T3)	15% PEG (T4)	20% PEG (T5)	Mean
CA-960	0.41	0.38	0.38	0.32	0.30	0.36
LCA-625	0.47	0.42	0.35	0.23	0.28	0.35
Dallae	0.42	0.39	0.38	0.27	0.27	0.35
LCA-334	0.43	0.56	0.51	0.41	0.35	0.45
G4	0.47	0.39	0.36	0.31	0.30	0.37
Arka Lohit	0.49	0.55	0.59	0.49	0.46	0.52
LCA-353	0.46	0.31	0.30	0.21	0.00	0.26
Arka Mohini	0.46	0.35	0.36	0.25	0.21	0.32
Mean	0.45	0.42	0.40	0.31	0.27	

Figure 4.9 Proline (µg g–1FW) of different genotypes at different concentration of PEG

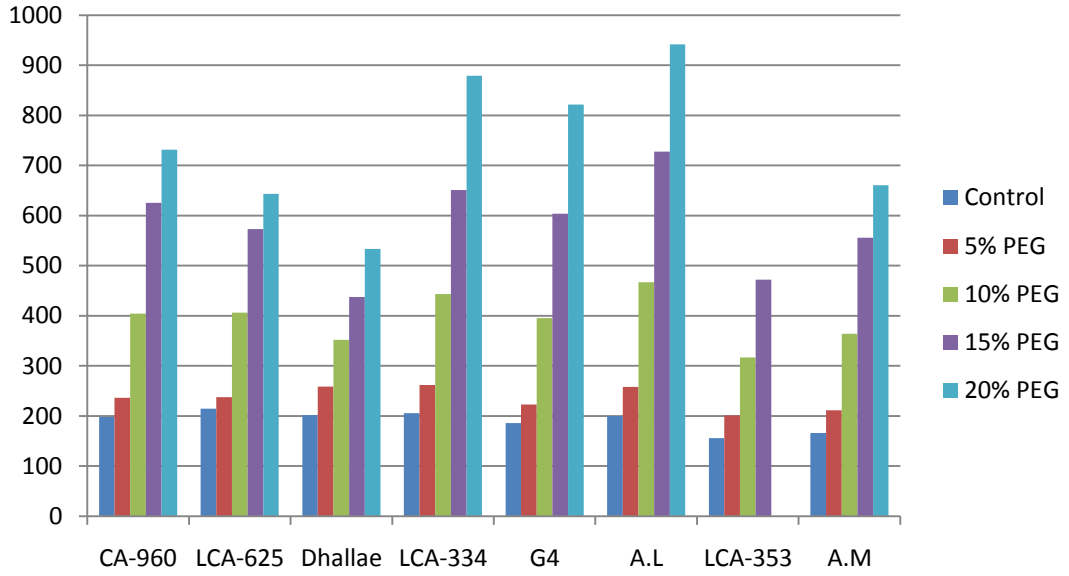

Table 4.10 Proline (µg g–1FW) different genotypes at different concentration of PEG

Cultivars	Control (T1)	5% PEG (T2)	10% PEG (T3)	15% PEG (T4)	20% PEG (T5)	Mean
CA-960	198.0	236.7	404.7	625.3	731.7	439.3
LCA-625	215.0	238.0	406.3	573.0	643.3	415.1
Dallae	202.0	259.0	352.0	438.0	533.7	356.9
LCA-334	206.0	262.0	443.3	651.0	879.3	488.3
G4	186.0	223.0	395.7	604.0	822.0	446.1
Arka Lohit	200.0	258.0	467.3	728.0	942.0	519.1
LCA-353	156.0	201.0	316.7	472.0	0.0	229.1
Arka Mohini	166.0	211.7	364.3	556.0	661.0	391.8
Mean	191.1	236.2	393.8	580.9	651.6	

4.2 Ionomics

In addition to *in vitro* screening of eight chilli cultivars based on morpho-physiological characters, ionome profiling was done to find out responsible elements for drought stress. For ionome profiling, pooled samples were collected from each cultivar of three biological replicates. To better understand how ionome profiling (Table 4.11-4.18) interact with morpho-physiological characters and to group the cultivars based on tolerance to drought, principle component analysis was done.

Principle component analysis

In the present investigation PCA analysis was done after auto-scaling the data of 39 parameters of eight chilli cultivars grown under different induced drought conditions. Those parameters were concentrations of 28 elements, values of 10 morpho-physiological and 1 biochemical characters obtained in response to different drought conditions. Results of PCA are discussed here.

4.2.1 Control (T1)

In the control condition (0% PEG) principal component analysis data (Fig. 4.10 and Table 4.19**)** revealed that first two principal components having eigenvalue more than one accounting for 78.0% of total variation. It was found that first principal component contributed 65.4% whereas second principal component contributed 12.6% of total variation analysed. The physiological traits root dry weight, fresh shoot weight, shoot dry weight, fresh root weigh, no. of internodes, no. of leaves, leaf area, root length, shoot length, proline, root to shoot dry weight and the concentrations of elements Co, Cu, P, Sn, Ni, Zn, Ca, Mo had positive contribution to PC1. Whereas Ga, S, V, Cs, Zr, Ba, B, Mn, Al, Li, K, Ce, Fe, Ti, Na, I, Sr, Cr, Rb, Mg, leaf area, root to shoot dry weight positively contributed to PC2. Mg, Ti, Sr, Rb, Fe, Na, Ce, Al,

Table 4.11 Ionome profiling of LCA-625 in different regimes of drought stress Induced by PEG-6000 (mean± SD)

Ionome	T1 (ppm)	T2 (ppm)	T3 (ppm)	T4 (ppm)	T5 (ppm)
Ca	9.53±0.064	8.97±0.163	7.23±0.005	14.48±0.314	12.52±0.01
S	34.07±0.329	27.49±0.839	24.13±3.532	19.09±0.559	11.26±2.83
K	588.87±1.94	435.61±0.761	310.57±1.23	262.16±8.067	134.52±3.078
Mg	57.44±0.255	59.42±1.406	74.27±0.143	101.95±1.738	116.16±2.645
P	50.9±0.488	90.76±1.383	126.41±2.413	195.05±0.552	150.2±4.646
Cu	3.2±0.045	3.32±0.031	3.9±0.057	3.11±0.158	4.74±0.156
Fe	18.1±0.29	16.16±0.382	13.43±0.374	11.52±0.053	10.16±0.422
Mn	24.77±0.027	26.13±0.009	23.23±0.001	16.23±0.105	10.3±0.417
Mo	0.59±0.032	0.64±0.037	0.54±0.016	0.71±0.018	0.64±0.011
B	7.35±0.072	3.08±0.066	2.44±0.032	0.73±0.026	0.52±0.007
Zn	0.5±0.009	0.58±0.042	0.61±0.004	0.92±0.074	1.26±0.019
Ba	6.1±0.008	2.29±0.069	1.9±0.156	3±0.018	1.98±0.452
Al	22.44±0.612	5.5±0.193	4.5±0.007	5.78±0.17	1.39±0.025
Co	0.17±0.003	0.18±0.01	0.15±0.001	0.18±0.008	0.17±0.004
Cr	0.77±0.027	26.65±0.059	28.3±0.007	35.59±0.25	12.78±0.625
Cs	0.03±0	0.02±0.001	0.02±0.001	0.02±0.001	0.01±0.001
Ga	1.16±0.091	0.53±0.052	0.46±0.004	0.73±0.213	0.4±0.185
Li	0.36±0.009	0.33±0.007	0.24±0.002	0.26±0.008	0.29±0.015
Na	8.36±0.1	7.8±0.039	5.93±0.002	7.07±0.152	3.57±0.045
Ni	5.16±0.057	5.3±0.101	4.65±0.178	5.41±0.186	4.82±0.059
Rb	1.46±0.044	2.47±0.129	2.43±0.004	2.72±0.082	8.56±0.147
Sr	0.67±0.009	0.87±0.024	0.89±0.032	0.91±0.01	6.17±0.19
Ti	23.63±0.392	23.73±0.871	19.95±0.007	25.22±1.302	15.24±0.316
V	118.54±0.576	184.2±9.022	41.28±2.449	169.41±9.434	68.29±7.31
Zr	0.44±0.025	0.13±0.002	0.07±0.003	0.14±0	0.01±0.006
Sn	7.56±0.597	18.6±1.152	10.58±0.353	11.52±0.429	7.16±0.547
I	17.92±0.454	14.48±0.839	15.48±2.555	9.26±2.017	8.94±2.109
Ce	0.2±0.009	0.08±0.003	0.11±0.003	0.12±0.001	0.02±0.003

Table 4.12 Ionome profiling of Dallae Khurasani in different regimes of drought stress Induced by PEG-6000 (mean± SD)

Ionome	T1(ppm)	T2(ppm)	T3(ppm)	T4(ppm)	T5(ppm)
Ca	16.29±0.4	9.01±0.071	8.97±0.466	7.83±0.302	7.69±0.52
S	29.18±0.183	17.83±2.932	17.07±1.363	17.12±0.477	21.68±0.549
K	697.8±13.2	522.75±11.708	510.25±26.209	227.68±6.437	192.6±7.625
Mg	137.21±2.44	85.24±1.588	86.5±0.085	46.76±0.209	44.12±1.948
P	202.05±2.675	154.22±3.597	154.64±1.612	76.58±0.835	80.16±1.127
Cu	12.43±0.206	9.38±0.413	9.33±0.075	4.09±0.122	2.17±0.106
Fe	41.83±1.208	39.09±0.095	38.59±1.165	34.62±0.41	11.54±0.412
Mn	28.15±0.028	28.35±1.344	27.97±0.478	17.21±0.08	6.32±0.122
Mo	2±0.028	1.92±0.132	1.86±0.048	1.53±0.007	2.55±0.043
B	8.63±0.266	0.24±0.068	0.28±0.141	0.54±0.849	0.62±0.022
Zn	1±0.01	0.92±0.019	0.92±0.014	0.36±0.025	1.03±0.013
Ba	3.35±0.05	2.93±0.186	2.88±0.076	2.01±0.07	0.75±0.013
Al	22.93±0.38	6.97±0.326	7.02±0.212	3.9±1.089	1.11±0.028
Co	0.47±0.019	0.65±0.013	0.64±0.011	0.19±0.01	0.14±0.01
Cr	29.5±0.203	19.66±0.497	19.03±0.036	11.01±0.296	12.87±0.692
Cs	0.02±0.003	0.02±0.002	0.02±0.001	0.01±0.003	0±0.01
Ga	0.52±0.34	0.42±0.03	0.39±0.001	0.42±0.155	0.41±0.103
Li	0.85±0.011	0.86±0.026	0.85±0.012	0.24±0.044	0.13±0.003
Na	15.46±0.038	10.42±0.135	9.42±0.237	9.65±0.529	3.05±0.148
Ni	15.68±0.285	20.27±0.238	9.76±0.06	5.41±0.132	4.68±0.361
Rb	2.68±0.082	8.19±0.096	8.17±0.154	2.78±0.043	1.45±0.09
Sr	1.91±0.014	3.75±0.05	3.7±0.082	1.44±0.04	0.25±0.032
Ti	37.05±0.159	28.18±2.224	7.11±0.518	11.99±0.376	16.18±0.594
V	82.68±10.505	23.95±4.075	24.36±0.684	60.21±188.629	120.42±8.364
Zr	0.2±0.003	0.04±0.007	0.04±0.003	0.04±0.002	0.03±0.002
Sn	19.7±0.992	20.85±1.06	11.38±0.631	18.08±0.151	9.2±0.244
I	5.4±0.403	19.92±1.443	18.28±0.676	15.92±1.621	11.43±0.542
Ce	0.32±0.009	0.37±0.001	0.37±0.008	0.12±0.016	0.02±0.001

Table 4.13 Ionome profiling of LCA-334 in different regimes of drought stress Induced by PEG-6000 (mean± SD)

Ionome	T1 (ppm)	T2 (ppm)	T3 (ppm)	T4 (ppm)	T5 (ppm)
Ca	13.61±0.235	16.3±0.003	15.24±0.144	12.69±0.027	5.71±0.025
S	42.37±3.818	40.22±0.075	31.08±3.228	25.22±1.177	14.14±1.037
K	888.29±5.334	748.27±7.74	601.39±12.738	310.56±0.631	178.4±1.584
Mg	131.14±1.44	117.87±1.641	109.05±0.346	106.06±0.715	41.42±0.862
P	234.3±9.342	184.13±0.461	288.55±1.714	248.46±1.626	83.57±0.23
Cu	8.31±0.155	9.26±0.019	9.66±0.067	6.43±0.113	4.29±0.017
Fe	55.57±1.422	41.29±0.62	33.8±0.601	29.36±0.24	14.01±1.357
Mn	35.71±0.352	30.31±0.013	23.57±0.688	13.57±0.078	14.15±0.395
Mo	0.69±0.07	1.96±0.031	2.05±0.055	2.13±0.025	0.71±0.031
B	13.29±0.185	12.04±0.04	3.38±0.022	3.55±0.044	26.24±0.709
Zn	0.4±0.022	0.49±0.002	0.6±0.007	0.82±0.01	0.28±0.012
Ba	4.74±0.089	4.56±0.094	4.26±0.04	5.28±0.666	6.04±0.086
Al	34.76±0.464	10.52±0.012	6.28±0.022	32.82±0.022	4.88±1.883
Co	0.37±0.012	0.6±0.012	0.52±0.018	0.28±0.01	0.26±0.006
Cr	25.32±0.637	29.62±0.113	37.88±0.117	21.09±0.197	12.73±0.06
Cs	0.03±0.001	0.03±0.001	0.03±0.001	0.04±0	0.03±0.002
Ga	1.05±0.035	0.65±0.094	0.49±0.101	0.98±0.071	0.9±0.001
Li	1.84±0.023	1.03±0.015	0.75±0.024	2.2±0.005	1±0.015
Na	16.78±0.084	14.45±0.261	8.76±0.169	9.29±0.03	7.25±0.14
Ni	8.76±0.097	20.21±0.52	5.9±0.304	7.32±0.109	4.96±0.124
Rb	2.7±0.042	7.83±0.288	8.15±0.08	8.15±0.047	1.58±0.067
Sr	2.2±0.008	2.77±0.082	3.2±0.051	4.66±0.006	1.57±0.032
Ti	45.31±0.846	59.05±0.184	11.92±0.126	16.35±0.345	24.57±0.015
V	590.66±3.469	79.35±0.317	16.25±0.337	444.82±2.849	91.71±0.74
Zr	0.42±0.003	0.15±0.002	0.1±0	0.31±0.005	0.3±0.023
Sn	16.29±0.185	20.89±1.438	13.78±1.11	14.06±0.387	8.26±0.336
I	5.8±1.918	12.13±0.403	8.22±0.191	1.81±1.682	18.39±1.088
Ce	0.35±0.002	0.19±0.013	0.31±0.011	0.35±0.008	0.7±0.014

Table 4.14 Ionome profiling of LCA-353 in different regimes of drought stress Induced by PEG-6000 (mean± SD)

Ionome	T1 (ppm)	T2 (ppm)	T3 (ppm)	T4 (ppm)	T5 (ppm)
Ca	4.29±0.088	4.15±0.064	3.59±0.033	4.15±0.043	5.99±0.117
S	30.45±0.357	28.56±1.241	27.11±1.18	25.16±1.455	25.16±4.718
K	244.96±2.795	186.93±6.333	173.81±2.213	173.81±0.537	157.41±35.24
Mg	36.66±0.077	34.57±1.327	32.05±0.24	34.57±0.377	50.75±9.302
P	52.7±0.658	76.44±0.333	79.95±0.472	76.44±1.794	110.29±23.81
Cu	4.83±0.121	3.39±22.837	2.87±0.012	2.67±0.012	2.67±0.014
Fe	21.97±0.312	16.25±0.595	13.42±0.473	10.87±0.478	10.43±0.505
Mn	22.01±0.046	20.62±0.198	19.8±0.256	17.41±0.061	17.41±0.245
Mo	0.57±0.014	0.57±0.032	0.51±0	0.53±0.012	0.53±0.057
B	7.1±0.015	1.21±0.169	1.04±0.031	1.04±0.009	0.59±0.052
Zn	0.92±0.014	0.88±0.015	0.1±0.002	0.1±0.003	0.38±0.005
Ba	1.32±0	4.24±0.272	0.77±0.033	1.02±0.145	1.02±0.944
Al	4.62±0.064	23.29±0.142	1.18±0.002	1.54±0.016	1.54±0.079
Co	0.15±0.007	0.18±0.003	0.13±0.006	0.15±0.004	0.15±0.001
Cr	43.53±0.458	31.83±0.589	11.03±0.236	11.07±0.241	11.07±0.189
Cs	0.02±0.003	0.02±0	0.01±0	0±0	0±0.001
Ga	0.54±0.286	0.77±0.095	0.44±0.044	0.6±0.202	0.6±0.031
Li	0.22±0.008	0.36±0.003	0.15±0.007	0.18±0.003	0.18±0.006
Na	5.64±0.059	8.02±0.109	4.59±0.063	3.03±0.04	3.03±0.666
Ni	5.06±0.267	4.62±0.156	4.09±0.188	4.29±0.167	4.29±0.046
Rb	1.42±0.008	2.72±0.025	2.05±0.012	3.81±0.113	3.81±0.352
Sr	0.39±0	1.57±0.021	0.55±0.004	1.51±0.053	1.51±0.089
Ti	18.39±0.388	20.4±0.654	16.7±0.527	17.53±0.132	17.53±0.617
V	128.78±1.973	115.74±4.111	23.31±0.69	123.87±0.198	123.87±5.076
Zr	0.13±0.003	0.19±0.004	0.11±0.007	0.02±0	0.02±0.01
Sn	7.59±0.118	8.47±0.572	6.39±0.084	9.88±0.092	9.88±0.277
I	12.84±0.856	9.03±1.727	13.9±0.812	12.19±2.402	12.19±2.146
Ce	0.07±0.001	0.38±0.009	0.03±0.005	0.03±0.001	0.03±0.002

Table 4.15 Ionome profiling of Arka Lohit in different regimes of drought stress Induced by PEG-6000 (mean± SD)

Ionomes	T1 (ppm)	T2 (ppm)	T3 (ppm)	T4 (ppm)	T5 (ppm)
Ca	7.24±0.319	15.84±0.468	26.84±0.06	31.61±0.544	34.96±0.088
S	39.5±0.757	25.16±4.268	16.71±2.294	14.74±0.359	13.34±6.211
K	973.4±11.737	769.52±22.827	653.79±0.788	388.66±S	214.25±64.143
Mg	190.26±2.171	224.12±5.819	259.63±0.972	310.52±4.43	373.55±10.062
P	231.37±7.05	245.63±2.506	283.18±1.797	318.97±4.107	315.23±25.15
Cu	10.32±0.206	11.28±0.422	13.16±0.006	14.88±0.072	15.01±0.882
Fe	56.08±2.049	58.55±0.897	65.82±0.304	68.95±1.018	69.8±0.582
Mn	38.77±1.352	37.94±0.06	42.57±0.078	71.35±1.115	85.85±0.144
Mo	1.09±0.051	1.36±0.042	1.75±0.03	2.3±0.053	2.88±0.812
B	11.19±1.122	3.25±0.066	2.18±0.006	1.11±0.028	0.76±0.351
Zn	1.33±0.013	1.33±0.005	0.9±0.003	0.32±0.016	0.17±0.08
Ba	7.19±0.106	3.75±0.161	8.36±0.253	4.16±0.068	15.27±20.587
Al	48.69±2.482	29.15±0.12	4.97±0.058	27.87±0.148	3.1±2.74
Co	0.44±0.013	0.71±0.016	0.19±0.007	0.92±0.018	0.13±0.016
Cr	29.87±0.641	51.81±0.417	12.37±0.317	65.6±0.23	10.39±2.322
Cs	0.04±0	0.02±0.001	0.01±0.001	0.03±0.003	0.02±0.012
Ga	1.52±0.23	0.5±0.034	1.06±0.007	0.69±0.055	1.86±2.155
Li	2.35±0.121	0.71±0.03	0.24±0	0.68±0.012	0.18±0.041
Na	32.37±0.195	12.75±0.336	8.81±0.044	11.36±0.116	4.83±0.301
Ni	10.2±0.015	22.7±0.408	6.16±0.145	27.14±0.654	3.85±0.809
Rb	3.14±0.006	6±0.055	9.79±0.024	8.65±0.118	7.59±0.284
Sr	3.85±0.048	3.17±0.008	0.53±0.001	5.24±0.004	0.49±0.568
Ti	60.09±1.317	36.6±1.092	21.74±0.094	18.93±0.74	13.06±4.785
V	294.73±3.771	48.54±0.711	61.29±3.077	62.1±1.062	74.17±41.715
Zr	0.73±0.025	0.12±0.001	0.04±0.003	0.26±0.033	0.19±0.236
Sn	12.62±0.252	21.28±0.126	12.43±0.807	34.53±0.984	8.01±2.338
I	1.16±0.184	11.82±0.812	8.6±3.446	8.23±0.505	8.7±4.695
Ce	0.76±0.019	0.28±0.013	0.17±0.012	0.3±0	0.07±0.076

Table 4.16 Ionome profiling of G4 in different regimes of drought stress Induced by PEG-6000 (mean± SD)

Ionome	T1 (ppm)	T2 (ppm)	T3 (ppm)	T4 (ppm)	T5 (ppm)
Ca	6.07±0.071	5.18±0.151	5.14±0.149	4.35±0	4.27±0.171
S	25.21±0.072	26.41±1.144	28.37±0.367	28.88±0.06	30.52±1.61
K	175.7±3.629	172.68±5.29	171.29±3.545	185.3±0.002	215.55±6.027
Mg	41.63±0.231	47.28±0.766	46.74±0.85	52.8±0.019	77.11±2.404
P	98.91±0.055	83.28±0.814	81.7±0.937	1.98±0.083	117.28±3.254
Cu	2.44±0.007	2.13±0.009	2.12±0.021	2.22±0	2.3±0.053
Fe	9.65±0.131	10.61±0.037	10.9±0.155	11.2±0.01	11.73±0.312
Mn	6.4±0.184	11.85±0.14	12.11±0.147	11.28±0.005	11.75±0.154
Mo	0.5±0.002	0.55±0.006	0.53±0.03	0.42±0	0.46±0.004
B	0.45±0.017	0.47±0.006	0.48±0.032	0.61±0.007	1.01±0.003
Zn	0.1±0.008	0.08±0.002	0.09±0.011	0.01±0	0.16±0.009
Ba	0.61±0.049	0.67±0.12	0.63±0.007	0.04±0.013	1.87±0.144
Al	1.34±0.006	1.05±0.003	1.03±0.029	0.16±0.003	1.42±0.015
Co	0.15±0.002	0.12±0	0.13±0.006	0±0	0.15±0.004
Cr	11.76±0.227	11.29±0.033	11.71±0.419	0.15±0.003	10.72±0.027
Cs	0±0	0±0.001	0±0	0±0	0.01±0.002
Ga	0.35±0.006	0.42±0.044	0.49±0.254	0.01±0.003	0.43±0.032
Li	0.23±0.002	0.16±0.003	0.16±0.009	0.01±0	0.19±0.011
Na	5.16±0.059	4.79±0.102	4.81±0.044	0.13±0.001	5.68±0.143
Ni	4.74±0.015	4.22±0.176	4.2±0.027	0.07±0.001	3.85±0.013
Rb	0.78±0.032	1.01±0.005	0.96±0.059	0.03±0	7.49±0.282
Sr	0.17±0.003	0.13±0.009	0.14±0.004	0.01±0.001	5.17±0.126
Ti	17.15±0.112	16.26±0.528	15.96±0.556	0.33±0.01	18.36±0.206
V	146.83±3.45	123.31±2.353	135±3.942	54.25±0.081	105.32±5.2
Zr	0.03±0.001	0.03±0.005	0.02±0.001	0.01±0	0.11±0.008
Sn	7.34±0.076	6.27±0.227	6.68±0.479	0.15±0.017	4.79±0.092
I	10.23±2.45	9.71±1.747	12.84±1.191	0.2±0.038	10.89±1.6
Ce	0.02±0	0.01±0.002	0.01±0	0±0	0.15±0

Table 4.17 Ionome profiling of Arka Mohini in different regimes of drought stress Induced by PEG-6000 (mean± SD)

Ionome	T1 (ppm)	T2 (ppm)	T3 (ppm)	T4 (ppm)	T5 (ppm)
Ca	2.75±0.124	6.18±0.074	4.43±0.058	19.23±0.487	11.45±0.089
S	20.08±0.009	20.09±0.568	23.14±1.511	21.04±3.49	24.33±2.388
K	749.07±0.691	625.11±1.016	221.69±2.556	203.45±17.853	200.13±1.951
Mg	27.21±0.551	38.51±0.282	44.91±0.342	83.9±3.662	160.23±0.589
P	97.46±0.436	98.48±0.161	97.29±2.242	347.53±4	227.35±0.003
Cu	2.85±0.073	2.95±0.108	2.93±0.067	4.49±0.029	4.02±0.121
Fe	11.2±0.201	12.95±0.084	15.54±0.17	17.92±0.299	18.64±0.216
Mn	9.29±0.24	12.08±0.079	16.18±0.047	25.92±0.242	32.87±0.002
Mo	0.64±0.023	0.68±0.058	0.67±0.04	0.69±0.006	0.78±0.027
B	0.41±0.055	0.52±0.034	0.72±0.003	1.61±0.037	0.89±0.001
Zn	0.24±0.01	0.25±0.014	0.44±0.001	0.59±0.001	0.65±0.003
Ba	0.72±0.023	1.58±0.04	0.92±0.029	2.21±0.028	1.28±0.043
Al	1.63±0.005	5.31±0.022	1.97±0	1.99±0.042	2.3±0.046
Co	0.17±0.003	0.21±0.003	0.17±0.006	0.23±0.003	0.17±0.002
Cr	13.71±0.149	13.77±0.148	14.3±0.092	13.6±0.158	14.1±0.248
Cs	0±0.001	0.01±0	0.01±0.001	0.02±0	0.01±0.002
Ga	0.51±0.1	0.56±0.068	0.49±0.114	0.42±0.066	0.46±0.128
Li	0.53±0.021	0.44±0.007	0.41±0.015	0.51±0.004	0.34±0.016
Na	4.08±0.156	5.95±0.063	3.46±0.088	5.46±0.096	4.51±0.048
Ni	5.11±0.217	5.27±0.081	5.33±0.146	5.39±0.024	5.26±0.096
Rb	1.14±0.064	3.76±0.007	3.68±0.018	6.32±0.174	6.34±0.092
Sr	0.11±0.006	1.07±0.048	0.55±0.012	4.31±0.083	2.67±0.037
Ti	15.67±0.005	21.39±0.523	17.17±0.135	23.15±0.28	21.53±0.132
V	104.67±0.828	109.91±6.166	21.02±1.316	98.83±3.258	110.78±0.311
Zr	0.02±0	0.25±0.003	0.04±0.001	0.04±0.009	0.03±0.001
Sn	4.94±0.109	4.39±0.05	4.52±0.32	8.7±0.353	8.83±0.076
I	16.52±0.989	13.9±2.265	18.36±0.761	15.76±1.641	14.72±1.406
Ce	0.02±0.002	0.15±0.01	0.05±0.004	0.03±0.002	0.08±0.002

Table 4.18 Ionome profiling of CA-960 in different regimes of drought stress Induced by PEG-6000 (mean± SD)

Ionome	T1 (ppm)	T2 (ppm)	T3 (ppm)	T4 (ppm)	T5 (ppm)
Ca	3.38±0.047	3.04±0.148	4.18±0.001	8.41±0.369	14.92±0.084
S	15.62±0.353	20.05±0.06	28.67±0.645	26.6±0.211	21.41±2.182
K	488±0.733	244.48±0.286	182.86±2.078	155.35±3.964	159.41±0.289
Mg	28.3±0.353	31.3±0.462	33.62±0.74	53.17±2.314	102.71±2.126
P	98.63±2.265	96.66±0.692	93.01±0.973	107.74±4.057	148.76±0.247
Cu	2.95±0.037	3.12±0.036	3.15±0.087	3.31±0.186	3.66±0.164
Fe	11.45±0.603	12.71±0.024	14.18±0.501	15.72±0.105	16.38±0.183
Mn	11.4±0.436	12.84±0.275	17.4±0.549	18.19±0.771	23.97±0.196
Mo	0.55±0.029	0.61±0.002	0.65±0.031	0.73±0.001	0.75±0.07
B	0.08±0.035	1.01±0.039	2.16±0.034	2.52±0.038	1.89±0.049
Zn	0.57±0.018	0.56±0.041	0.32±0.037	0.24±0.002	0.21±0.001
Ba	1.19±0.06	1.5±0.08	1.49±0.016	3.54±0.666	2.01±0.041
Al	1.23±0.026	3.6±0.124	4.2±0.145	2.87±0.02	4.58±0.067
Co	0.15±0.001	0.15±0.002	0.17±0.005	0.19±0.005	0.19±0.007
Cr	10.02±0.6	16.42±0.363	17.48±0.296	11.46±0.317	14.09±0.086
Cs	0±0.001	0.02±0.002	0.02±0.001	0.01±0.001	0.02±0
Ga	0.22±0.009	0.35±0.047	0.56±0.108	0.67±0.054	0.43±0.016
Li	0.14±0.014	0.18±0.001	0.27±0.017	0.41±0.016	0.37±0.015
Na	3.44±0.065	5.75±0.002	6.07±0.009	5.07±0.095	6.13±0.01
Ni	4.82±0.101	4.5±0.132	5.23±0.171	5.01±0.147	5.35±0.241
Rb	0.84±0.027	2.11±0.008	1.4±0.007	3.25±0.007	7.13±0.083
Sr	0.12±0.009	0.49±0.021	0.28±0.009	1.51±0.052	4.02±0.011
Ti	17.29±0.001	19.79±0.864	22.03±0.84	23.45±1.578	19.54±0.466
V	65.8±1.316	86.22±4.691	181.1±9.392	113.04±7.698	115.46±0.477
Zr	0.02±0.001	0.11±0.002	0.11±0.004	0.35±0.009	0.04±0.002
Sn	5.96±0.017	10.24±0.496	7.06±0.118	10.72±0.597	17.93±0.059
I	15.86±0.795	18.01±1.023	1.18±1.747	5.28±2.15	10.55±1.402
Ce	0.02±0.002	0.52±0.454	0.1±0.001	0.06±0.007	0.04±0.001

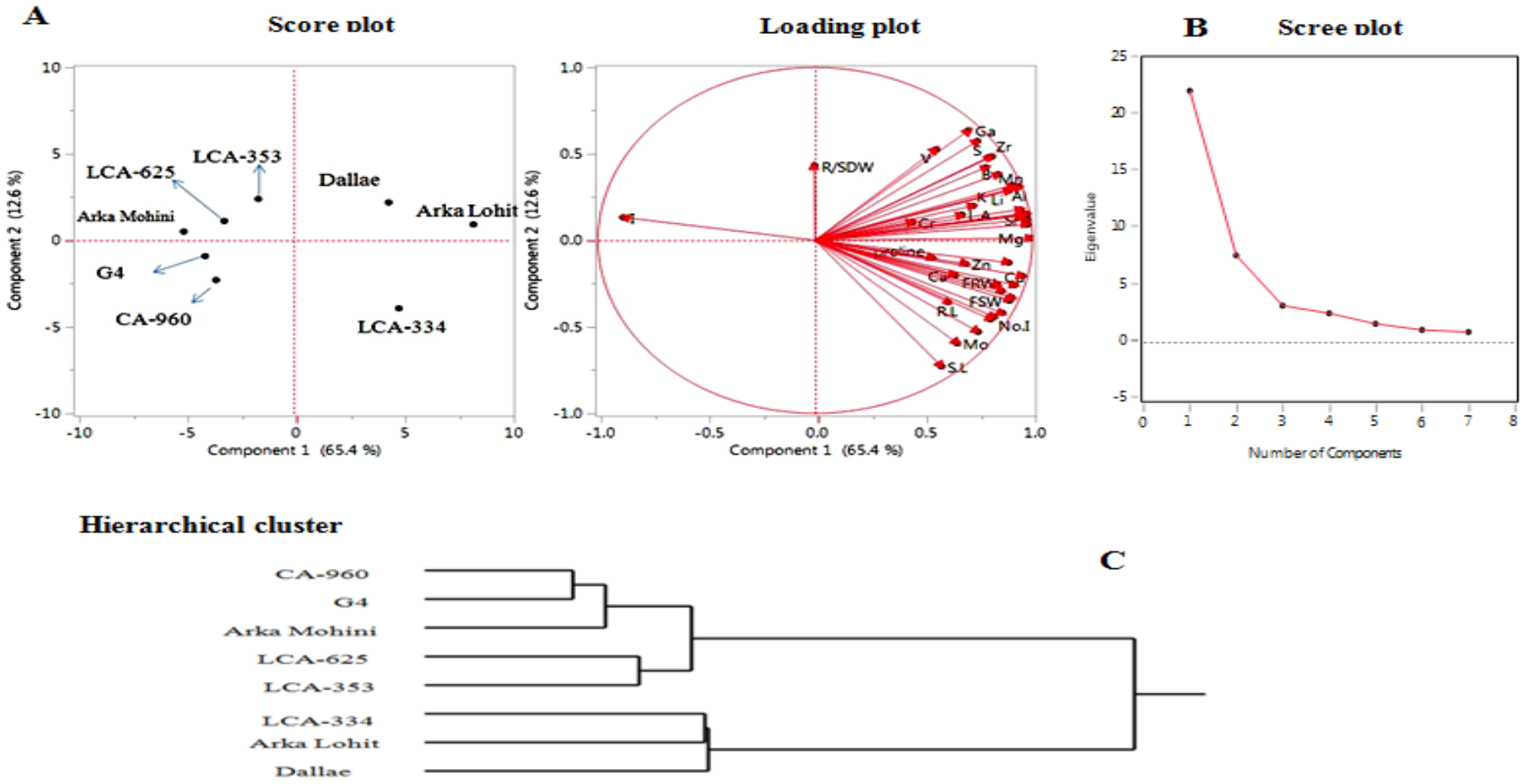

Figure 4.10 A. PCA score and loading plots B. Scree plot C. Hierarchical cluster in control condition

Table 4.19 Eigen values, proportion of the total variance, cumulative per cent variance and component loading of different variables of chilli cultivars under controlled condition.

	PC1	PC2
Eigen values (variance)	**25.5756**	**4.9075**
Proportion of variance (%)	**65.579**	**12.583**
Cumulative variance (%)	**65.579**	**78.162**
Ca	0.13498	-0.06567
S	0.14773	0.25966
K	0.14331	0.09116
Mg	0.19638	0.00499
P	0.17541	-0.05520
Cu	0.18133	-0.11560
Fe	0.18926	0.07125
Mn	0.17942	0.14360
Mo	0.12917	-0.26839
B	0.16639	0.17400
Zn	0.13690	-0.06635
Ba	0.15570	0.18713
Al	0.18564	0.13829
Co	0.18808	-0.09134
Cr	0.08815	0.04808
Cs	0.15837	0.21510
Ga	0.13961	0.28299
Li	0.17591	0.12948
Na	0.18726	0.06065
Ni	0.15946	-0.20479
Rb	0.19242	0.04128
Sr	0.19267	0.05232
Ti	0.19289	0.06769
V	0.10958	0.24316
Zr	0.16125	0.21509
Sn	0.16545	-0.11227
I	-0.17436	0.05968
Ce	0.18680	0.07734
Shoot Length	0.11494	-0.33182
Root Length	0.12120	-0.16637
No. of Leaves	0.14849	-0.24274
No. of Internodes	0.16274	-0.20090
Leaf Area	0.13298	0.07223
Fresh shoot weight	0.17649	-0.15669
Shoot dry weight	0.16995	-0.18972
Fresh root weight	0.16929	-0.13452
Root dry weight	0.17866	-0.15120
Root to shoot dry weight	0.00011	0.18711
Proline	0.10710	-0.04712

Mn, Li, B, Zr, Cs, Ba,S, K, Ga, V, Cr, leaf area and root shoot to dry weight contributed to both PC1 and PC2. Score plot and hierarchical cluster analysis revealed that eight capsicum cultivars separated into three groups. Arka Lohit, LCA-334 and Dalle khurasani cultivars were grouped together as a one group. Second group of cultivars comprised of LCA-353 and LCA-625 and third group had CA-960, G4 and Arka Mohini.

4.2.2 Treatment 2 (5% PEG)

At 5% PEG induced drought stress condition principal component analysis data (Fig. 4.11 and Table 4.20**)** revealed that first two principal components having eigenvalue more than one accounting for 76.3% of total variation. It was found that PC1 contributed 56.7% whereas PC2 contributed 19.6% of total variation. Cu, Ni, Fe, Co, fresh root weight, P, proline, Mn, Na, Mg, Sn, Sr, Mo, Ca, Rb, root dry weight, Li, root to shoot dry weight, fresh shoot weight, no. of internodes, leaf area, root length, shoot dry weight, K, Ti, Zn, Cr, shoot length, Ba, Cs, no. of leaves, Al, B, I, Ce, S contributed positively in descending order to PC1 and V, Zr, Ga elements contributed negatively to PC1. On the other hand, Ga, S, Ba, Zr, B, Ti, Cs, Na, Al, Li, Ca, Cr, K, Mn, Rb, V, Sr, Mo, Fe, Cu, Co, Ni, Sn, P, Mg, Zn positively contributed to PC2 and all physiological characters and I and Ce were negatively contributed to PC2. Score plot and hierarchical cluster analysis revealed that eight capsicum cultivars could be separated into three groups. Dalle khurasani, Arka Lohit and LCA-334 grouped together and fell under the positive direction of PC1. Second group cultivars consisted of LCA- 625, Arka Mohini and G4 fell on the positive side of PC2. Cultivars CA-960 and LCA-353 formed third group in the quadrant where PC1 and PC2 were negative.

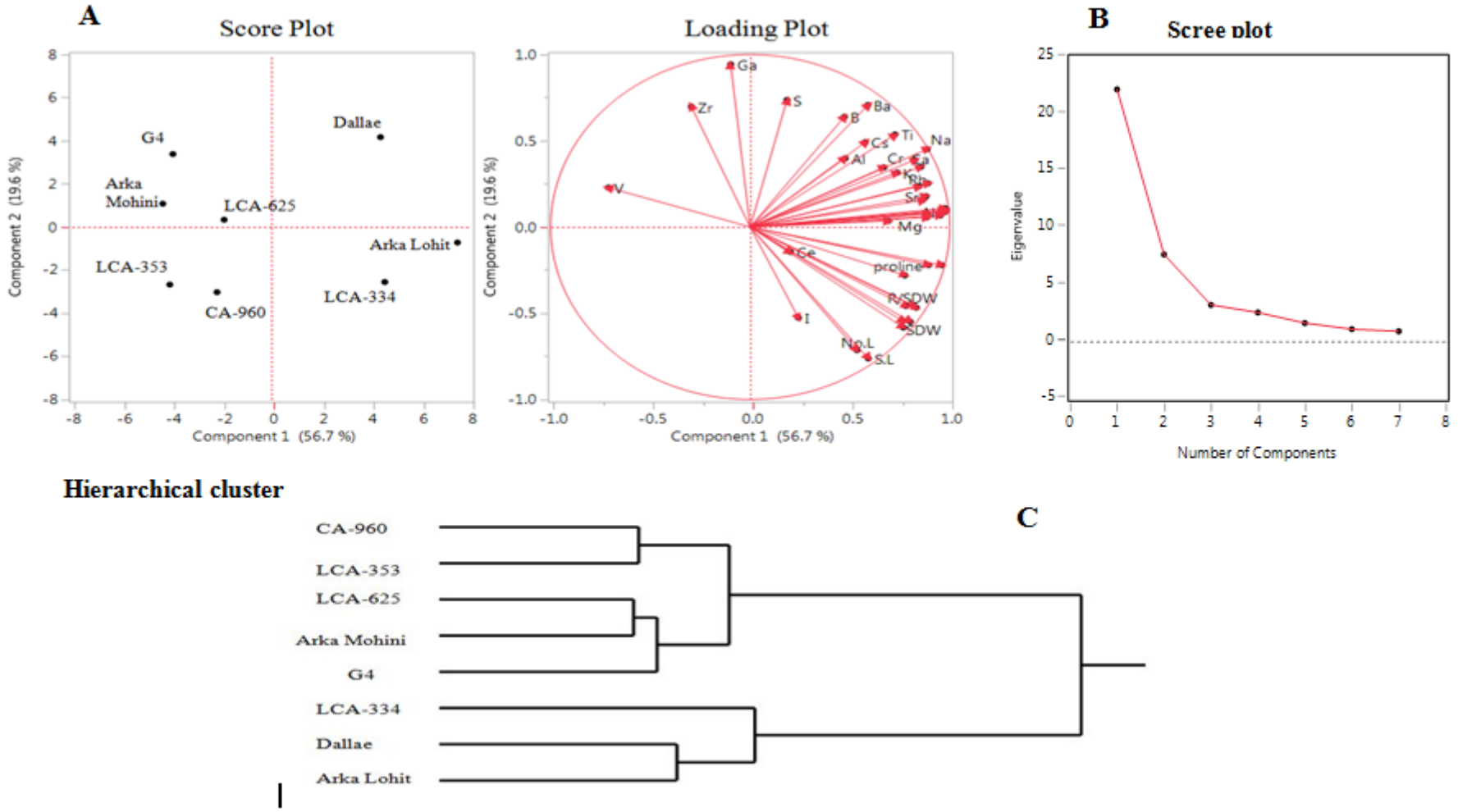

Figure 4.11 A. PCA score and loading plots B. Scree plot C. Hierarchical cluster in 5% PEG 6000 Drought stress condition

Table 4.20 Eigen values, proportion of the total variance, cumulative per cent variance and component loading of different variables of chilli cultivars under 5% PEG condition.

	PC1	PC2
Eigen values (variance)	**22.1276**	**7.6251**
Proportion of variance (%)	**56.738**	**19.551**
Cumulative variance (%)	**56.738**	**76.289**
Ca	0.18191	0.12629
S	0.03923	0.26653
K	0.15659	0.11422
Mg	0.18802	0.01998
P	0.20245	0.02402
Cu	0.20946	0.03641
Fe	0.20742	0.03951
Mn	0.18951	0.09167
Mo	0.18357	0.05631
B	0.10032	0.23117
Zn	0.14723	0.01273
Ba	0.12550	0.25616
Al	0.10126	0.14432
Co	0.20614	0.03331
Cr	0.14229	0.12507
Cs	0.12273	0.17807
Ga	-0.02073	0.34128
Li	0.17541	0.14085
Na	0.18851	0.16339
Ni	0.20770	0.03176
Rb	0.17967	0.08519
Sr	0.18730	0.06467
Ti	0.15423	0.19413
V	-0.15097	0.08256
Zr	-0.06267	0.25277
Sn	0.18786	0.02706
I	0.05138	-0.19057
Ce	0.04297	-0.05237
Shoot Length	0.12589	-0.27592
Root Length	0.16325	-0.21107
No. of Leaves	0.11378	-0.25847
No. of Internodes	0.16587	-0.16594
Leaf Area	0.16477	-0.10147
Fresh shoot weight	0.17062	-0.19903
Shoot dry weight	0.16261	-0.19749
Fresh root weight	0.20405	-0.07999
Root dry weight	0.17693	-0.16929
Root to shoot dry weight	0.17389	-0.16435
Proline	0.19001	-0.07984

4.2.3 Treatment 3 (10 % PEG)

At 10% PEG induced drought stress condition principal component analysis data (Fig. 4.12 and Table 4.21**)** revealed that first two principal components having eigenvalue more than one accounting for 75.2% of total variation. It was found that PC1 contributed 53.7% whereas PC2 contributed 21.5 % of total variation. Root to shoot dry weight, Cu, Fe, K, Rb, root dry weight, Na, Zn, Ba, Mn, leaf area, Mg, Ca, Mo, root length, shoot length, fresh root weight, P, Sn, proline, fresh shoot weight, Al, shoot dry weight, Ce, Ni, no. of leaves, no. of internodes, Ga, Co, Sr, Li, Cs, B, Cr positively contributed in descending order to PC1 and S, Zr, Ti, V, I negatively contributed to PC1. Whereas PC2 was positively contributed in the descending order by Cr, Cs, Sr, Li, Co, Ce, Al, Zr, Mo, Sn, B, Na, P, K, S, Rb, Ni, Zn, Cu, I and Ti, V, no. of internodes, no. of leaves, Ga, root length, shoot length, fresh root weight, proline, shoot dry weight, fresh shoot weight, root dry weight, leaf area, root to shoot dry weight, Mg, Mn, Ca, Ba, Fe, I negatively contributed to PC2. Score plot and hierarchical cluster analysis revealed that eight capsicum cultivars separated in to three groups. Dallae, LCA-334 and Arka Lohit grouped together and fell under positive direction of PC1, second group cultivars LCA- 625, Arka Mohini and G4 fell near to origin. Cultivars CA-960 and LCA-353 formed third group in the quadrant where PC1 and PC2 were at negative side.

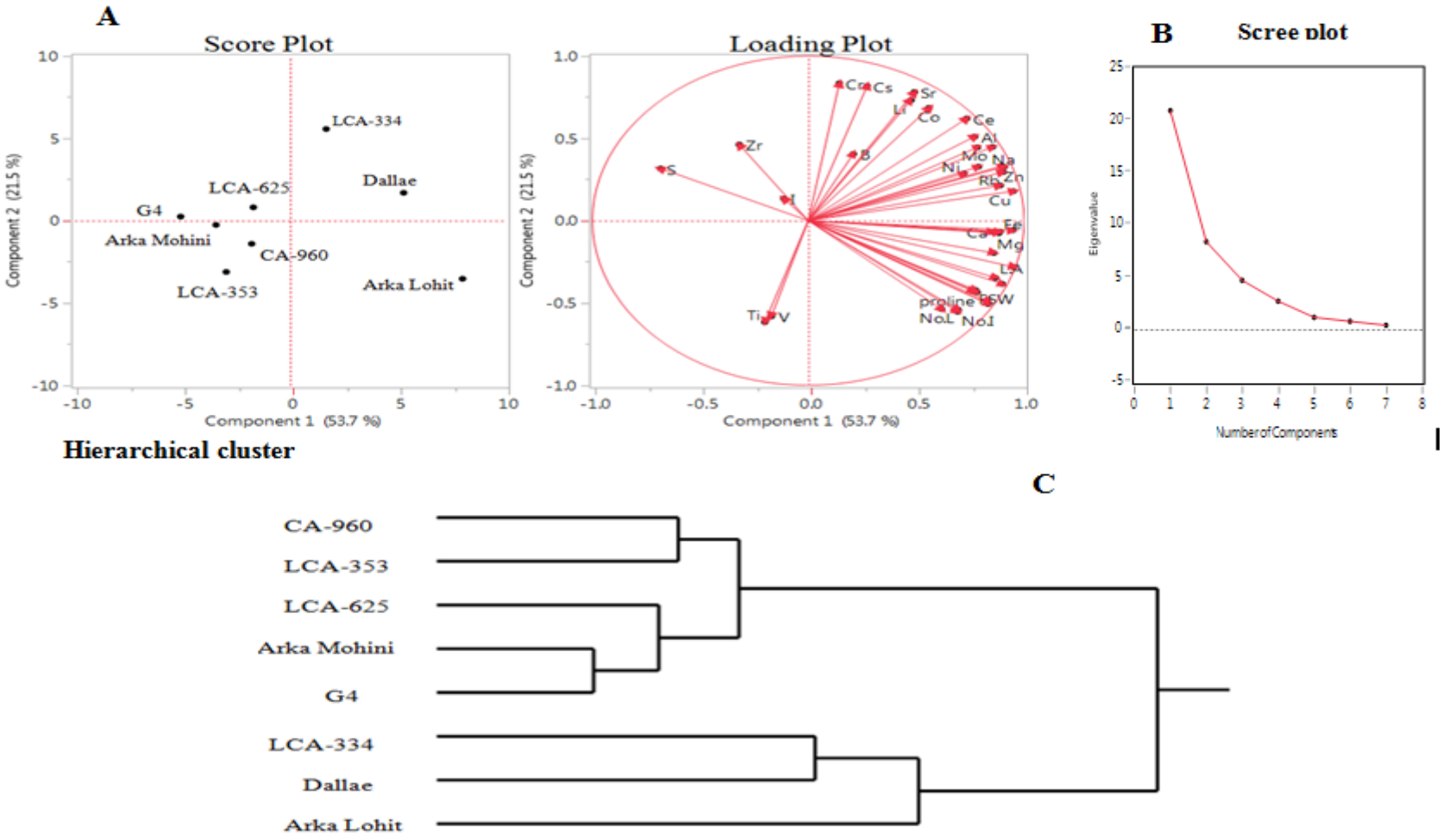

Figure 4.12 A. PCA score and loading plots B. Scree plot C. Hierarchical cluster in 10% PEG 6000 Drought stress condition

Table 4.21 Eigen values, proportion of the total variance, cumulative per cent variance and component loading of different variables of chilli cultivars under 10%PEG condition.

	PC1	PC2
Eigen values (variance)	**20.9340**	**8.3919**
Proportion of variance (%)	**53.677**	**21.518**
Cumulative variance (%)	**53.677**	**75.195**
Ca	0.18694	-0.02357
S	-0.14937	0.10919
K	0.19835	0.11271
Mg	0.18774	-0.06777
P	0.17232	0.11318
Cu	0.20777	0.06169
Fe	0.20690	-0.02006
Mn	0.19286	-0.02638
Mo	0.18655	0.15446
B	0.04562	0.13965
Zn	0.19436	0.07375
Ba	0.19409	-0.02280
Al	0.16853	0.17617
Co	0.12217	0.23629
Cr	0.03152	0.28780
Cs	0.05964	0.28204
Ga	0.13500	-0.18532
Li	0.10404	0.25266
Na	0.19602	0.11454
Ni	0.15746	0.09897
Rb	0.19729	0.10177
Sr	0.10753	0.26961
Ti	-0.04390	-0.21258
V	-0.03717	-0.20044
Zr	-0.06977	0.15940
Sn	0.17122	0.15368
I	-0.02459	0.04603
Ce	0.16047	0.21422
Shoot Length	0.18191	-0.17423
Root Length	0.18193	-0.17435
No. of Leaves	0.15178	-0.18677
No. of Internodes	0.15126	-0.19086
Leaf Area	0.18953	-0.11992
Fresh shoot weight	0.16918	-0.14525
Shoot dry weight	0.16682	-0.14769
Fresh root weight	0.18182	-0.16904
Root dry weight	0.19625	-0.13241
Root to shoot dry weight	0.20784	-0.09714
Proline	0.17097	-0.15004

4.2.4 T4 Treatment (15% PEG)

At 15% PEG induced drought stress condition, principle component analysis data (Fig. 4.13 and Table 4.22**)** revealed that first two principal components having eigen value more than one accounting for 78.3% of total variation. It was found that PC1 contributed 53.1% whereas PC2 contributed 25.2 % of total variation. Fe, Cu, Sn, Co, Ni, fresh root weight, Mg, K, Mo, Cr, Mn, root length, Na, Ca, root dry weight, shoot dry weight, root to shoot dry weight, fresh shoot weight, Ce, Al, leaf area, Rb, Sr, shoot length, Cs, Ba, no. of internodes, no. of leaves, proline, P, Zr, Ga, Li, Ti, B, Zn, V, I contributed positively in descending order to PC1. V, Zn, Li, Ga, B, Cs, Ba, Rb, P, Ti, Sr, Al, Ce, Zr, Na, Mo, K, Ca, S, Cr, Cu, Co, Mg positively contributed to PC2 in descending order and no. of leaves, proline, no. of internodes, shoot length, leaf area, root to shoot dry weight, fresh shoot weight, root dry weight, root length, fresh root weight, shoot dry weight, Mn, Fe, I, Ni, Sn contributed negatively to PC2. Score and Hierarchical cluster analysis revealed that eight capsicum cultivars could be separated in to three groups. Arka Lohit and LCA-334 cultivars grouped together and fell under the positive direction of PC1. LCA- 625, G4 and Arka Mohini fell near to origin formed second group cultivars. Cultivars CA-960, LCA-353 and Dallae formed third group and got placed where below average values of PC1 and PC2 were plotted.

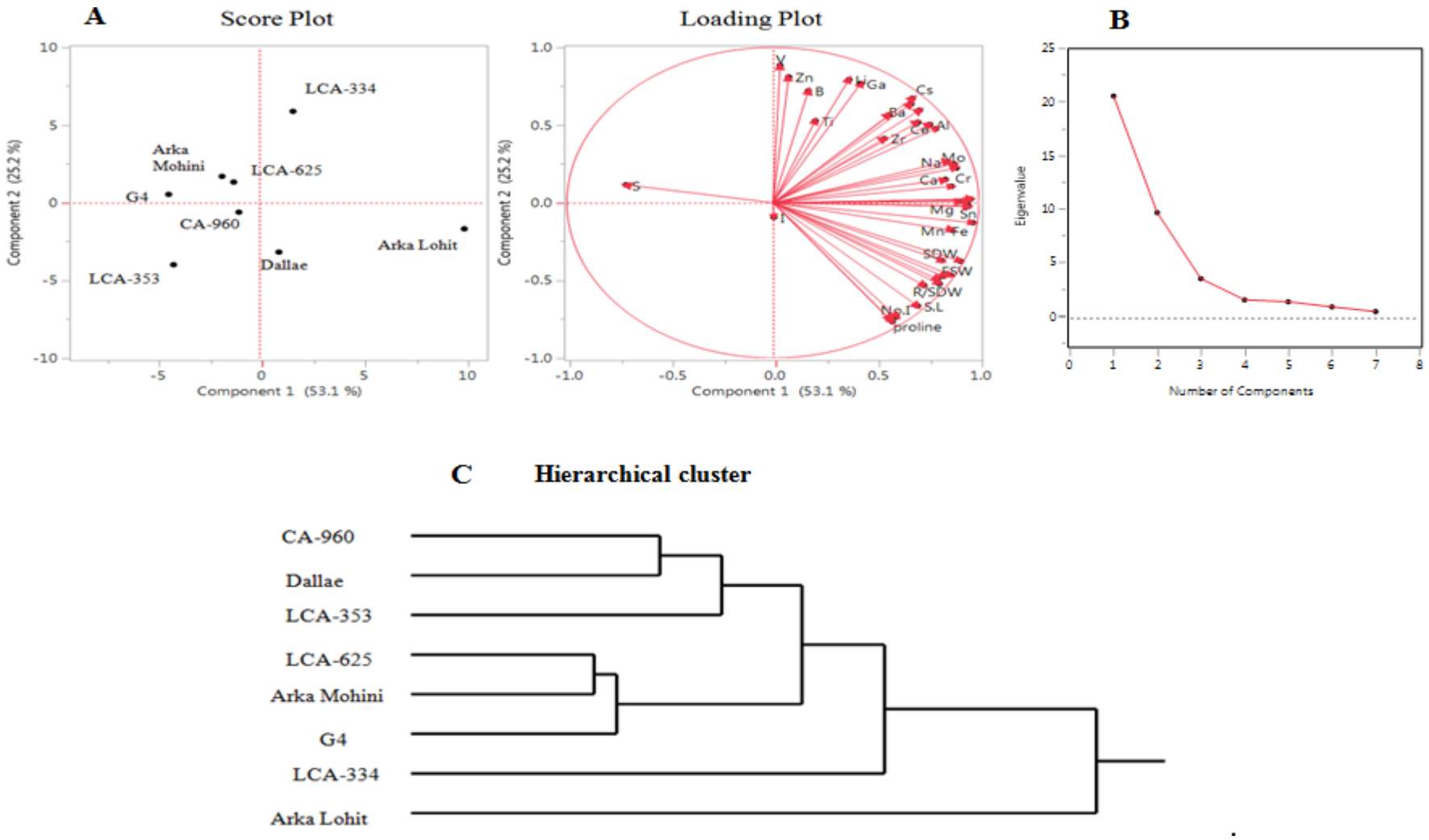

Figure 4.13 A. PCA score and loading plots B. Scree plot C. Hierarchical cluster in 15% PEG 6000 Drought stress condition

Table 4.22 Eigen values, proportion of the total variance, cumulative per cent variance and component loading of different variables of chilli cultivars under 15% PEG condition.

	PC1	PC2
Eigen values (variance)	**20.7174**	**9.8311**
Proportion of variance (%)	**53.122**	**25.208**
Cumulative variance (%)	**53.122**	**78.330**
Ca	0.18363	0.04779
S	-0.15657	0.03675
K	0.19545	0.07126
Mg	0.19887	0.00271
P	0.12372	0.18058
Cu	0.21062	0.00766
Fe	0.21300	-0.04088
Mn	0.19010	-0.05606
Mo	0.19311	0.07950
B	0.03767	0.22952
Zn	0.01674	0.25813
Ba	0.14712	0.20274
Al	0.16710	0.16079
Co	0.20808	0.00495
Cr	0.19032	0.03365
Cs	0.14869	0.21448
Ga	0.09347	0.24471
Li	0.08098	0.25278
Na	0.18545	0.08440
Ni	0.20774	-0.00775
Rb	0.15622	0.19006
Sr	0.15445	0.16530
Ti	0.04509	0.16795
V	0.00752	0.28169
Zr	0.11862	0.13161
Sn	0.20833	-0.00643
I	0.00098	-0.02967
Ce	0.17360	0.15205
Shoot Length	0.15419	-0.21202
Root Length	0.18891	-0.14817
No. of Leaves	0.12730	-0.24425
No. of Internodes	0.13176	-0.23433
Leaf Area	0.16100	-0.16983
Fresh shoot weight	0.17550	-0.15759
Shoot dry weight	0.17992	-0.11846
Fresh root weight	0.19982	-0.11990
Root dry weight	0.18174	-0.15085
Root to shoot dry weight	0.17737	-0.16657
Proline	0.12380	-0.23804

4.2.5 T5 Treatment (20% PEG)

At 20% PEG induced drought stress condition LCA-353 cultivar did not survive. Hence, LCA-353 was designated as highly susceptible to drought stress. Principle component analysis (Fig. 4.14 and Table 4.23**)** revealed that first two principal components having eigenvalue more than one accounting 69.9% of total variation. It was found that PC1 contributed 44.8% whereas PC2 contributed 25.1% of total variation. Root dry weight, Ba, shoot length, Ga, root length, root to shoot dry weight, fresh root weight, shoot dry weight, fresh shoot weight, Cu, Fe, leaf area, proline, Mn, Ca, Mg, Zr, Cs, P, no. of leaves, Mo, Al, no. of internodes, Na, K, Ce, B, Li positively contributed in descending order to PC1 and Cr, V, Zn, Ni, S, Sr, Ti, I, Sn, Co, Rb negatively contributed to PC1. Whereas Co, Li, B, Ce, Ti, I, Na, Al, Cs, Zr, Ni, Cr, fresh shoot weight, shoot dry weight, proline, fresh root weight, root length, leaf area, Sn, V, root dry weight, shoot length positively contributed to PC2 and Rb, Mg, P, Ca, Mo, Fe, Mn, Cu, Zn, no. of internodes, K, Ba, Ga, S, Sr, no. of leaves, root to shoot dry weight were negatively contributed to PC2. Score and hierarchical cluster analysis revealed that seven capsicum cultivars separated into two groups *viz.* Arka Lohit on the extreme positive side of PC1 followed by LCA-334 as a one group. Remaining all other cultivars fell near to origin and formed another group.

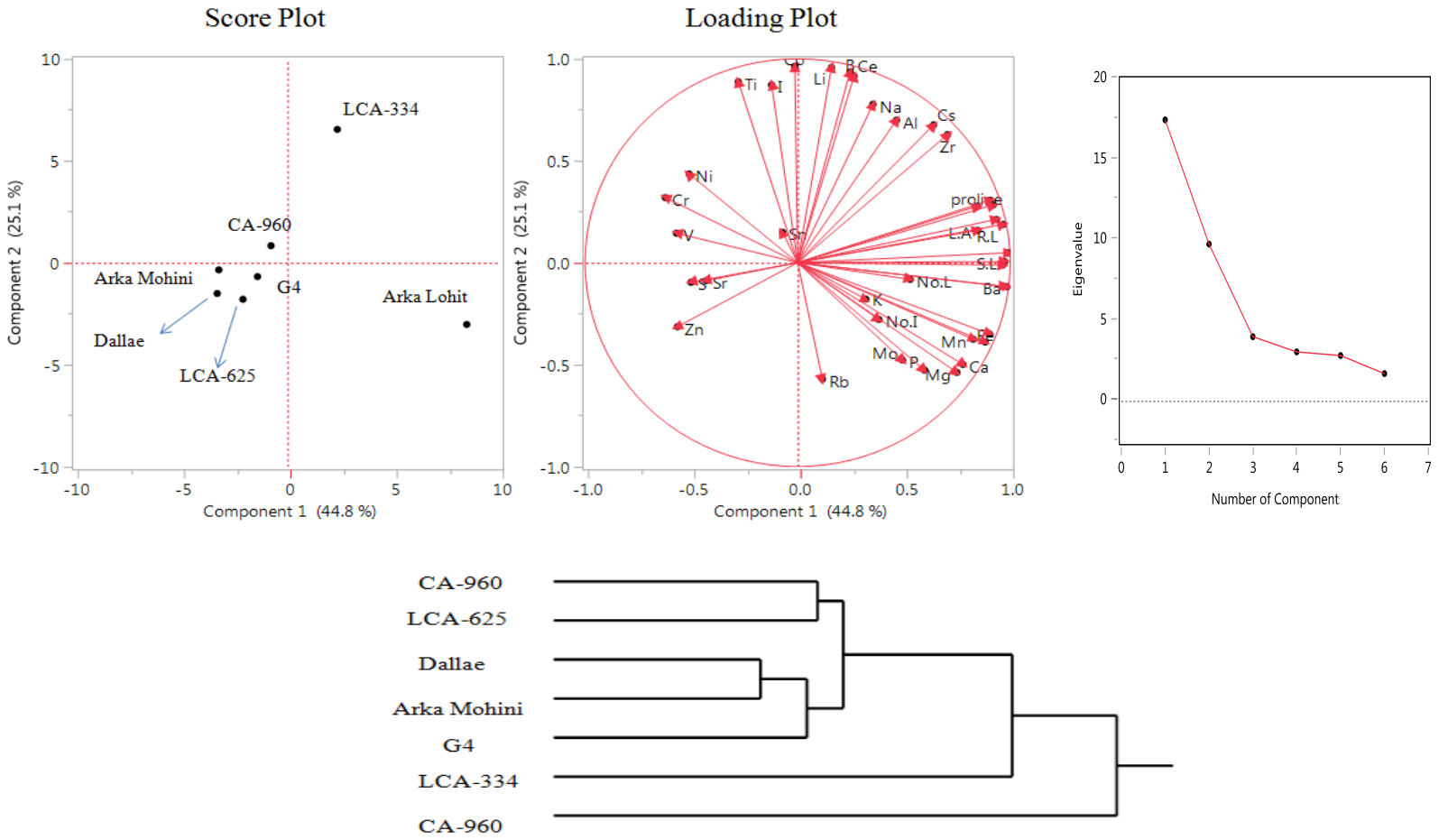

Figure 4.14 A. PCA score and loading plots B. Scree plot C. Hierarchical cluster in 20% PEG 6000 Drought stress condition

Table 4.23 Eigen values, proportion of the total variance, cumulative per cent variance and component loading of different variables of chilli cultivars under 20% PEG condition.

	PC1	PC2
Eigen values (variance)	**17.4857**	**9.7783**
Proportion of variance (%)	**44.835**	**25.073**
Cumulative variance (%)	**44.835**	**69.908**
Ca	0.18596	-0.15959
S	-0.11986	-0.03077
K	0.07667	-0.05721
Mg	0.17895	-0.17197
P	0.14265	-0.16837
Cu	0.21576	-0.11169
Fe	0.21088	-0.12459
Mn	0.19748	-0.12000
Mo	0.11790	-0.15282
B	0.05897	0.29903
Zn	-0.13524	-0.10042
Ba	0.23536	-0.03778
Al	0.11116	0.22341
Co	-0.00313	0.30770
Cr	-0.14876	0.10206
Cs	0.15302	0.21632
Ga	0.23212	-0.03724
Li	0.03807	0.30604
Na	0.08464	0.24837
Ni	-0.12199	0.13914
Rb	0.02784	-0.18233
Sr	-0.10297	-0.02817
Ti	-0.06683	0.28376
V	-0.13628	0.04627
Zr	0.16858	0.20084
Sn	-0.01690	0.04757
I	-0.02927	0.27877
Ce	0.06340	0.29241
Shoot Length	0.23270	0.00169
Root Length	0.23122	0.06004
No. of Leaves	0.12652	-0.02625
No. of Internodes	0.09139	-0.08913
Leaf Area	0.20250	0.05074
Fresh shoot weight	0.21686	0.09806
Shoot dry weight	0.21986	0.09038
Fresh root weight	0.22353	0.06795
Root dry weight	0.23693	0.01549
Root to shoot dry weight	0.23055	-0.00372
Proline	0.20208	0.08846

4.3 Profile of ions in the susceptible and tolerant germplasm lines

Screening of eight chilli cultivars based on morpho-physiological characters and ionome profiling it has been found that Arka Lohit showed better tolerance to drought stress and LCA-353 exhibited susceptible. Comparison of ionome profiling of drought tolerant cultivar and susceptible cultivars in control and highest stress condition, could pave the way to understand which ionome has positive role towards drought tolerance. Though highest drought stress condition in the present study was 20% PEG treatment, the samples were collected from 15% PEG treated material, as the most susceptible cultivar LCA-353 did not survive in 20% PEG. Hence, to have a meaningful comparison, 15% PEG treatment was used along with control for comparative ionomics study.

From the results obtained from the comparative ionomics analysis (Table 4.24) the following findings could be made

- Concentration of Ca, Mg, Cu, Fe, Mn, Mo, Ni, Sn increased in stress condition as compared to control in drought tolerant cultivar (Arka Lohit) whereas, in drought susceptible cultivar (LCA-353) these elements got reduced.
- Concentration of P, Rb, Sr increased in stress condition in both drought tolerant and susceptible cultivars as compared to control.
- Concentration of elements like K, S, B, Zn, Ba, Al, Li, Na, Ti, Zr, Cr, Ce decreased in both drought tolerant and susceptible cultivars as compared to control.
- Compared to control the concentration of Ga, decreased in stress condition in drought tolerant cultivar whereas in susceptible cultivar it was at par both in control and stress condition.

Table 4.24 Ionome comparison of drought tolerant and drought susceptible cultivars (ppm±sd) in control and the highest drought condition

Ionome	Arka Lohit(T1)	Arka Lohit(T4)	LCA-353(T1)	LCA-353 (T4)
Ca	7.24±0.319	31.61±0.544	4.29±0.088	4.15±0.043
S	39.5±0.757	14.74±0.359	30.45±0.357	25.16±1.455
K	973.4±11.737	388.66±S	244.96±2.795	173.81±0.537
Mg	190.26±2.171	310.52±4.43	36.66±0.077	34.57±0.377
P	231.37±7.05	318.97±4.107	52.7±0.658	76.44±1.794
Cu	10.32±0.206	14.88±0.072	4.83±0.121	2.67±0.012
Fe	56.08±2.049	68.95±1.018	21.97±0.312	10.87±0.478
Mn	38.77±1.352	71.35±1.115	22.01±0.046	17.41±0.061
Mo	1.09±0.051	2.3±0.053	0.57±0.014	0.53±0.012
B	11.19±1.122	1.11±0.028	7.1±0.015	1.04±0.009
Zn	1.33±0.013	0.32±0.016	0.92±0.014	0.1±0.003
Ba	7.19±0.106	4.16±0.068	1.32±0.012	1.02±0.145
Al	48.69±2.482	27.87±0.148	4.62±0.064	1.54±0.016
Co	0.14±0.013	0.32±0.018	0.015±0.007	0.015±0.004
Cr	0.087±0.641	0.06±0.23	0.53±0.458	0.07±0.241
Cs	0.04±0.03	0.03±0.003	0.02±0.003	0.01±0.03
Ga	1.52±0.23	0.69±0.055	0.54±0.286	0.6±0.202
Li	1.35±0.121	0.68±0.012	0.22±0.008	0.18±0.003
Na	32.37±0.195	11.36±0.116	5.64±0.059	3.03±0.04
Ni	1.02±0.015	2.14±0.065	1.06±0.0267	0.29±0.0167
Rb	1.14±0.006	1.65±0.118	1.02±0.008	1.71±0.113
Sr	0.85±0.048	1.024±0.004	0.39±0.042	0.51±0.053
Ti	1.09±0.0317	0.093±0.074	0.39±0.388	0.053±0.02
V	0.73±3.771	0.1±1.062	0.65±1.973	0.38±0.198
Zr	0.73±0.025	0.26±0.033	0.13±0.003	0.02±0.01
Sn	0.62±0.0252	0.83±0.0984	0.59±0.118	0.188±0.092
I	1.16±0.184	2.23±0.05	1.084±0.08	1.089±0.04
Ce	0.76±0.019	0.3±0.01	0.07±0.001	0.03±0.001

- In drought tolerant cultivar concentration of Co, I increased in stress condition compare to control whereas in susceptible cultivar they were at par both in control and stress conditions with same concentrations.

4.4 Proteomics

Sampling

Eight capsicum *annuum* cultivars were screened *in vitro* condition and based on results of morpho-physiological characters obtained from these cultivars under different drought stress conditions, it was found that Arka Lohit was drought tolerant and LCA- 353 was susceptible cultivars. To find out responsible proteins for stress tolerance label free protein quantification was done. For label free protein quantification pooled samples of three biological replicates of drought tolerant and susceptible cultivars were collected from control and highest drought stress condition for further analysis. Though highest drought stress condition in the present study was 20% PEG treatment, the samples were collected from 15% PEG treated material, as the most susceptible cultivar LCA-353 did not survive in 20% PEG. Hence, to have a meaningful comparision 15% PEG treatment was used along with control in proteomics study. Protein differentiations was done with help of PLGS software.

4.4.1 Constitutively expressed proteins of tolerant genotype (C1) over susceptible (C2) under control condition

In the control condition the comparison on differential proteins between drought tolerant cultivar and drought susceptible cultivar showed total of 36 proteins from 2 to 5.58 of fold change (Table 4.25). The up regulated proteins interactions were analysed using string analysis and the network given in the figure (Fig.4.15). The string analysis showed the function enrichment of the proteins coding for the

Table 4.25 Protein Profile of drought tolerant cultivar VS Drought susceptible cultivar in the control condition (C1 VS C2)

Accession	Protein Name	Fold Change
A0A1U8EFC0	B3 domain-containing transcription repressor VAL2-like isoform X2 OS=Capsicum annuum GN=LOC107845911 PE=4 SV=1	2.01
A0A1U8H4J3	probable serine/threonine protein kinase IREH1 isoform X3 OS=Capsicum annuum GN=LOC107875972 PE=4 SV=1	2.03
A0A1U8HLL0	probable serine/threonine-protein kinase At1g54610 isoform X1 OS=Capsicum annuum GN=LOC107879687 PE=4 SV=1	2.03
Q4ZIQ4	Pin-II type proteinase inhibitor 8 OS=Capsicum annuum GN=PI-8 PE=2 SV=1	2.05
T1PZB5	Pin-II type proteinase inhibitor 54 OS=Capsicum annuum PE=2 SV=1	2.05
T1PZE6	Pin-II type proteinase inhibitor 25 OS=Capsicum annuum PE=2 SV=1	2.08
T1PZA9	Pin-II type proteinase inhibitor 24 OS=Capsicum annuum PE=2 SV=1	2.08
A0A1U8EW09	uncharacterized protein LOC107850987 isoform X2 OS=Capsicum annuum GN=LOC107850987 PE=4 SV=1	2.08
A0A097J9C0	Pin-II type proteinase inhibitor 72 (Fragment) OS=Capsicum annuum GN=PI-72 PE=2 SV=1	2.10
T1PZB0	Pin-II type proteinase inhibitor 29 OS=Capsicum annuum PE=2 SV=1	2.10
P56615	Proteinase inhibitor PSI-1.1 OS=Capsicum annuum PE=1 SV=1	2.12
A0A1U8FJX1	LOW QUALITY PROTEIN: ARF guanine-nucleotide exchange factor GNOM-like OS=Capsicum annuum GN=LOC107858761 PE=4 SV=1	2.12
Q4ZIQ5	Pin-II type proteinase inhibitor 32 OS=Capsicum annuum GN=PI-7 PE=2 SV=1	2.14
Q4U5Z5	PinII-type proteinase inhibitor 11 OS=Capsicum annuum PE=2 SV=1	2.16
Q4U5Z4	PinII-type proteinase inhibitor 6 (Fragment) OS=Capsicum annuum PE=2 SV=1	2.18
T1PZF6	Pin-II type proteinase inhibitor 50 OS=Capsicum annuum PE=2 SV=1	2.18
Q4ZIQ2	Pin-II type proteinase inhibitor 10 OS=Capsicum annuum GN=PI-10 PE=2 SV=1	2.20
D2CH22	Pin-II type proteinse inhibitor 23 OS=Capsicum annuum PE=2 SV=1	2.25
D2CGT7	Pin-II type proteinase inhibitor 20 OS=Capsicum annuum PE=2 SV=1	2.27
A0A1U8ESY4	homeobox-DDT domain protein RLT1 OS=Capsicum annuum GN=LOC107850366 PE=4 SV=1	2.27
D2CGT5	Pin-II type proteinase inhibitor 18 OS=Capsicum annuum PE=2 SV=1	2.29
A0A1U8HFJ8	probable serine/threonine-protein kinase At1g54610 isoform X2 OS=Capsicum annuum GN=LOC107879687 PE=4 SV=1	2.36

T1PZB7	Pin-II type proteinase inhibitor 64 OS=Capsicum annuum PE=2 SV=1	2.46
A0A1U8H6Q1	LOW QUALITY PROTEIN: putative late blight resistance protein homolog R1A-3 OS=Capsicum annuum GN=LOC107873699 PE=3 SV=1	2.53
A0A1U8HBR2	seed biotin-containing protein SBP65-like OS=Capsicum annuum GN=LOC107878410 PE=4 SV=1	2.59
A0A1U8HGP1	uncharacterized protein LOC107877497 OS=Capsicum annuum GN=LOC107877497 PE=4 SV=1	2.89
A0A1U8G423	pleiotropic drug resistance protein 1 OS=Capsicum annuum GN=LOC107864282 PE=3 SV=1	2.97
A0A1U8GDR2	uncharacterized protein LOC107864352 isoform X2 OS=Capsicum annuum GN=LOC107864352 PE=4 SV=1	3.29
A0A1U8G4Z4	uncharacterized protein LOC107864352 isoform X3 OS=Capsicum annuum GN=LOC107864352 PE=4 SV=1	3.29
A0A1U8GA20	LOW QUALITY PROTEIN: E3 ubiquitin-protein ligase SHPRH OS=Capsicum annuum GN=LOC107863480 PE=4 SV=1	3.32
A0A1U8F7I3	LOW QUALITY PROTEIN: ABC transporter B family member 11-like OS=Capsicum annuum GN=LOC107851247 PE=4 SV=1	3.39
A0A1U8GYN2	ABC transporter A family member 7-like OS=Capsicum annuum GN=LOC107873937 PE=4 SV=1	3.49
A0A1U8GBV7	uncharacterized protein LOC107864352 isoform X1 OS=Capsicum annuum GN=LOC107864352 PE=4 SV=1	3.67
A0A1U8H3Y0	uncharacterized protein LOC107875835 isoform X1 OS=Capsicum annuum GN=LOC107875835 PE=4 SV=1	4.31
A0A1U8GMI2	histone acetyltransferase HAC1-like OS=Capsicum annuum GN=LOC107870442 PE=4 SV=1	4.44
A0A1U8EHK8	uncharacterized protein LOC107845877 isoform X1 OS=Capsicum annuum GN=LOC107845877 PE=4 SV=1	5.47
A0A1U8EPR9	uncharacterized protein LOC107845877 isoform X2 OS=Capsicum annuum GN=LOC107845877 PE=4 SV=1	5.58

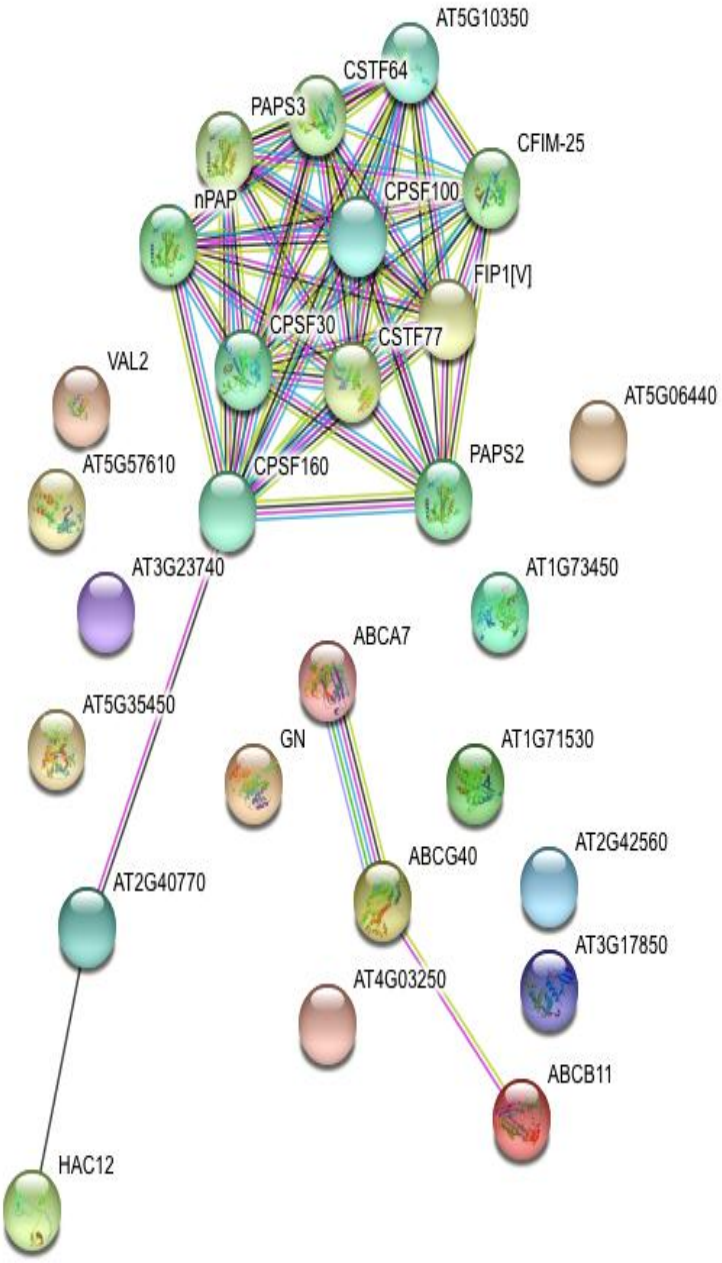

Figure 4.15 String analysis network for Drought tolerant (C1) Vs Drought susceptible (C2) in control condition

molecular functions such as mRNA binding, polynucleotide adenyltransferase activity, RNA binding. The protein function enrichment was only found to be in the transcriptional level. Since, the analyzed proteins were derived from the control condition of the tolerant and susceptible genotypes. Out of 36 proteins 10 up regulated proteins with above 3 fold expressions namely E3 ubiquitin-protein ligase, ABC transporter B family member 11-like, ABC transporter A family member 7-like, histone acetyltransferase HAC1-like, uncharacterized protein (6 proteins).

When comparing tolerant genotypes over susceptible the above mentioned signature proteins which constitute highly expressing were attributed to tolerant nature of Arka Lohit, since, the differential protein profiling was done in the control condition without drought induction from Arka Lohit and LCA-353.

4.4.2 Drought responsive proteins of tolerant germplasm under control (C1) and high drought condition (T1)

Comparison on differential proteins between drought tolerant cultivar in control and stress conditions showed total 171 proteins from 2 to 25.03 fold change (Table 4.26). The up regulated proteins interactions were analysed using string analysis and the network is given in the figure (Fig.4.16). The string analysis showed the function enrichment of the proteins coding for the molecular functions such as nucleoside binding, ribonucleoside binding, ATP binding, small molecule binding and nucleotide binding. Out of 171 proteins, 118 up regulated proteins with above 3 fold expressions were grouped into heat shock proteins/CHAPERONS (heat shock 70 kDa protein-like, heat shock cognate 70 kDa protein, heat shock cognate 70 kDa protein 2), ROS proteins, R gene proteins, Chromatin modify proteins, transporters, wound response proteins and other proteins like nuclear pore Anchor (2) DNA-directed RNA

Table 4.26 Protein profile of drought tolerant cultivar in control (C1) Vs Drought tolerant cultivar in highest drought stress treatment (T1)

Accession	Protein Name	Fold Change
A0A1U8GRA7	LOW QUALITY PROTEIN: late blight resistance protein R1-A-like OS=Capsicum annuum GN=LOC107871299 PE=3 SV=1	2.01
A0A1U8H395	protein WVD2-like 3 OS=Capsicum annuum GN=LOC107873274 PE=4 SV=1	2.01
A0A1U8HJB8	G-type lectin S-receptor-like serine/threonine-protein kinase At4g27290 OS=Capsicum annuum GN=LOC107878718 PE=4 SV=1	2.01
A0A1U8EPS7	seed biotin-containing protein SBP65-like OS=Capsicum annuum GN=LOC107845520 PE=4 SV=1	2.03
A0A1U8FPQ9	protein furry homolog-like OS=Capsicum annuum GN=LOC107860387 PE=4 SV=1	2.05
A0A1U8F075	LOW QUALITY PROTEIN: DExH-box ATP-dependent RNA helicase DExH12-like OS=Capsicum annuum GN=LOC107848814 PE=4 SV=1	2.08
A0A1U8FQF4	ABC transporter B family member 15-like OS=Capsicum annuum GN=LOC107860581 PE=4 SV=1	2.08
A0A1U8HEU1	uncharacterized protein LOC107879373 OS=Capsicum annuum GN=LOC107879373 PE=4 SV=1	2.08
A0A1U8FPC2	DNA polymerase OS=Capsicum annuum GN=LOC107860285 PE=3 SV=1	2.08
A0A1U8EAB8	uncharacterized protein LOC107844228 OS=Capsicum annuum GN=LOC107844228 PE=4 SV=1	2.10
A0A1U8F9J3	protein CROWDED NUCLEI 1-like isoform X2 OS=Capsicum annuum GN=LOC107855486 PE=4 SV=1	2.12
A0A1U8FI47	protein CROWDED NUCLEI 1-like isoform X3 OS=Capsicum annuum GN=LOC107855486 PE=4 SV=1	2.14
A0A1U8HEG0	uncharacterized protein LOC107879249 OS=Capsicum annuum GN=LOC107879249 PE=4 SV=1	2.14
A0A1U8HGY9	uncharacterized protein LOC107877591 isoform X3 OS=Capsicum annuum GN=LOC107877591 PE=4 SV=1	2.16
A0A1U8GYM1	uncharacterized protein LOC107873940 OS=Capsicum annuum GN=LOC107873940 PE=4 SV=1	2.16
A0A1U8EAX4	uncharacterized protein LOC107841665 OS=Capsicum annuum GN=LOC107841665 PE=4 SV=1	2.18
A0A1U8H8C6	uncharacterized protein LOC107877591 isoform X1 OS=Capsicum annuum GN=LOC107877591 PE=4 SV=1	2.18
A0A1U8ECC0	uncharacterized protein LOC107841612 OS=Capsicum annuum GN=LOC107841612 PE=4 SV=1	2.20
A0A1U8F9I9	protein CROWDED NUCLEI 1-like isoform X1 OS=Capsicum annuum GN=LOC107855486 PE=4 SV=1	2.20
A0A1U8GYD3	Histone H4 OS=Capsicum annuum GN=LOC107873824 PE=3 SV=1	2.20
A0A1U8G1X3	protein NETWORKED 1D-like OS=Capsicum annuum GN=LOC107863791 PE=4 SV=1	2.20
A0A1U8GX93	Histone H4 OS=Capsicum annuum GN=LOC107873518 PE=3 SV=1	2.23

Q71V09	Histone H4 OS=Capsicum annuum PE=3 SV=3	2.25
A0A1U8E1T0	Histone H4 OS=Capsicum annuum GN=LOC107838933 PE=3 SV=1	2.25
A0A1U8FA50	uncharacterized protein LOC107855692 isoform X1 OS=Capsicum annuum GN=LOC107855692 PE=4 SV=1	2.27
A0A1U8FIP7	uncharacterized protein LOC107855692 isoform X2 OS=Capsicum annuum GN=LOC107855692 PE=4 SV=1	2.27
A0A1U8HF28	uncharacterized protein LOC107877591 isoform X2 OS=Capsicum annuum GN=LOC107877591 PE=4 SV=1	2.27
A0A1U8FY19	pentatricopeptide repeat-containing protein At5g16860 OS=Capsicum annuum GN=LOC107862859 PE=4 SV=1	2.29
A0A1U8F7M1	putative U-box domain-containing protein 50 OS=Capsicum annuum GN=LOC107851285 PE=4 SV=1	2.29
A0A1U8EPR3	proteasome-associated protein ECM29 homolog isoform X4 OS=Capsicum annuum GN=LOC107845889 PE=4 SV=1	2.44
A0A1U8EHU0	protein ROS1-like OS=Capsicum annuum GN=LOC107843192 PE=4 SV=1	2.44
A0A1U8GEG6	Protein translocase subunit SecA OS=Capsicum annuum GN=LOC107867797 PE=3 SV=1	2.46
A0A1U8H6L6	Histone H4 OS=Capsicum annuum GN=LOC107874440 PE=3 SV=1	2.51
A0A1U8FR89	DNA-directed RNA polymerase subunit beta OS=Capsicum annuum GN=LOC107858033 PE=3 SV=1	2.51
A0A1U8EUZ8	protein RNA-directed DNA methylation 3 OS=Capsicum annuum GN=LOC107850304 PE=4 SV=1	2.53
A0A1U8GS00	methyltransferase-like protein 1 OS=Capsicum annuum GN=LOC107871490 PE=3 SV=1	2.56
A0A1U8ENT4	sarcoplasmic reticulum histidine-rich calcium-binding protein OS=Capsicum annuum GN=LOC107848615 PE=4 SV=1	2.59
A0A1U8EF64	proteasome-associated protein ECM29 homolog isoform X2 OS=Capsicum annuum GN=LOC107845889 PE=4 SV=1	2.61
A0A1U8ER90	proteasome-associated protein ECM29 homolog isoform X1 OS=Capsicum annuum GN=LOC107845889 PE=4 SV=1	2.61
A0A1U8EHL3	proteasome-associated protein ECM29 homolog isoform X5 OS=Capsicum annuum GN=LOC107845889 PE=4 SV=1	2.61
A0A1U8GZU8	uncharacterized protein LOC107874345 isoform X2 OS=Capsicum annuum GN=LOC107874345 PE=4 SV=1	2.64
A0A1U8H0W0	DDB1- and CUL4-associated factor homolog 1 OS=Capsicum annuum GN=LOC107871649 PE=4 SV=1	2.80
A0A1U8HIG0	uncharacterized protein LOC107878476 OS=Capsicum annuum GN=LOC107878476 PE=4 SV=1	2.80
A0A1U8H6Q0	LOW QUALITY PROTEIN: putative late blight resistance protein homolog R1B-13 OS=Capsicum annuum GN=LOC107874492 PE=3 SV=1	2.80
A0A1U8EF91	proteasome-associated protein ECM29 homolog isoform X3 OS=Capsicum annuum GN=LOC107845889 PE=4 SV=1	2.83
A0A1U8FWW0	pentatricopeptide repeat-containing protein At1g06710, mitochondrial OS=Capsicum annuum GN=LOC107859642 PE=4 SV=1	2.83
A0A060BIE4	DNA-directed RNA polymerase subunit beta OS=Capsicum annuum var. glabriusculum GN=rpoB PE=3 SV=1	2.83

A0A1U8G4P1	pleiotropic drug resistance protein 1 OS=Capsicum annuum GN=LOC107864281 PE=3 SV=1	2.89
A0A1U8EIN1	methyl-CpG-binding domain-containing protein 9 OS=Capsicum annuum GN=LOC107847389 PE=4 SV=1	2.89
J7H6R6	DNA-directed RNA polymerase subunit beta OS=Capsicum annuum GN=rpoB PE=3 SV=1	2.92
A0A1U8EFY3	DExH-box ATP-dependent RNA helicase DExH1 isoform X2 OS=Capsicum annuum GN=LOC107846195 PE=4 SV=1	2.92
A0A1U8FZA6	pentatricopeptide repeat-containing protein At3g18110, chloroplastic OS=Capsicum annuum GN=LOC107862962 PE=4 SV=1	2.97
A0A0M4JAE6	DNA-directed RNA polymerase subunit beta OS=Capsicum annuum var. annuum GN=rpoB PE=3 SV=1	2.97
A0A1U8GFK7	protein CROWDED NUCLEI 1-like isoform X2 OS=Capsicum annuum GN=LOC107868051 PE=4 SV=1	3.00
A0A0M4JLD2	DNA-directed RNA polymerase subunit beta OS=Capsicum annuum var. glabriusculum GN=rpoB PE=3 SV=1	3.00
A0A1U8EIB6	DExH-box ATP-dependent RNA helicase DExH1 isoform X1 OS=Capsicum annuum GN=LOC107846195 PE=4 SV=1	3.00
A0A1U8G6X2	uncharacterized protein LOC107863157 OS=Capsicum annuum GN=LOC107863157 PE=4 SV=1	3.06
A0A1U8DUV1	uncharacterized protein LOC107839095 OS=Capsicum annuum GN=LOC107839095 PE=4 SV=1	3.10
T1PZK4	Pin-II type proteinase inhibitor 57 OS=Capsicum annuum PE=2 SV=1	3.19
Q4U5Z5	PinII-type proteinase inhibitor 11 OS=Capsicum annuum PE=2 SV=1	3.22
Q4ZIQ2	Pin-II type proteinase inhibitor 10 OS=Capsicum annuum GN=PI-10 PE=2 SV=1	3.22
T1PZF6	Pin-II type proteinase inhibitor 50 OS=Capsicum annuum PE=2 SV=1	3.22
T1PZ90	Pin-II type proteinase inhibitor 58 OS=Capsicum annuum PE=2 SV=1	3.25
Q4ZIQ5	Pin-II type proteinase inhibitor 32 OS=Capsicum annuum GN=PI-7 PE=2 SV=1	3.32
O49146	Wound-induced proteinase inhibitor 2 OS=Capsicum annuum GN=PIN2 PE=2 SV=1	3.35
T1PZ86	Pin-II type proteinase inhibitor 38 OS=Capsicum annuum PE=2 SV=1	3.35
D2CGT6	Pin-II type proteinase inhibitor 19 OS=Capsicum annuum PE=2 SV=1	3.39
T1PZK7	Pin-II type proteinase inhibitor 62 OS=Capsicum annuum PE=2 SV=1	3.42
T1PZJ7	Pin-II type proteinase inhibitor 37 OS=Capsicum annuum PE=2 SV=1	3.42
T1PZF9	Pin-II type proteinase inhibitor 65 OS=Capsicum annuum PE=2 SV=1	3.42
A0A1U8F6C7	uncharacterized protein LOC107851324 OS=Capsicum annuum GN=LOC107851324 PE=4 SV=1	3.42
Q4U5Z3	PinII-type proteinase inhibitor 12 (Fragment) OS=Capsicum annuum PE=2 SV=1	3.46
P56615	Proteinase inhibitor PSI-1.1 OS=Capsicum annuum PE=1 SV=1	3.46

A0A097J9C1	Pin-II type proteinase inhibitor 75 (Fragment) OS=Capsicum annuum GN=PI-75 PE=2 SV=1	3.46
T1PZK0	Pin-II type proteinase inhibitor 47 OS=Capsicum annuum PE=2 SV=1	3.46
T1PZJ8	Pin-II type proteinase inhibitor 42 OS=Capsicum annuum PE=2 SV=1	3.46
T1PZE6	Pin-II type proteinase inhibitor 25 OS=Capsicum annuum PE=2 SV=1	3.46
T1PZB4	Pin-II type proteinase inhibitor 49 OS=Capsicum annuum PE=2 SV=1	3.46
T1PZB2	Pin-II type proteinase inhibitor 39 OS=Capsicum annuum PE=2 SV=1	3.46
Q4Z8K2	Pin-II type proteinase inhibitor 4 OS=Capsicum annuum GN=PI-4 PE=2 SV=1	3.46
T1PZ85	Pin-II type proteinase inhibitor 33 OS=Capsicum annuum PE=2 SV=1	3.46
T1PZ75	Pin-II type proteinase inhibitor 56 OS=Capsicum annuum PE=2 SV=1	3.46
T1PZ72	Pin-II type proteinase inhibitor 46 OS=Capsicum annuum PE=2 SV=1	3.46
A0A1U8EIW2	E3 ubiquitin-protein ligase PRT6-like OS=Capsicum annuum GN=LOC107843479 PE=4 SV=1	3.46
A0A1U8FLQ0	DNA-directed RNA polymerase subunit OS=Capsicum annuum GN=LOC107856811 PE=3 SV=1	3.49
D2CGT4	Pin-II type proteinase inhibitor 17 OS=Capsicum annuum PE=2 SV=1	3.49
A0A097J9D6	Pin-II type proteinase inhibitor 74 (Fragment) OS=Capsicum annuum GN=PI-74 PE=2 SV=1	3.49
A0A097J9D3	Pin-II type proteinase inhibitor 78 (Fragment) OS=Capsicum annuum GN=PI-78 PE=2 SV=1	3.49
D2CGQ2	Pin-II type proteinase inhibitor 16 OS=Capsicum annuum PE=2 SV=1	3.49
A0A097J9C5	Pin-II type proteinase inhibitor 77 (Fragment) OS=Capsicum annuum GN=PI-77 PE=2 SV=1	3.49
T1PZL0	Pin-II type proteinase inhibitor 67 OS=Capsicum annuum PE=2 SV=1	3.49
T1PZJ5	Pin-II type proteinase inhibitor 27 OS=Capsicum annuum PE=2 SV=1	3.49
T1PZF8	Pin-II type proteinase inhibitor 60 OS=Capsicum annuum PE=2 SV=1	3.49
T1PZF7	Pin-II type proteinase inhibitor 55 OS=Capsicum annuum PE=2 SV=1	3.49
T1PZF0	Pin-II type proteinase inhibitor 35 OS=Capsicum annuum PE=2 SV=1	3.49
T1PZB6	Pin-II type proteinase inhibitor 59 OS=Capsicum annuum PE=2 SV=1	3.49
T1PZB5	Pin-II type proteinase inhibitor 54 OS=Capsicum annuum PE=2 SV=1	3.49
A0A1U8F7U0	Phospholipid-transporting ATPase OS=Capsicum annuum GN=LOC107851337 PE=3 SV=1	3.49
T1PZ89	Pin-II type proteinase inhibitor 53 OS=Capsicum annuum PE=2 SV=1	3.49

A0A097J9D7	Pin-II type proteinase inhibitor 76 (Fragment) OS=Capsicum annuum GN=PI-76 PE=2 SV=1	3.53
A0A097J9B6	Pin-II type proteinase inhibitor 68 OS=Capsicum annuum GN=PI-68 PE=2 SV=1	3.53
A0A1U8G7B9	wound-induced proteinase inhibitor 2-like OS=Capsicum annuum GN=LOC107862768 PE=4 SV=1	3.53
Q4ZIQ6	Pin-II type proteinase inhibitor 5 OS=Capsicum annuum GN=PI-5 PE=2 SV=1	3.53
Q4ZIQ4	Pin-II type proteinase inhibitor 8 OS=Capsicum annuum GN=PI-8 PE=2 SV=1	3.53
Q4ZIQ3	Pin-II type proteinase inhibitor 9 OS=Capsicum annuum GN=PI-9 PE=2 SV=1	3.53
T1PZB0	Pin-II type proteinase inhibitor 29 OS=Capsicum annuum PE=2 SV=1	3.53
T1PZA9	Pin-II type proteinase inhibitor 24 OS=Capsicum annuum PE=2 SV=1	3.53
Q4Z8K3	Pin-II type proteinase inhibitor 3 OS=Capsicum annuum GN=PI-3 PE=2 SV=1	3.53
T1PZ91	Pin-II type proteinase inhibitor 43 OS=Capsicum annuum PE=2 SV=1	3.53
T1PZ84	Pin-II type proteinase inhibitor 28 OS=Capsicum annuum PE=2 SV=1	3.53
T1PZ73	Pin-II type proteinase inhibitor 51 OS=Capsicum annuum PE=2 SV=1	3.53
D2CGU0	Pin-II type proteinase inhibitor 14 OS=Capsicum annuum PE=2 SV=1	3.56
T1PZK2	Pin-II type proteinase inhibitor 52 OS=Capsicum annuum PE=2 SV=1	3.56
T1PZF5	Pin-II type proteinase inhibitor 45 OS=Capsicum annuum PE=2 SV=1	3.56
T1PZF2	Pin-II type proteinase inhibitor 40 OS=Capsicum annuum PE=2 SV=1	3.56
T1PZE8	Pin-II type proteinase inhibitor 30 OS=Capsicum annuum PE=2 SV=1	3.56
T1PZB3	Pin-II type proteinase inhibitor 44 OS=Capsicum annuum PE=2 SV=1	3.56
T1PZB1	Pin-II type proteinase inhibitor 34 OS=Capsicum annuum PE=2 SV=1	3.56
T1PZ78	Pin-II type proteinase inhibitor 66 OS=Capsicum annuum PE=2 SV=1	3.56
T1PZ76	Pin-II type proteinase inhibitor 61 OS=Capsicum annuum PE=2 SV=1	3.56
T1PZ64	Pin-II type proteinase inhibitor 31 OS=Capsicum annuum PE=2 SV=1	3.56
Q9SDL4	Putative uncharacterized protein CapinII OS=Capsicum annuum GN=CapinII PE=2 SV=1	3.56
A0A1U8H6V0	uncharacterized protein LOC107873723 isoform X1 OS=Capsicum annuum GN=LOC107873723 PE=4 SV=1	3.56
A0A1U8GFL5	protein CROWDED NUCLEI 1-like isoform X1 OS=Capsicum annuum GN=LOC107868051 PE=4 SV=1	3.60
T1PZ68	Pin-II type proteinase inhibitor 41 OS=Capsicum annuum PE=2 SV=1	3.60

T1PZ65	Pin-II type proteinase inhibitor 36 OS=Capsicum annuum PE=2 SV=1	3.60
A0A1U8DXZ0	probable pre-mRNA-splicing factor ATP-dependent RNA helicase DEAH5 OS=Capsicum annuum GN=LOC107840517 PE=4 SV=1	3.74
A0A1U8E8X8	miraculin-like OS=Capsicum annuum GN=LOC107843776 PE=4 SV=1	3.74
A0A1U8E7U4	heat shock cognate 70 kDa protein 2 OS=Capsicum annuum GN=LOC107842953 PE=3 SV=1	3.74
A0A1U8F2J7	nuclear-pore anchor isoform X1 OS=Capsicum annuum GN=LOC107853094 PE=4 SV=1	3.78
A0A1U8F2B0	nuclear-pore anchor isoform X2 OS=Capsicum annuum GN=LOC107853094 PE=4 SV=1	3.78
A0A1U8GTB5	protein MIZU-KUSSEI 1-like OS=Capsicum annuum GN=LOC107872247 PE=4 SV=1	3.86
A0A1U8FU98	1-phosphatidylinositol-3-phosphate 5-kinase FAB1B-like isoform X1 OS=Capsicum annuum GN=LOC107861644 PE=4 SV=1	4.01
A0A1U8H0H9	uncharacterized protein LOC107874632 OS=Capsicum annuum GN=LOC107874632 PE=4 SV=1	4.10
A0A1U8H8U2	uncharacterized protein LOC107877681 isoform X2 OS=Capsicum annuum GN=LOC107877681 PE=4 SV=1	4.14
A0A1U8FTC3	1-phosphatidylinositol-3-phosphate 5-kinase FAB1B-like isoform X2 OS=Capsicum annuum GN=LOC107861644 PE=4 SV=1	4.22
A0A1U8H8F6	heat shock 70 kDa protein-like OS=Capsicum annuum GN=LOC107877565 PE=3 SV=1	4.26
A0A1U8H8P5	uncharacterized protein LOC107877681 isoform X1 OS=Capsicum annuum GN=LOC107877681 PE=4 SV=1	4.31
A0A1U8FWL6	soluble starch synthase 3, chloroplastic/amyloplastic OS=Capsicum annuum GN=LOC107859587 PE=3 SV=1	4.44
A0A1U8EIB5	heat shock cognate 70 kDa protein 2 OS=Capsicum annuum GN=LOC107843340 PE=3 SV=1	4.62
A0A1U8HBR5	ABC transporter G family member 28-like OS=Capsicum annuum GN=LOC107875597 PE=4 SV=1	4.71
A0A1U8GFS5	ATP-dependent DNA helicase mph1 isoform X2 OS=Capsicum annuum GN=LOC107867972 PE=4 SV=1	4.71
A0A1U8GMJ9	uncharacterized protein LOC107867972 isoform X5 OS=Capsicum annuum GN=LOC107867972 PE=4 SV=1	4.76
A0A1U8HB16	uncharacterized protein LOC107875937 isoform X2 OS=Capsicum annuum GN=LOC107875937 PE=4 SV=1	4.81
A0A1U8F2B4	SRR1-like protein OS=Capsicum annuum GN=LOC107849738 PE=4 SV=1	4.81
A0A1U8GPD2	ATP-dependent DNA helicase mph1 isoform X1 OS=Capsicum annuum GN=LOC107867972 PE=4 SV=1	4.81
A0A1U8GF78	ATP-dependent DNA helicase mph1 isoform X4 OS=Capsicum annuum GN=LOC107867972 PE=4 SV=1	4.85
A0A1U8H4E4	uncharacterized protein LOC107875937 isoform X1 OS=Capsicum annuum GN=LOC107875937 PE=4 SV=1	4.90
A0A1U8EQE1	protein SENSITIVITY TO RED LIGHT REDUCED 1-like OS=Capsicum annuum GN=LOC107849279 PE=4 SV=1	4.95

A0A1U8GF87	ATP-dependent DNA helicase mph1 isoform X3 OS=Capsicum annuum GN=LOC107867972 PE=4 SV=1	5.00
A0A1U8H2F8	uncharacterized protein LOC107875432 isoform X2 OS=Capsicum annuum GN=LOC107875432 PE=4 SV=1	5.00
A0A1U8FTK2	uncharacterized protein LOC107861690 OS=Capsicum annuum GN=LOC107861690 PE=4 SV=1	5.05
A0A1U8G423	pleiotropic drug resistance protein 1 OS=Capsicum annuum GN=LOC107864282 PE=3 SV=1	5.10
A0A1U8H2N6	uncharacterized protein LOC107875432 isoform X1 OS=Capsicum annuum GN=LOC107875432 PE=4 SV=1	5.26
A0A1U8H2T0	heat shock cognate 70 kDa protein 1 OS=Capsicum annuum GN=LOC107875543 PE=3 SV=1	5.99
A0A1U8H6W5	uncharacterized protein LOC107876932 OS=Capsicum annuum GN=LOC107876932 PE=4 SV=1	5.99
A0A1U8F5H5	heat shock cognate 70 kDa protein 2-like OS=Capsicum annuum GN=LOC107854185 PE=3 SV=1	6.96
A0A1U8GYV9	uncharacterized protein LOC107873871 OS=Capsicum annuum GN=LOC107873871 PE=4 SV=1	7.24
A0A1U8F849	restin homolog OS=Capsicum annuum GN=LOC107851405 PE=4 SV=1	8.25
A0A1U8GMJ7	probable helicase senataxin isoform X2 OS=Capsicum annuum GN=LOC107867548 PE=4 SV=1	8.50
A0A1U8EYP3	LOW QUALITY PROTEIN: pleiotropic drug resistance protein 1-like OS=Capsicum annuum GN=LOC107851743 PE=3 SV=1	8.67
A0A1U8GKP5	probable helicase senataxin isoform X1 OS=Capsicum annuum GN=LOC107867548 PE=4 SV=1	8.67
A0A1U8EAM1	protein ROS1-like OS=Capsicum annuum GN=LOC107843637 PE=4 SV=1	9.12
A0A1U8E3E2	dnaJ homolog subfamily C member 2-like OS=Capsicum annuum GN=LOC107839368 PE=4 SV=1	9.12
A0A1U8F0E5	late blight resistance protein R1-A-like OS=Capsicum annuum GN=LOC107851572 PE=3 SV=1	9.68
A0A1U8GSM6	vinorine synthase-like OS=Capsicum annuum GN=LOC107871937 PE=4 SV=1	10.38
A0A1U8F464	uncharacterized protein LOC107850448 OS=Capsicum annuum GN=LOC107850448 PE=4 SV=1	15.18
A0A1U8GYI5	uncharacterized protein LOC107873723 isoform X2 OS=Capsicum annuum GN=LOC107873723 PE=4 SV=1	15.33
A0A1U8ED77	uncharacterized protein LOC107844446 OS=Capsicum annuum GN=LOC107844446 PE=4 SV=1	17.64
A0A1U8F0Y6	protein CHROMATIN REMODELING 8 isoform X1 OS=Capsicum annuum GN=LOC107852603 PE=4 SV=1	20.49
A0A1U8EJE6	Phospholipid-transporting ATPase OS=Capsicum annuum GN=LOC107847530 PE=3 SV=1	20.49
A0A1U8F3Q4	uncharacterized protein LOC107853531 OS=Capsicum annuum GN=LOC107853531 PE=4 SV=1	25.03

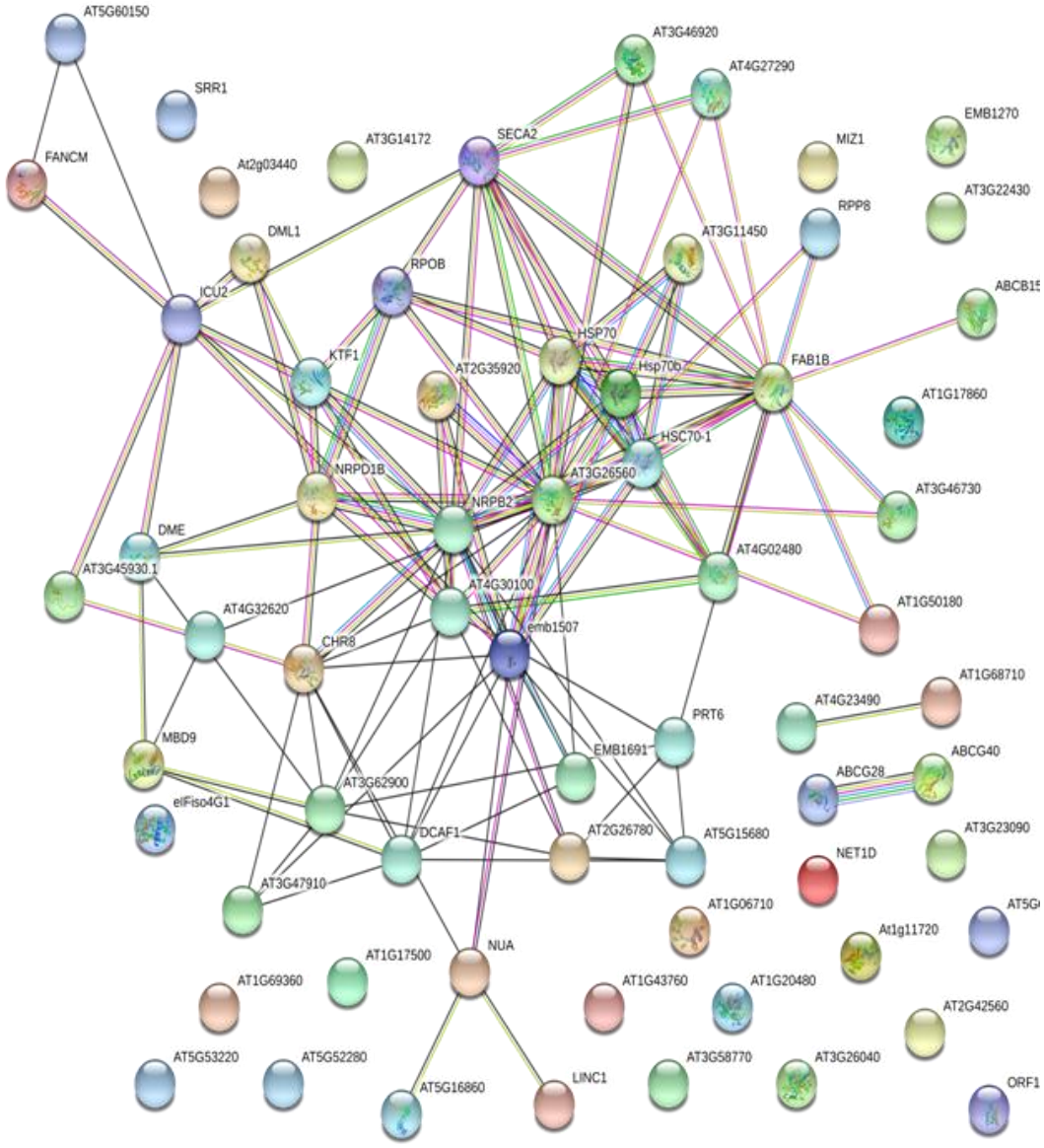

Figure 4.16 String analysis network for Drought tolerant in control (C1) Vs Drought tolerant (T1) in stress condition

polymerase subunit beta, E3 ubiquitin-protein ligase PRT6-like, vinorine synthase, and uncharacterised proteins (19 proteins).

4.4.3 Drought tolerant proteins in tolerant germplasm (T1) and susceptible germplasm (T2) under high stress condition

The comparative profile of the induced proteins in T1 Vs T2 showed total of 103 proteins from 2 to 27.94 fold change (Table 4.27). The up regulated proteins interactions were analysed using string analysis and the network is given in the figure (Fig.4.17). The string analysis showed the function enrichment of the proteins coding for the molecular functions such as ADP binding, organic cyclic compound binding, heterocyclic compound binding. Out of 103 proteins 53 up regulated proteins with above 3 fold expressions were grouped into histones, photosynthetic related, signalling, cell wall components and other proteins *viz.* auxilin-like protein, nuclear pore complex protein, nuclear-pore anchor, putative late blight resistance, WPP domain-associated protein, tetratricopeptide repeat protein, uncharacterized proteins, exocyst complex component EXO70A1-like, late blight resistance protein R1-A-like and zinc finger CCCH domain.

4.4.4 Drought responsible proteins in susceptible germplasm under control (C2) and high stress condition (T2)

Comparison on differential proteins between drought susceptible cultivar in control and stress conditions showed total 109 proteins from 2 to 33.78 fold change (Table 4.28). The up regulated proteins interactions were analysed using string analysis and the network is given in the figure (Fig.4.18). Out of 109 proteins 33 up regulated proteins with above 3 fold expressions were grouped into chromatin modified proteins, R genes, signalling and other proteins *viz.* protein TSS isoform, E3

Table 4.27 Protein profile of drought tolerant cultivar Vs drought susceptible cultivar in highest drought stress condition (T1 VsT2)

Accession	Protein Name	Fold Change
A0A1U8F1I0	Clathrin heavy chain OS=Capsicum annuum GN=LOC107852781 PE=3 SV=1	2.01
A0A1U8GN39	uncharacterized protein LOC107870610 OS=Capsicum annuum GN=LOC107870610 PE=4 SV=1	2.03
A0A1U8F6U7	pleiotropic drug resistance protein 1-like OS=Capsicum annuum GN=LOC107851073 PE=3 SV=1	2.03
A0A1U8EZZ9	uncharacterized protein LOC107852240 OS=Capsicum annuum GN=LOC107852240 PE=4 SV=1	2.08
A0A1U8E6H8	protein ALWAYS EARLY 3-like isoform X2 OS=Capsicum annuum GN=LOC107842611 PE=4 SV=1	2.10
A0A1U8FLQ0	DNA-directed RNA polymerase subunit OS=Capsicum annuum GN=LOC107856811 PE=3 SV=1	2.14
A0A1U8EV32	type I inositol polyphosphate 5-phosphatase 12-like isoform X1 OS=Capsicum annuum GN=LOC107850325 PE=4 SV=1	2.14
A0A1U8E8N4	digalactosyldiacylglycerol synthase 1, chloroplastic-like OS=Capsicum annuum GN=LOC107843714 PE=4 SV=1	2.16
A0A1U8FU17	ABC transporter G family member 36-like OS=Capsicum annuum GN=LOC107861796 PE=3 SV=1	2.16
A0A1U8H6Q1	LOW QUALITY PROTEIN: putative late blight resistance protein homolog R1A-3 OS=Capsicum annuum GN=LOC107873699 PE=3 SV=1	2.18
A0A1U8GAH8	uncharacterized protein LOC107863995 OS=Capsicum annuum GN=LOC107863995 PE=4 SV=1	2.20
A0A1U8GIY7	ABC transporter G family member 17-like OS=Capsicum annuum GN=LOC107866541 PE=4 SV=1	2.20
A0A1U8DYB4	LOW QUALITY PROTEIN: separase OS=Capsicum annuum GN=LOC107839972 PE=4 SV=1	2.25
A0A1U8EEY7	LOW QUALITY PROTEIN: endoribonuclease Dicer homolog 1 OS=Capsicum annuum GN=LOC107845244 PE=3 SV=1	2.27
A0A1U8F936	LOW QUALITY PROTEIN: putative late blight resistance protein homolog R1B-8 OS=Capsicum annuum GN=LOC107852210 PE=3 SV=1	2.27
A0A1U8GF89	uncharacterized protein LOC107865699 isoform X2 OS=Capsicum annuum GN=LOC107865699 PE=4 SV=1	2.27
A0A1U8GF84	uncharacterized protein LOC107865699 isoform X1 OS=Capsicum annuum GN=LOC107865699 PE=4 SV=1	2.27
A0A1U8GY65	putative late blight resistance protein homolog R1B-23 OS=Capsicum annuum GN=LOC107870917 PE=3 SV=1	2.29
A0A1U8GWT6	putative disease resistance protein At3g14460 isoform X1 OS=Capsicum annuum GN=LOC107871019 PE=3 SV=1	2.29
A0A1U8ESP8	type I inositol polyphosphate 5-phosphatase 12-like isoform X2 OS=Capsicum annuum GN=LOC107850325 PE=4 SV=1	2.29
A0A1U8ESX8	type I inositol polyphosphate 5-phosphatase 12-like isoform X3 OS=Capsicum annuum GN=LOC107850325 PE=4 SV=1	2.32
A0A1U8GYV5	callose synthase 9 OS=Capsicum annuum GN=LOC107874024 PE=4 SV=1	2.36

A0A1U8GQS5	pleiotropic drug resistance protein 1-like OS=Capsicum annuum GN=LOC107871249 PE=3 SV=1	2.41
A0A1U8GR43	pleiotropic drug resistance protein 1-like OS=Capsicum annuum GN=LOC107871251 PE=3 SV=1	2.41
A0A1U8FWV9	uncharacterized protein LOC107862285 OS=Capsicum annuum GN=LOC107862285 PE=4 SV=1	2.46
A0A1U8ESE6	LOW QUALITY PROTEIN: DEAD-box ATP-dependent RNA helicase 42-like OS=Capsicum annuum GN=LOC107850256 PE=4 SV=1	2.48
A0A1U8GAS0	kinesin-like protein NACK1 isoform X1 OS=Capsicum annuum GN=LOC107866914 PE=3 SV=1	2.48
A0A1U8ENK5	uncharacterized protein LOC107845197 OS=Capsicum annuum GN=LOC107845197 PE=4 SV=1	2.53
A0A1U8F009	uncharacterized protein LOC107849434 OS=Capsicum annuum GN=LOC107849434 PE=4 SV=1	2.56
A0A1U8EDU8	protein ALWAYS EARLY 3-like isoform X1 OS=Capsicum annuum GN=LOC107842611 PE=4 SV=1	2.61
A0A1U8ESM7	DEAD-box ATP-dependent RNA helicase 42-like OS=Capsicum annuum GN=LOC107850255 PE=4 SV=1	2.61
A0A1U8EUZ8	protein RNA-directed DNA methylation 3 OS=Capsicum annuum GN=LOC107850304 PE=4 SV=1	2.69
A0A1U8GK12	kinesin-like protein NACK1 isoform X2 OS=Capsicum annuum GN=LOC107866914 PE=3 SV=1	2.69
A0A1U8E9Z0	LOW QUALITY PROTEIN: probable disease resistance protein At4g27220 OS=Capsicum annuum GN=LOC107844070 PE=3 SV=1	2.69
A0A1U8EJ34	uncharacterized protein LOC107846556 OS=Capsicum annuum GN=LOC107846556 PE=4 SV=1	2.75
A0A1U8H4N6	myosin-6-like isoform X1 OS=Capsicum annuum GN=LOC107873632 PE=3 SV=1	2.77
A0A1U8GDB3	LOW QUALITY PROTEIN: putative disease resistance RPP13-like protein 1 OS=Capsicum annuum GN=LOC107867553 PE=3 SV=1	2.77
A0A1U8FUX3	uncharacterized protein At1g65710 OS=Capsicum annuum GN=LOC107859661 PE=4 SV=1	2.77
A0A1U8GC73	LOW QUALITY PROTEIN: putative late blight resistance protein homolog R1A-3 OS=Capsicum annuum GN=LOC107864462 PE=3 SV=1	2.77
A0A1U8GAW4	protein RCC2 OS=Capsicum annuum GN=LOC107866922 PE=4 SV=1	2.77
A0A1U8H6I1	myosin-6-like isoform X2 OS=Capsicum annuum GN=LOC107873632 PE=3 SV=1	2.80
A0A1U8EQM1	LOW QUALITY PROTEIN: uncharacterized protein LOC107846258 OS=Capsicum annuum GN=LOC107846258 PE=4 SV=1	2.83
A0A1U8HEU1	uncharacterized protein LOC107879373 OS=Capsicum annuum GN=LOC107879373 PE=4 SV=1	2.83
A0A1U8E3I8	LOW QUALITY PROTEIN: uncharacterized protein LOC107842468 OS=Capsicum annuum GN=LOC107842468 PE=4 SV=1	2.83
A0A1U8EQD1	LOW QUALITY PROTEIN: uncharacterized protein LOC107845648 OS=Capsicum annuum GN=LOC107845648 PE=4 SV=1	2.86
A0A1U8EW09	uncharacterized protein LOC107850987 isoform X2 OS=Capsicum annuum GN=LOC107850987 PE=4 SV=1	2.89

A0A1U8GDD9	uncharacterized protein LOC107864252 OS=Capsicum annuum GN=LOC107864252 PE=4 SV=1	2.94
A0A1U8G0F5	paired amphipathic helix protein Sin3-like 2 OS=Capsicum annuum GN=LOC107860583 PE=4 SV=1	2.94
A0A1U8EEP0	uncharacterized protein LOC107842834 OS=Capsicum annuum GN=LOC107842834 PE=4 SV=1	2.97
A0A1U8GYV3	uncharacterized protein LOC107874050 OS=Capsicum annuum GN=LOC107874050 PE=4 SV=1	3.00
A0A1U8GP73	uncharacterized protein LOC107870828 OS=Capsicum annuum GN=LOC107870828 PE=4 SV=1	3.00
A0A1U8GP03	Alpha-1,4 glucan phosphorylase OS=Capsicum annuum GN=LOC107867882 PE=3 SV=1	3.03
A0A1U8EY11	uncharacterized protein LOC107851471 OS=Capsicum annuum GN=LOC107851471 PE=4 SV=1	3.19
A0A1U8FI23	uncharacterized protein LOC107858660 OS=Capsicum annuum GN=LOC107858660 PE=4 SV=1	3.19
A0A1U8HJM6	LOW QUALITY PROTEIN: uncharacterized protein LOC107878287 OS=Capsicum annuum GN=LOC107878287 PE=4 SV=1	3.19
A0A1U8GET6	Alpha-1,4 glucan phosphorylase OS=Capsicum annuum GN=LOC107867882 PE=3 SV=1	3.22
A0A1U8FWM5	LOW QUALITY PROTEIN: uncharacterized protein LOC107860118 OS=Capsicum annuum GN=LOC107860118 PE=4 SV=1	3.22
A0A1U8EIN1	methyl-CpG-binding domain-containing protein 9 OS=Capsicum annuum GN=LOC107847389 PE=4 SV=1	3.25
A0A1U8GKA5	auxilin-like protein 1 OS=Capsicum annuum GN=LOC107866979 PE=4 SV=1	3.25
A0A1U8HBE5	callose synthase 12 OS=Capsicum annuum GN=LOC107878332 PE=4 SV=1	3.29
A0A1U8FJX1	LOW QUALITY PROTEIN: ARF guanine-nucleotide exchange factor GNOM-like OS=Capsicum annuum GN=LOC107858761 PE=4 SV=1	3.29
A0A1U8GAA5	pentatricopeptide repeat-containing protein At3g46790, chloroplastic-like OS=Capsicum annuum GN=LOC107863540 PE=4 SV=1	3.32
A0A1U8G6N1	LOW QUALITY PROTEIN: uncharacterized protein LOC107863103 OS=Capsicum annuum GN=LOC107863103 PE=4 SV=1	3.35
A0A1U8FU33	nuclear pore complex protein NUP155-like OS=Capsicum annuum GN=LOC107858930 PE=4 SV=1	3.42
A0A1U8GQZ9	LOW QUALITY PROTEIN: putative late blight resistance protein homolog R1A-3 OS=Capsicum annuum GN=LOC107871300 PE=3 SV=1	3.53
A0A1U8F6F0	kinase-interacting protein 1-like OS=Capsicum annuum GN=LOC107850966 PE=4 SV=1	3.56
A0A1U8G1D0	Formin-like protein OS=Capsicum annuum GN=LOC107863649 PE=3 SV=1	3.63
A0A1U8FJH3	WPP domain-associated protein OS=Capsicum annuum GN=LOC107859043 PE=4 SV=1	3.71
A0A1U8EVW8	uncharacterized protein LOC107850987 isoform X1 OS=Capsicum annuum GN=LOC107850987 PE=4 SV=1	3.78
A0A1U8FB74	tetratricopeptide repeat protein 7A OS=Capsicum annuum GN=LOC107855435 PE=4 SV=1	3.78

A0A1U8F2J7	nuclear-pore anchor isoform X1 OS=Capsicum annuum GN=LOC107853094 PE=4 SV=1	3.90
A0A1U8F2B0	nuclear-pore anchor isoform X2 OS=Capsicum annuum GN=LOC107853094 PE=4 SV=1	3.90
A0A1U8FUZ5	Phytochrome OS=Capsicum annuum GN=LOC107859147 PE=3 SV=1	4.35
A0A1U8FHC9	ATP synthase subunit beta OS=Capsicum annuum GN=LOC107854659 PE=3 SV=1	4.35
A0A1U8FAB7	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107855779 PE=3 SV=1	4.35
A0A1U8GR74	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107868436 PE=3 SV=1	4.39
A0A1U8FC47	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107855771 PE=3 SV=1	4.39
A0A1U8DUQ9	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107839061 PE=3 SV=1	4.48
A0A1U8FIV4	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107855768 PE=3 SV=1	4.48
A0A1U8FJ29	trichohyalin isoform X1 OS=Capsicum annuum GN=LOC107855293 PE=4 SV=1	4.48
A0A1U8EU42	Cellulose synthase OS=Capsicum annuum GN=LOC107850563 PE=3 SV=1	4.48
A0A1U8FAS3	zinc finger CCCH domain-containing protein 13 isoform X2 OS=Capsicum annuum GN=LOC107855293 PE=4 SV=1	4.48
A0A1U8FKG7	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107855776 PE=3 SV=1	4.53
A0A1U8G1X3	protein NETWORKED 1D-like OS=Capsicum annuum GN=LOC107863791 PE=4 SV=1	4.53
A0A1U8FIW4	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107855782 PE=3 SV=1	4.53
A0A1U8DSA3	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107839061 PE=3 SV=1	4.53
A0A1U8FAC3	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107855780 PE=3 SV=1	4.53
A0A1U8FAB3	Chlorophyll a-b binding protein, chloroplastic OS=Capsicum annuum GN=LOC107855767 PE=3 SV=1	4.53
A0A1U8GPV1	kinase-interacting protein 1-like OS=Capsicum annuum GN=LOC107870972 PE=4 SV=1	4.57
A0A1U8EU17	Cellulose synthase OS=Capsicum annuum GN=LOC107850544 PE=3 SV=1	4.76
A0A1U8GEA7	Cellulose synthase OS=Capsicum annuum GN=LOC107867632 PE=3 SV=1	4.85
A0A1U8FG97	exocyst complex component EXO70A1-like OS=Capsicum annuum GN=LOC107854278 PE=4 SV=1	5.00
A0A1U8GG24	cytochrome P450 71A3-like OS=Capsicum annuum GN=LOC107866002 PE=3 SV=1	6.11
A0A1U8E029	late blight resistance protein R1-A-like OS=Capsicum annuum GN=LOC107841140 PE=3 SV=1	6.17
A0A1U8E450	myosin-6-like OS=Capsicum annuum GN=LOC107842607 PE=3 SV=1	6.42
A0A1U8E6Y9	uncharacterized protein LOC107839919 OS=Capsicum annuum GN=LOC107839919 PE=4 SV=1	6.69

A0A1U8H8U2	uncharacterized protein LOC107877681 isoform X2 OS=Capsicum annuum GN=LOC107877681 PE=4 SV=1	16.44
A0A1U8H8P5	uncharacterized protein LOC107877681 isoform X1 OS=Capsicum annuum GN=LOC107877681 PE=4 SV=1	18.73
A0A1U8H3Y0	uncharacterized protein LOC107875835 isoform X1 OS=Capsicum annuum GN=LOC107875835 PE=4 SV=1	23.57
A0A1U8GC06	Histone H2A OS=Capsicum annuum GN=LOC107864401 PE=3 SV=1	25.28
A0A1U8EYL7	Histone H2A OS=Capsicum annuum GN=LOC107848286 PE=3 SV=1	25.28
A0A1U8FD88	Histone H2A OS=Capsicum annuum GN=LOC107856188 PE=3 SV=1	26.31
A0A1U8EFA0	Histone H2A OS=Capsicum annuum GN=LOC107842970 PE=3 SV=1	27.94

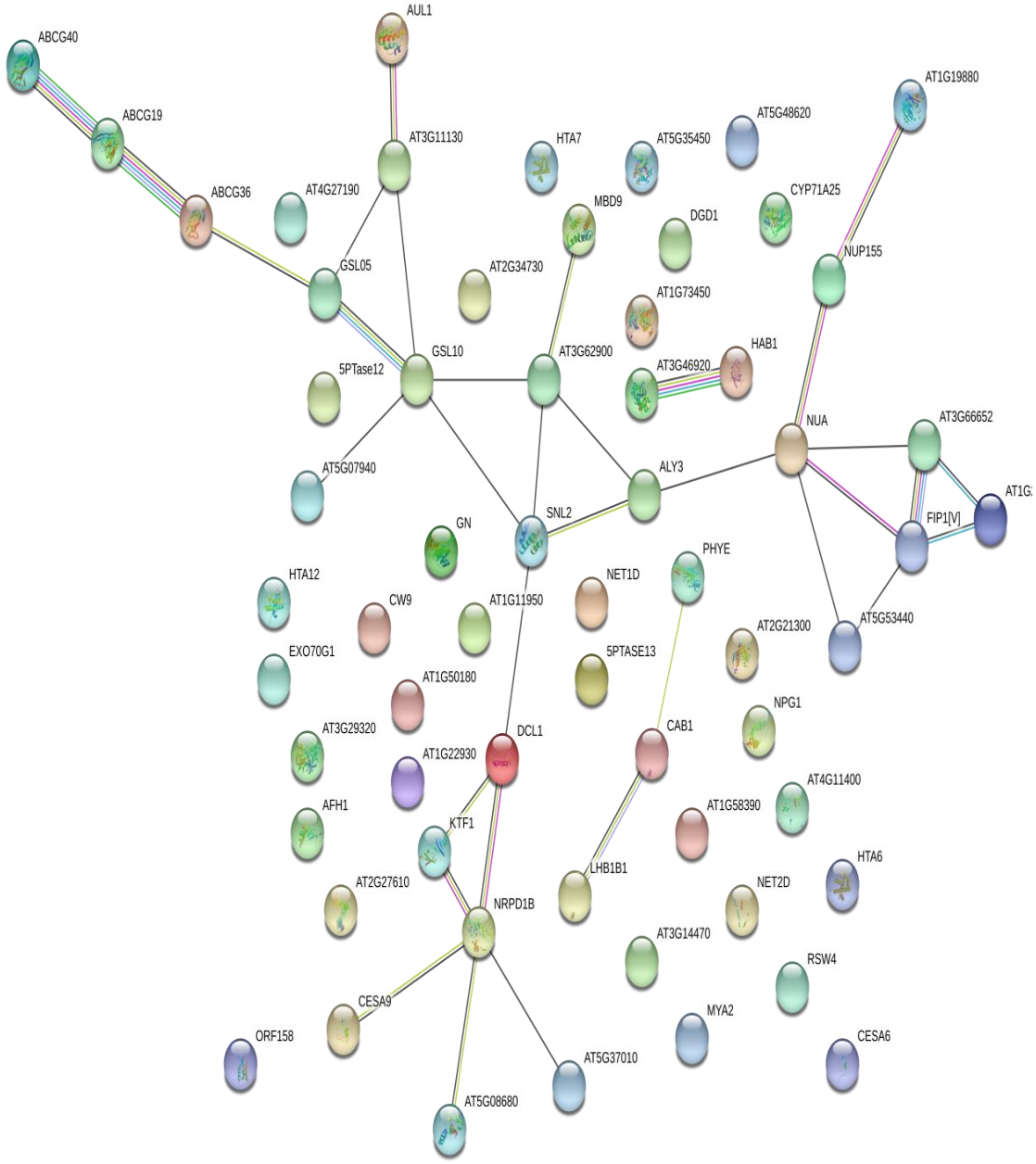

Figure 4.17 String analysis network for Drought tolerant (T1) Vs Drought susceptible (T2) in stress condition

Table 4.28 Protein profile of drought susceptible cultivar in control (C2) VS Drought susceptible cultivar in highest drought stress treatment (T2)

Accession	Protein Name	Fold change
A0A1U8E7A9	uncharacterized protein LOC107842804 isoform X2 OS=Capsicum annuum GN=LOC107842804 PE=4 SV=1	2.01
A0A1U8EVG1	uncharacterized protein LOC107850399 OS=Capsicum annuum GN=LOC107850399 PE=4 SV=1	2.01
A0A1U8H8N6	dicer-like protein 4 OS=Capsicum annuum GN=LOC107877671 PE=3 SV=1	2.03
A0A1U8H3Q5	ubiquitin carboxyl-terminal hydrolase 13-like OS=Capsicum annuum GN=LOC107875701 PE=3 SV=1	2.05
A0A1U8F243	protein FLOWERING LOCUS T-like OS=Capsicum annuum GN=LOC107849676 PE=4 SV=1	2.05
A0A1U8FQP9	histone-lysine N-methyltransferase CLF-like isoform X4 OS=Capsicum annuum GN=LOC107860273 PE=4 SV=1	2.05
A0A1U8FIK3	paired amphipathic helix protein Sin3-like 2 isoform X6 OS=Capsicum annuum GN=LOC107858800 PE=4 SV=1	2.08
A0A1U8H3Y0	uncharacterized protein LOC107875835 isoform X1 OS=Capsicum annuum GN=LOC107875835 PE=4 SV=1	2.10
A0A1U8GAH8	uncharacterized protein LOC107863995 OS=Capsicum annuum GN=LOC107863995 PE=4 SV=1	2.12
A0A1U8HAQ0	uncharacterized protein LOC107875848 OS=Capsicum annuum GN=LOC107875848 PE=4 SV=1	2.14
A0A1U8HER7	transcription initiation factor TFIID subunit 1 isoform X1 OS=Capsicum annuum GN=LOC107877504 PE=4 SV=1	2.14
A0A1U8GBL4	flavonoid 3'-monooxygenase-like OS=Capsicum annuum GN=LOC107867103 PE=3 SV=1	2.16
A0A1U8F9T2	myosin-12 isoform X2 OS=Capsicum annuum GN=LOC107855568 PE=3 SV=1	2.16
A0A1U8F9S8	myosin-12 isoform X1 OS=Capsicum annuum GN=LOC107855568 PE=3 SV=1	2.16
A0A1U8HAR8	chromatin modification-related protein EAF1 B-like OS=Capsicum annuum GN=LOC107875340 PE=4 SV=1	2.18
A0A1U8G5L1	pre-mRNA-splicing factor ATP-dependent RNA helicase DEAH1-like isoform X1 OS=Capsicum annuum GN=LOC107862291 PE=4 SV=1	2.23
A0A1U8HDF5	uncharacterized protein LOC107876877 OS=Capsicum annuum GN=LOC107876877 PE=4 SV=1	2.23
A0A1U8ET32	sister chromatid cohesion protein PDS5 homolog A isoform X2 OS=Capsicum annuum GN=LOC107850353 PE=4 SV=1	2.23
A0A1U8FID5	myosin-12 isoform X3 OS=Capsicum annuum GN=LOC107855568 PE=3 SV=1	2.25
A0A1U8FTI9	paired amphipathic helix protein Sin3-like 2 isoform X3 OS=Capsicum annuum GN=LOC107858800 PE=4 SV=1	2.25
A0A1U8GY65	putative late blight resistance protein homolog R1B-23 OS=Capsicum annuum GN=LOC107870917 PE=3 SV=1	2.27
A0A1U8DU88	protein PLASTID MOVEMENT IMPAIRED 1-RELATED 2-like OS=Capsicum annuum GN=LOC107838947 PE=4 SV=1	2.29

A0A1U8ESV8	sister chromatid cohesion protein PDS5 homolog A isoform X1 OS=Capsicum annuum GN=LOC107850353 PE=4 SV=1	2.29
A0A1U8FY25	chaperone protein ClpB3, chloroplastic OS=Capsicum annuum GN=LOC107860476 PE=3 SV=1	2.32
A0A1U8GEG6	Protein translocase subunit SecA OS=Capsicum annuum GN=LOC107867797 PE=3 SV=1	2.32
A0A1U8GBM5	geraniol 8-hydroxylase-like OS=Capsicum annuum GN=LOC107867114 PE=3 SV=1	2.34
A0A1U8FK21	paired amphipathic helix protein Sin3-like 2 isoform X4 OS=Capsicum annuum GN=LOC107858800 PE=4 SV=1	2.36
A0A1U8GD58	protein NETWORKED 2A isoform X2 OS=Capsicum annuum GN=LOC107867350 PE=4 SV=1	2.36
A0A1U8F936	LOW QUALITY PROTEIN: putative late blight resistance protein homolog R1B-8 OS=Capsicum annuum GN=LOC107852210 PE=3 SV=1	2.36
A0A1U8GLP4	protein NETWORKED 2A isoform X1 OS=Capsicum annuum GN=LOC107867350 PE=4 SV=1	2.36
A0A1U8F724	afadin- and alpha-actinin-binding protein-like isoform X1 OS=Capsicum annuum GN=LOC107854612 PE=4 SV=1	2.36
A0A1U8EDP7	uncharacterized protein LOC107842579 OS=Capsicum annuum GN=LOC107842579 PE=4 SV=1	2.39
A0A1U8FIJ8	paired amphipathic helix protein Sin3-like 2 isoform X1 OS=Capsicum annuum GN=LOC107858800 PE=4 SV=1	2.39
A0A1U8FRP9	paired amphipathic helix protein Sin3-like 2 isoform X2 OS=Capsicum annuum GN=LOC107858800 PE=4 SV=1	2.39
A0A1U8GIM9	uncharacterized protein LOC107867017 isoform X1 OS=Capsicum annuum GN=LOC107867017 PE=4 SV=1	2.39
A0A1U8FZ26	uncharacterized protein LOC107863125 OS=Capsicum annuum GN=LOC107863125 PE=4 SV=1	2.41
A0A1U8FLQ0	DNA-directed RNA polymerase subunit OS=Capsicum annuum GN=LOC107856811 PE=3 SV=1	2.41
A0A1U8F2C1	sister chromatid cohesion protein PDS5 homolog A isoform X3 OS=Capsicum annuum GN=LOC107850353 PE=4 SV=1	2.41
A0A1U8HGP9	transcription initiation factor TFIID subunit 1 isoform X2 OS=Capsicum annuum GN=LOC107877504 PE=4 SV=1	2.41
A0A1U8FAX9	putative late blight resistance protein homolog R1A-3 OS=Capsicum annuum GN=LOC107852308 PE=3 SV=1	2.41
A0A1U8ET89	uncharacterized protein LOC107850389 OS=Capsicum annuum GN=LOC107850389 PE=4 SV=1	2.41
A0A1U8GBS2	uncharacterized protein LOC107867017 isoform X2 OS=Capsicum annuum GN=LOC107867017 PE=4 SV=1	2.44
A0A1U8GBK3	flavonoid 3'-monooxygenase-like OS=Capsicum annuum GN=LOC107867094 PE=3 SV=1	2.44
A0A1U8GMI2	histone acetyltransferase HAC1-like OS=Capsicum annuum GN=LOC107870442 PE=4 SV=1	2.44
A0A1U8GUV4	villin-2 isoform X2 OS=Capsicum annuum GN=LOC107870039 PE=4 SV=1	2.44
A0A1U8FIK9	paired amphipathic helix protein Sin3-like 2 isoform X5 OS=Capsicum annuum GN=LOC107858800 PE=4 SV=1	2.46
A0A1U8EJF2	uncharacterized protein LOC107847543 isoform X1 OS=Capsicum annuum GN=LOC107847543 PE=4 SV=1	2.48
A0A1U8H9L5	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2-like OS=Capsicum annuum GN=LOC107877880	2.48

	PE=4 SV=1	
A0A1U8FZE0	uncharacterized protein LOC107863225 isoform X2 OS=Capsicum annuum GN=LOC107863225 PE=4 SV=1	2.51
A0A1U8FMI4	oxygen-evolving enhancer protein 2, chloroplastic OS=Capsicum annuum GN=LOC107856476 PE=4 SV=1	2.51
A0A1U8H3V5	uncharacterized protein LOC107873423 isoform X3 OS=Capsicum annuum GN=LOC107873423 PE=4 SV=1	2.51
A0A1U8H365	uncharacterized protein LOC107873252 OS=Capsicum annuum GN=LOC107873252 PE=4 SV=1	2.53
A0A1U8EBU5	LOW QUALITY PROTEIN: probable disease resistance protein At4g27220 OS=Capsicum annuum GN=LOC107843959 PE=3 SV=1	2.53
A0A1U8E5B9	uncharacterized protein LOC107839502 OS=Capsicum annuum GN=LOC107839502 PE=4 SV=1	2.53
A0A1U8FFL9	afadin- and alpha-actinin-binding protein-like isoform X2 OS=Capsicum annuum GN=LOC107854612 PE=4 SV=1	2.56
A0A1U8F8U8	afadin- and alpha-actinin-binding protein-like isoform X4 OS=Capsicum annuum GN=LOC107854612 PE=4 SV=1	2.56
A0A1U8GKX4	villin-2 isoform X1 OS=Capsicum annuum GN=LOC107870039 PE=4 SV=1	2.56
A0A1U8FWL6	soluble starch synthase 3, chloroplastic/amyloplastic OS=Capsicum annuum GN=LOC107859587 PE=3 SV=1	2.59
A0A1U8GWY6	uncharacterized protein LOC107873423 isoform X1 OS=Capsicum annuum GN=LOC107873423 PE=4 SV=1	2.61
A0A1U8G099	uncharacterized protein LOC107863225 isoform X1 OS=Capsicum annuum GN=LOC107863225 PE=4 SV=1	2.61
A0A1U8F714	afadin- and alpha-actinin-binding protein-like isoform X5 OS=Capsicum annuum GN=LOC107854612 PE=4 SV=1	2.66
A0A1U8FH74	afadin- and alpha-actinin-binding protein-like isoform X3 OS=Capsicum annuum GN=LOC107854612 PE=4 SV=1	2.69
A0A1U8FA13	uncharacterized protein At1g04910-like OS=Capsicum annuum GN=LOC107855642 PE=4 SV=1	2.72
A0A1U8FC62	Formin-like protein OS=Capsicum annuum GN=LOC107856334 PE=3 SV=1	2.75
B1PDJ8	Chitin binding protein OS=Capsicum annuum PE=2 SV=1	2.77
A0A1U8EJH0	uncharacterized protein LOC107847543 isoform X2 OS=Capsicum annuum GN=LOC107847543 PE=4 SV=1	2.77
Q8W2B2	Antifungal protein OS=Capsicum annuum PE=2 SV=1	2.77
A0A1U8GDA0	kinesin-like protein BC2 OS=Capsicum annuum GN=LOC107867373 PE=3 SV=1	2.80
A0A1U8G7H3	uncharacterized protein LOC107863296 OS=Capsicum annuum GN=LOC107863296 PE=4 SV=1	2.83
A0A1U8GWX8	uncharacterized protein LOC107873423 isoform X2 OS=Capsicum annuum GN=LOC107873423 PE=4 SV=1	2.86
A0A1U8H5R4	uncharacterized protein LOC107873423 isoform X4 OS=Capsicum annuum GN=LOC107873423 PE=4 SV=1	2.89
A0A1U8E924	kinesin-related protein 11-like isoform X2 OS=Capsicum annuum GN=LOC107840466 PE=3 SV=1	2.92
A0A1U8FRP5	putative ankyrin repeat protein RF_0381 OS=Capsicum annuum GN=LOC107860949 PE=4 SV=1	2.92

A0A1U8E7L7	kinesin-related protein 11-like isoform X1 OS=Capsicum annuum GN=LOC107840466 PE=3 SV=1	2.97
A0A1U8GXW1	Ubiquitinyl hydrolase 1 OS=Capsicum annuum GN=LOC107871273 PE=3 SV=1	2.97
A0A1U8FEG4	protein TSS isoform X1 OS=Capsicum annuum GN=LOC107854227 PE=4 SV=1	3.03
A0A1U8GXE0	uncharacterized protein LOC107873423 isoform X5 OS=Capsicum annuum GN=LOC107873423 PE=4 SV=1	3.06
A0A1U8GN39	uncharacterized protein LOC107870610 OS=Capsicum annuum GN=LOC107870610 PE=4 SV=1	3.13
A0A1U8GPV1	kinase-interacting protein 1-like OS=Capsicum annuum GN=LOC107870972 PE=4 SV=1	3.25
A0A1U8E4K1	pre-mRNA-splicing factor 38 OS=Capsicum annuum GN=LOC107839656 PE=4 SV=1	3.25
A0A1U8FG36	protein TSS isoform X2 OS=Capsicum annuum GN=LOC107854227 PE=4 SV=1	3.29
A0A1U8G6C5	uncharacterized protein LOC107862487 isoform X3 OS=Capsicum annuum GN=LOC107862487 PE=4 SV=1	3.32
A0A1U8F8K3	DNA ligase 1 isoform X1 OS=Capsicum annuum GN=LOC107855147 PE=4 SV=1	3.32
A0A1U8DZL1	putative late blight resistance protein homolog R1A-10 isoform X2 OS=Capsicum annuum GN=LOC107840990 PE=3 SV=1	3.35
A0A1U8EVK9	Non-specific serine/threonine protein kinase OS=Capsicum annuum GN=LOC107847581 PE=3 SV=1	3.39
A0A1U8E217	putative late blight resistance protein homolog R1A-10 isoform X1 OS=Capsicum annuum GN=LOC107840990 PE=3 SV=1	3.42
A0A1U8G0I3	Structural maintenance of chromosomes protein OS=Capsicum annuum GN=LOC107863516 PE=3 SV=1	3.42
A0A1U8DVR1	protein TSS OS=Capsicum annuum GN=LOC107839324 PE=4 SV=1	3.46
A0A1U8E3H3	E3 ubiquitin-protein ligase listerin isoform X2 OS=Capsicum annuum GN=LOC107842460 PE=4 SV=1	3.46
A0A1U8E3G0	E3 ubiquitin-protein ligase listerin isoform X3 OS=Capsicum annuum GN=LOC107842460 PE=4 SV=1	3.53
A0A1U8F8J9	DNA ligase 1 isoform X2 OS=Capsicum annuum GN=LOC107855147 PE=4 SV=1	3.56
A0A1U8E5T4	E3 ubiquitin-protein ligase listerin isoform X1 OS=Capsicum annuum GN=LOC107842460 PE=4 SV=1	3.67
A0A1U8F990	DExH-box ATP-dependent RNA helicase DExH14 isoform X1 OS=Capsicum annuum GN=LOC107855400 PE=4 SV=1	4.31
A0A1U8FI95	LOW QUALITY PROTEIN: putative late blight resistance protein homolog R1A-3 OS=Capsicum annuum GN=LOC107855523 PE=3 SV=1	4.53
A0A1U8H6Q1	LOW QUALITY PROTEIN: putative late blight resistance protein homolog R1A-3 OS=Capsicum annuum GN=LOC107873699 PE=3 SV=1	4.57
A0A1U8FHU9	DExH-box ATP-dependent RNA helicase DExH14 isoform X2 OS=Capsicum annuum GN=LOC107855400 PE=4 SV=1	4.81
A0A1U8E6D7	ABC transporter B family member 1 OS=Capsicum annuum GN=LOC107843126 PE=4 SV=1	5.47
A0A1U8E4A7	phagocyte signaling-impaired protein OS=Capsicum annuum GN=LOC107842646 PE=4 SV=1	5.93

A0A1U8G4F4	uncharacterized protein LOC107862487 isoform X2 OS=Capsicum annuum GN=LOC107862487 PE=4 SV=1	6.11
A0A1U8FWM3	uncharacterized protein LOC107862487 isoform X1 OS=Capsicum annuum GN=LOC107862487 PE=4 SV=1	6.75
A0A1U8EK97	Condensin complex subunit 1 OS=Capsicum annuum GN=LOC107843871 PE=3 SV=1	7.54
A0A1U8E8W1	alkane hydroxylase MAH1-like OS=Capsicum annuum GN=LOC107843772 PE=3 SV=1	8.00
A0A1U8E4J5	nardilysin-like isoform X1 OS=Capsicum annuum GN=LOC107842726 PE=3 SV=1	9.03
A0A1U8EEQ7	alkane hydroxylase MAH1-like OS=Capsicum annuum GN=LOC107845140 PE=3 SV=1	9.39
A0A1U8END8	alkane hydroxylase MAH1-like OS=Capsicum annuum GN=LOC107845139 PE=3 SV=1	9.49
A0A1U8EE79	nardilysin-like isoform X2 OS=Capsicum annuum GN=LOC107842726 PE=4 SV=1	9.58
A0A1U8EE49	uncharacterized protein LOC107842333 isoform X1 OS=Capsicum annuum GN=LOC107842333 PE=4 SV=1	10.70
A0A1U8E598	uncharacterized protein LOC107842333 isoform X2 OS=Capsicum annuum GN=LOC107842333 PE=4 SV=1	10.91
A0A1U8FZA6	pentatricopeptide repeat-containing protein At3g18110, chloroplastic OS=Capsicum annuum GN=LOC107862962 PE=4 SV=1	33.78

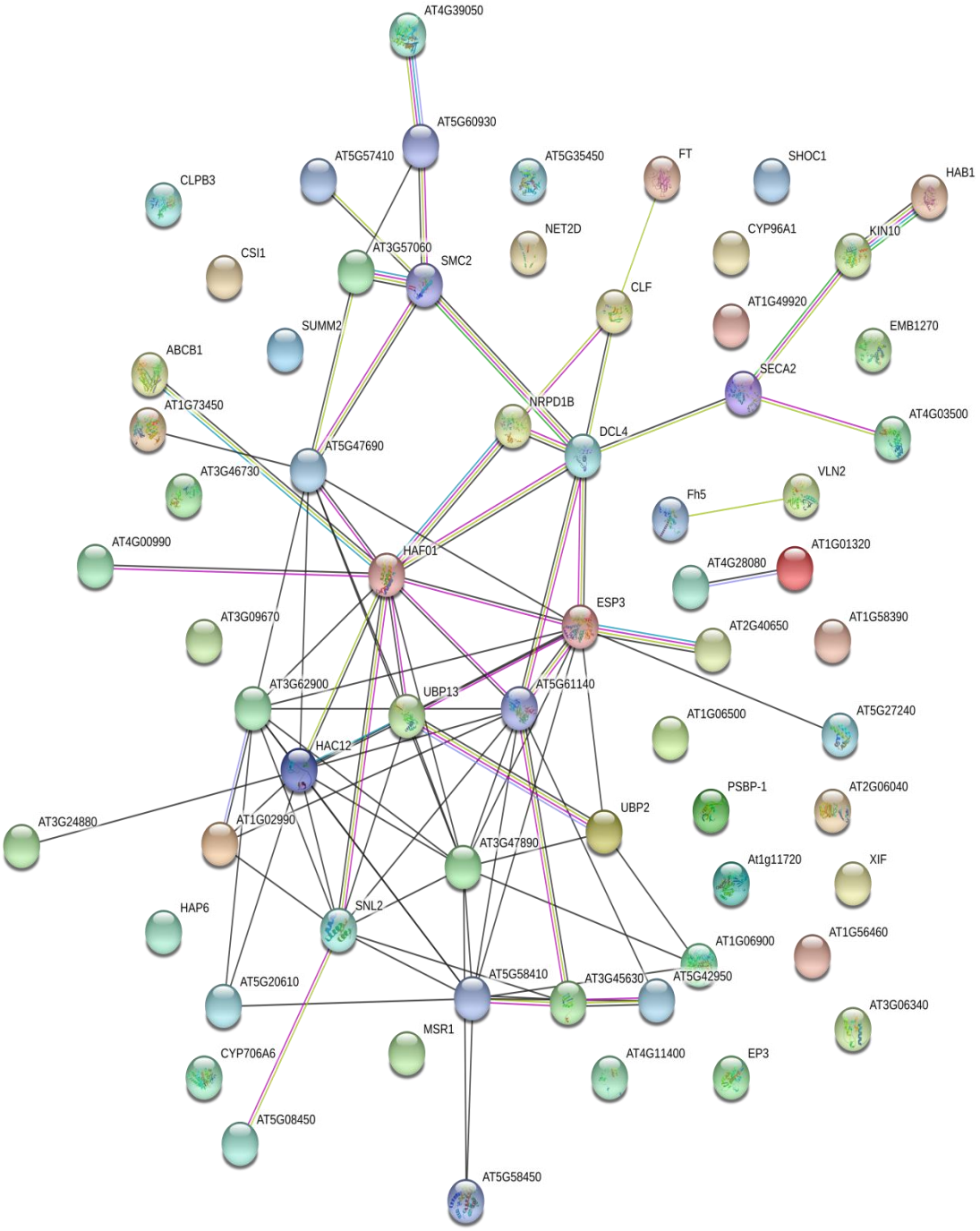

Figure 4.18 String analysis network for Drought suceptible in control (C2) Vs Drought susceptible (T2) in stress condition

ubiquitin-protein ligase listerin, phagocyte signaling-impaired protein, alkane hydroxylase, DNA ligase, ABC transporter B family member and uncharacterized protein.

4.5 Correlation of drought tolerant proteins and ionome

Ionome (Elements) are one of the essential building blocks of the living cell and are involved as cofactor, structural components of biological molecules. Mineral nutrient absorption by the plant and transport into the plant system and their function driven by many ion regulator proteins. Plants adjust to environment condtion by modifying metabolic process. Stress induced alteration of gene expression to modulate metabolic process leads to alteration of cellular protein abundance. In the present study drought tolerant proteins found through proteomics analysis and the elements possibly regulating them found through ionomics analysis are depicted in Table.4.29.

Table 4.29 Relationship of drought responsive proteins and ionome

Drought tolerant proteins	**Regulated ions**
Cell wall realted proteins	
Alpha-1,4 glucan phosphorylase,	Calcium and Copper
Callose synthase 12	
Cellulose synthase	
Trichohyalin isoform	
Protein NETWORKED	
Formin-like protein	
Photosynthesis related proteins	
Phytochrome	Magnesium, Manganese, Iron
ATP synthase subunit beta	
cytochrome P450 71A3	
Chlorophyll a-b binding protein	
pentatricopeptide repeat-containing protein	
Histone protiens	Iron
R-proteins (Resistence)	Nickel

CHAPTER-5

DISCUSSION

The results obtain during the course of experimentation as reported in the chapter 4 have been discussed in the present section. Chilli (*capsicum annuum* L.) is the important spice cum vegetable crop of solanaceae family. Drought stress is one of the major abiotic stresses which cause the drastic reduction of productivity in chilli due to reducing the number of flowers and flower and fruit drop. The basic approach for development of drought tolerant genotypes is to select germplasm containing genetic variability for high yield potential and drought adaptive traits. Therefore, estimation of the type and amount of total genetic variability associated with the target traits is equally important. Screening of cultivars is very crucial in the selection of genotype to grow successfully in stress conditions and which is based on physiological traits, morphological traits and biochemical traits. Identifying and understanding mechanisms of drought tolerance are crucial for the development of tolerant crop varieties.

5.1 Screening *Capsicum annuum* cultivars for drought tolerance

Present study had revealed that all the observed growth parameters had shown highly significant variation between treatments as well as among cultivars. In general, drought stress reduced all phenotypic expressions such as shoot length, number of leaves, number of internodes, leaf area and dry matter of the plants. Severity of drought stress was more in T5 (20%) PEG condition. In this condition, LCA-353 could survive only for seven days and this might be due to dehydration induced desiccation of the plant tissues which lead to th cellular death (Breda *et al*., 2006) as

well as stomatal closure, which occurred to prevent dehydration, caused reduction in the photosynthetic uptake of carbon ultimately leading to the plant starvation and death (McDowell *et al.,* 2008) .

5.1.1 Morpho-physiolgical characters response during PEG induced drought Stress condition

Shoot length was significantly reduced with increased drought stress in all cultivars compared to control. The mean shoot length was varying from 4.084 cm (LCA-353) to 8.94 cm (Arka Lohit). In all drought stress conditions, Arka Lohit performed better and showed least reduction of shoot length compared to control. At the highest concentration of PEG (20%), cultivars Arka Lohit, LCA-334, G4 recorded 6.5cm, 5.4cm, and 5.1cm of shoot length respectively, which were higher than other cultivars. These results indicated that Arka Lohit, LCA-334 and G4 showed better performance under drought stress as far as shoot length was concerned. Similar results reported by Kulkurni *et al.* (2007) and they observed that a drastic reduction in shoot growth in tomato with increased PEG concentration. However, the reduction was considerably lower in mutant derivatives and hybrids which were resistant. Radhouane *et al.* (2007) and Govindaraj *et al.* (2010) also found similar results in pearl millet in drought induced by polyethylene glycol.

Compared to control root length was significantly reduced with increased drought stress in all cultivars. The mean root length varied from 8.07 cm (LCA-353) to 14.24 cm (Arka Lohit). In all the stress condition it was observed that Arka Lohit showed higher mean root length and also shown least root length reductions at different water deficiency conditions, followed by LCA 334. Comparable results were found in tomato (Kulkurni *et al.* 2007) and in pearl millet (Radhouane *et al.,* 2007 and Govindaraj *et al.,* 2010). These results indicated that Arka Lohit produced more root

length in drought stress condition which was the most important character for drought tolerance. An important indication of drought tolerance is an early and rapid elongation of roots. A root system with longer root length at deeper layer is useful in extracting water in upland conditions (Narayan *et al.* 1991 and Kim *et al.*, 2001).

An increase in drought stress reduced the number of leaves. The mean value of the number of leaves varied from 4.13 (LCA-353) to 8.60 (Arka Lohit). Number of internees were also reduced by increasing drought stress. The highest number of internodes was recorded in Arka Lohit (4.67) whereas lowest no. of internodes was observed in LCA-353 (2.53). A clear difference was observed in leaf area among seven cultivars when plants were growing in the control condition (0% PEG). When comparing the effects of drought stress on leaf area, the highest leaf area was found in control, followed by T2 (5% PEG), while leaf area in T5 (20% PEG) was the least amongst survived cultivars, suggesting that severe drought stress decreased leaf area. From mean analysis, the highest leaf area was observed in Arka Lohit (47.38cm^2) and the lowest was in LCA-353 (24.15 cm^2). In the present experiment, there was a reduction of the number of leaves, number of internodes and leaf area with increasing drought stress. Similar results reported by other researchers during drought stress in chilli (Hortan *et al.*,1982 and Khan *et al.,* 2012) and cow pea (Abayomi *et al.,* 2009). Other workers had also shown that water deficit during the vegetative phase causes leaf and plant growth reductions (Kerbauy 2004). This was due to the onset of a water deficient condition reduces the plant-cell's water potential and turgor, which elevate the solutes' concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases, leading to reduction of leaf development and growth inhibition, which was reflected in shoot length, leaf area, number of leaves and number of internodes and other growth parameters (Seyed *et al*. 2012). Reduced leaf

area through the early leaf senescence profoundly reduced the photosynthetic activity of the plant. Reasonably good photosynthetic leaf area was maintained by the drought tolerant cultivars under stress condition compared to drought avoidant cultivars (Baker, 1989). Hence, based on the results of the present study, we conclude that Arka Lohit was tolerating drought stress by maintaining the photosynthetic leaf area.

When plants were subjected to drought stress, shoot dry weight decreased significantly in all treatments in all cultivars compared to control. Highest mean shoot dry weight was recorded in Arka Lohit (195.83 mg) followed by LCA 334 (174.68 mg) where as lowest dry weight was recorded in LCA-353 (95.98 mg). Similarly root dry weight was also reduced along with increasing drought stress. The mean performance depicted that highest root dry weight was recorded in Arka Lohit (100.67 mg) and the lowest was recorded in LCA-353 (35.69 mg). Among all the cultivars, Arka Lohit root dry weight reduction was least and even at the highest drought stress condition (20% PEG), root dry weight recorded was highest among all cultivars. Reduction in leaf area reduced transpiration surface (Namirembe *et al.,* 2009) and may be a drought avoidance strategy for the plants. On the other hand, the reduction of leaf area limits photosynthesis, and further decreases biomass production, this was the reason for the reduction of shoot dry weight and root dry weight along with increasing drought stress in the present experiment. Comparable results were found in tomato cultivars screened for water stress by (Yin *et al.,* 2005 and Nahar *et al.,* 2009). The results showed that root to shoot dry weight was decreased in all cultivars except LCA-334 and Arka Lohit. In LCA-334, at 0% PEG, 5% PEG, 10% PEG 15% PEG and 20% PEG drought stress conditions, the root to shoot dry weight ratio was measured at 0.43, 0.56, 0.51, 0.41 and 0.35 respectively, indicating that moderate drought condition increased root to shoot dry weight and in severe drought conditions

it decreased. Similarly, in Arka Lohit also root to shoot dry weight showed 0.49, 0.55, 0.59, 0.50 and 0.46. The result revealed that Arka Lohit till treatment 4 (15% PEG) root to shoot dry weight was increasing and again reduced in treatment 5 (20% PEG). It indicated that under moderate drought condition dry matter allocated to shoots was less compared to the roots. Plants in dry condition often decreased biomass production and divert more biomass to roots, maintaining a higher root to shoot ratio (Yin *et al.*, 2005; Martin *et al*, 2006; Villagra *et al.*, 2006 and Erice *et al.*, 2007) as an adaptation to drought resistance. In all drought stress conditions, Arka Lohit accumulated highest proline content than other cultivars, whereas the lowest was accumulated by LCA-353 followed by Dell Khursani. Genotypes which accumulate high proline concentration under stress environment are generally considered to be tolerant (Yamada *et al.* 2005; Vendruscolo *et al.*, 2007 and Abbas *et al.*, 2014). Similar type results reported in chickpea genotypes which performed better under drought showed significant levels of proline than that of genotypes which were sensitive under water deficit conditions (Kaur *et al.*, 2013). Sarma *et al.*, (2013) also reported increased proline content in leaves and roots than control in *Capsicum annuum* var. Solan Bharpur during PEG and NaCl induced stress. These results indicated that Arka Lohit tolerates drought stress. This may due to Proline reestablishes cellular redox balance by removing excess levels of ROS.

5.2 Ionomics

Based on ionome profiling of chilli cultivars, it was found that every cultivar had shown variation between treatments.

5.2.1 Principle component analysis

Since ionome profiling was a large data, principle component analysis was done. This is to draw the possible conclusions such as how cultivar behaved during particular stress conditon and how ionome profiling contributed towards stress tolerance.

Principal component analysis in combination with the ward's minimum variance method grouped the cultivars into different clusters based on contributed variables in the control condition. Since, almost all morpho-physiological characters contributed more positively to PC1, cultivars which fell in the positive direction of PC1 had better growth and development. Elements which contributed to PC1 either directly or indirectly involved for the development of these morpho-physiological characters. Therefore, Arka Lohit, Dallae khurasani and LCA-334 were the best performers under control condition since, these cultivars fell on positive direction of PC1. LCA-353 and LCA-625 cultivars may be considered as second best performers, since, they fell near to origin, followed by CA-960, Arka Mohani and G4, since, these cultivars grouped away from PC1 and in the opposite direction. Descending order of cultivars based on performance in the control condition Arka Lohit > Dali Khurasani > LCA-334 > LCA-625 > LCA-353 > Arka Mohini > G4 > CA-960.

In the 5% PEG induced drought stress condition also almost all physiological characters are more positively contributed to form PC1. Dallae khurasani Arka Lohit, and LCA-334 considered as drought tolerant cultivars since, they fell positive side of PC1. CA-960 and LCA-353 can considered as susceptible cultivars because, these cultivars fell opposite side and away from PC1. Since, LCA- 625, Arka Mohini and

G4 fell near to origin, they may be considered as moderately tolerant cultivars. Essential elements Cu, Fe, Mn, P, Ca, Mg, K and non-essential elements Na, Ni, Co, Li, Sr, Rb, Sn, and Ti contributed to PC1 and these elments may be considered drought tolerance contributing elements at 5% PEG induced drought stress level.

In the 10% PEG induced drought stress condition, almost all physiological characters were more positively contributed to PC1 and negatively contributed to PC2. Arka Lohit, Dallae khurasani and LCA-334 were considered as drought tolerant cultivars because they fell on the positive side of PC1. CA-960 and LCA-353 can be considered as susceptible to drought since, these cultivars fell where the least contribution of PC1 variables could be plotted. LCA- 625, Arka Mohini and G4 cultivars fell near to the origin and hence, they may be considered as moderately tolerant cultivars. Essential elements *viz*. K, Cu, Zn, P, Fe, Mn, Mg and non-essential elements *viz*. Rb, Mo, Na, Sn, Ba, Al and Ce contributed to PC1 and these elements may be considered as drought tolerance contributing elements in 10 % PEG induced drought stress condition.

In the 15% PEG induced drought stress condition, almost all physiological characters contributed most positively to PC1 and negatively to PC2. Since, Arka Lohit had high positive plotting from PC1 contributed variables it was considered as drought tolerant cultivar followed by LCA-334 which also fell in the positive direction of PC1. CA-960, LCA-353 and Dallae can considered as susceptible because these cultivars fell where the least contribution of PC1 and PC2 variables were plotted. Cultivars LCA- 625, Arka Mohini and G4 fell near to origin and hence, considered as moderately tolerant cultivars. The essential elements which contributed positively for PC1 were K, Cu, Mg, Ca, Fe, P, Mn and the non-essential elements

were Rb, Co, Ce, Mo, Al, Ni, Cs, Sn, Na, Sr, Cr, Ba and they may considered as drought tolerant contributing elements in 15% PEG induced drought stress condition.

In the 20% PEG induced drought stress condition LCA-353 could not survive. Thus, it was designated as highly susceptible to drought stress. Arka Lohit fell on the extreme positive side of PC1 and hence, considered as highly drought tolerant cultivar followed by LCA-334 which fell positive direction of PC1. Remaining six cultivars fell near to origin and were considered as moderate tolerant. Root to shoot dry weight, no. of internodes, no. of leaves Rb, Mg, P, Ca, Mo, Fe, Mn, Cu, Zn, K, Ba, Ga, S, Sr positively contributed PC1 and negatively contributed to PC2. These variables can considered as drought tolerance responsible variables.

Based on PCA analysis, it was found that Arka Lohit fell under the quarter where all major essential elements (Mg, Fe, Cu, Mn, Ca, P, K) and some of non essential elements (Rb, Co, Ce, Mo, Al, Ni, Cs, Sn, Na, Sr, Cr, Ba) showed positive correlation in all drought stress conditions, whereas LCA-334 fells under the quarter where maximum of these elements had positive association. Hence, Arka Lohit was found to be highly tolerant followed by LCA-334. LCA-353 could not survive in the severity of drought stress at T5 condition. Therefore, LCA-353 was found to be highly susceptible. Remaining cultivars had shown moderate drought tolerance.

5.2.2 Elements responsible for drought tolerance based on principle component analysis

Regarding elements, in 5% PEG drought stress condition Cu, Fe, Mn, P, Ca, Mg, K, Na, Ni, Co, Li, Sr, Rb, Sn, Ti were responsible for drought tolerance. In 10% PEG induced drought stress condition K, Cu, Zn, P, Fe, Mn, Mg, Rb, Mo, Na, Sn, Ba, Al, Ce were responsible for drought tolerance. In 15% PEG induced drought

stress condition K, Cu, Mg, Ca, Fe, P, Mn, Rb, Co, Ce, Mo, Al, Ni, Cs, Sn, Na, Sr, Cr, Ba were responsible for drought tolerance. In 20% PEG induced drought stress condition Mg, Fe, Cu, Mn, Ca, P, K, Ga, Ba, Mo, Rb, Zr were responsible for drought tolerance. Overall, essential elements Fe, Mg, P, K, Ca, Cu and non essential elements Rb, Mo, Al, Na, Ba, Sn were responsible for drought tolerance especially for the Arka Lohit and LCA-334.

5.2.3 Elements responsible for drought tolerance based on comparison of ionome profile in drought tolerant and susceptible cultivars.

By comparing ionome profiling of drought tolerant and susceptible cultivars in control and stress condtions it was found that the concentration of Ca, Mg, Cu, Fe, Mn, Mo, Ni, Sn increased in stress condition as compared to control in drought tolerant cultivar (Arka Lohit) whereas, in drought susceptible cultivar (LCA-353) these elements got reduced. P, Rb, Sr elements concentration increased in stress condition in both drought tolerant and susceptible cultivars as compared to control.

5.2.4 Drought tolerance response elements

Comparative analysis of ionome profile of drought tolerant and susceptible cultivars as well as principle component analysis on ionome profiles at different stress levels had yielded that Ca, Mg, Cu, Fe, P, Mo, Rb, Sr were the common elements which were responsible for drought tolerance in the tolerant germplasm. To understand the role of these elements for drought stress a discussion is made as under:

Calcium (Ca)

Calcium has a major role to protect the plant during and after the drought stress. During drought stress, rapid changes occur in cytosolic free calcium levels, this mediates the increased expression of drought responsive genes coding for proteins

and protect the plant (Knight *et al.*, 1998). Plasma membrane ATPases pump back nutrients which were lost during drought stress. Plasma membrane ATPases will be activated by calcium (Palta, 2000). Nayyar and Kaushal, (2002) found that a combination of calcium and ABA application effectively alleviated the drought stress in wheat. Upadhaya *et al*. (2011) found that foliar spray of calcium chloride reduced water stress induced changes in tea.

Iron (Fe)

Drought induced oxidative stress can be alleviated by iron nutrition because iron nutrition, increases the antioxidant enzymes such as Cu/Zn super oxide dismutase (Cu/ZnSOD), APX and POD(peroxidase). Lombardi *et al.*, (2003) reported in onion that iron supplementation improved antioxidant enzymes. Iron deficiency reduced activity of CAT (catalase) and PODs, the ubiquitous haem containing enzymes (Abadia *et al.*, 1999).

Copper (Cu)

Faize *et al.*, (2011) reported that over expression of cytosloic CuSOD/ZnSOD improved tolerance against water stress. Cu/ZnSOD reduced the effects of water stress through balancing water use efficiency and reduction of hydrogen peroxide generation and electrolyte leakage.

Magnesium (Mg)

Magnesium alleviated the water stress by increasing root growth and root surface area, that improved the uptake of water and nutrients. In addition, production and transport of photo assimilates increased, leading to mitigation of drought (Waraich *et al.,* 2011). Cakmak and Kirkby (2008) reported that foliar application of magnesium increased the yield and reduced drought stress.

Phosphorus (P)

Under drought stress, phosphorus application had the positive effects on plant growth, such as increase in root growth (Singh and Sale, 1998), leaf area and photosynthesis (Singh *et al.*, 2013), higher cell membrane stability and water relations (Singh *et al.*, 2006 and Kang *et al.*, 2014).

Molybdenum (Mo)

Molybdenum reduces the drought stress effects and increase water use efficiency due to its participation in enzymes of N metabolism, S metabolism and protein synthesis (Waraich et al., 2011).

5.3 Proteomics

5.3.1 Constitutively expressed proteins of tolerant genotype over susceptible C1 Vs C2

Comparing tolerant genotypes over susceptible the E3 ubiquitin-protein ligase, ABC transporter B family member 11-like, ABC transporter A family member 7-like, histone acetyltransferase HAC1-like, and uncharacterized proteins which constituted highly expressing proteins, were attributed to tolerant nature of Arka Lohit, since, the differential protein profiling was done for Arka Lohit and LCA-353 in the control condition without induction of drought. The high level expressions of these proteins might be the marker proteins specific for the Arka Lohit.

5.3.1.1. E3 ubiquitin-protein ligase

The upregulation of E3 ubiquitin-protein ligase from the tolerant (Arka Lohit) showed the ubiquitonation process was 3.32 fold higher than the susceptible. The E3 ubiquitin-protein ligase is a a well known protein involved in the ubiquition during

biotic and a biotic stress response (Li and Kim, 2011). Up regulation of ubiquitin-protein ligase from *Arabidopsis* often shown to enhance the drought tolerance (Liu, 2011).

5.3.1.2. ABC transport protein family

The ABC transport protein family is known as importers that confers tolerance to metals, so in *Arabidopsis* ABC-B type transport, expression was attributed to Cd and Pb tolerance (Kim 2006). In general, the specific transporters of the ABC receptors in the plants are not well known. In our study the over expression of ABC transporters A and B in Arka Lohit (tolerant genotype) suggest that the ABC transporters also play in drought tolerance.

5.3.1.3. Histone acetyltransferase HAC1-like

Histone acetyltransferase HAC1-like are the members of HAT, the enzymes catalyses Histone acetylation, that facilitates the transcription of the gene. The earlier study showed the involvement of HAT and HAT containing complexes in drought stress response (Fang *et al.,* 2013 and Yuan *et al.*, 2013). Histone variants in plants generally of two groups, the constitiutively expressed which is stable in nature and stress induced. In our study, the higher expression of the HAT in Arka Lohit clearly attributes its constitutive and stable expression to the tolerant genotype. This group of constitiutively and stably expressed HATs are the major variance in plants than the stress inducible minor variants. In *Arabidopsis* in unstressed condition H1.3 expression in guard cells was demonstrated to give the drought tolerance (Rutowicez *et al.,* 2015).

5.3.2 Drought responsive proteins for tolerance (C1 VS T1)

The proteins identified in this category were the drought inducible proteins at 30 days and are associated with the drought tolerance. The inducible proteins were belonging to Heat shock proteins/CHAPERONS, basal resistance (ROS proteins, R gene proteins), Chromatin modifying proteins, transporters and wound response proteins.

5.3.2.1. Heat Shock Proteins

The heat shock proteins are the important factors in plants to protect against the stress as they are involved in protein folding, stabilization of proteins and membranes. The increased level of heat shock proteins was demonstrated for its role in drought tolerance in barley (Kausar *et al.*, 2013). In present study, the up regulation of heat shock proteins *viz*. heat shock 70 kDa protein-like, heat shock cognate 70 kDa protein, and heat shock cognate 70 kDa protein 2 (3) showed its role in drought tolerance in Arka Lohit after 30 days of drought stress.

5.3.2.2. Basal resistance

The ROS related proteins have the scavenging cascade which assists the survival of plants under water deficient condition. The activity of this cascade increases under drought stress to meet the high energy demand of the plant (Dhindsa, 1991). The up regulation of Protein ROS1-like (2) showed the osmotic stress tolerance of Arka Lohit upon drought.

The drought stress, depressed the basal plant immunity (major R-genes), this was correlated with reduced ROS production. The basal resistance was shown to be

affected by drought to various degrees (Bidzinski *et al.*, 2016). The present study had shown that the up regulation of R-genes during drought at 30 days Arka Lohit, as fitness factor for this genotype, even the correlation of R-gene up regulation with increased ROS production was also observed**.**

5.3.2.3. Chromatin modifying proteins

Abiotic stress initiates a wide range of plant responses, including alteration of gene expression. The higher expression of chromatin modifying proteins from the present study suggested the protective role of these proteins against drought in the Arka Lohit.

In the present study, we observed proteins which are involved in signalling pathways. The SRR1 proteins were shown to regulate the expression of clock regulated genes such as CCA1 and TOC1 and also the Phytochrome B and PHYB-independent signalling pathways.

5.3.2.4. Transporters

1-phosphatidylinositol-3-phosphate 5-kinase (ATPIP5K1) is an important regulator for phosphatidylinositol signalling cascade in water stress response in plants (Thapa *et al.* 2015). In the present study, expression of this protein was induced significantly after 30 days of drought. This result corraborates with the earlier work of Thapa *et al.* (2015) in arbodopsis, where the ATPIP5K1 expression was induced during water stress. Reports suggest that the involvement 1-phosphatidylinositol-3-phosphate 5-kinase protein activates with increased the level of cytosolic Ca under stress (Mikumi *et al.*, 1998)

Phospholipid-transporting ATPase involved in transport of phospholipids these proteins involved in intracellular signalling, that are needed for normal growth and adaptation to varied growth environments (Poulsen *et al*. 2008).

Under the stress the condition plants need to maintain the concentration gradient of important metabolite by adjusting the activity of the membrane. In the present study, we identified above mentioned SRR1proteins, 1-phosphatidylinositol-3-phosphate 5-kinase, Phospholipid-transporting ATPase are the important signalling related proteins to maintain the normal cellular activity under drought condition.

The report on over expression of soluble starch synthase under heat stress correlated towards improving the grain yield in transgenic wheat (Tian *et al*. 2018) Hence, it can be correlated with increased expression of soluble starch synthase upon drought stress in chilli in the present study. The upregulation of starch synthase upon drought stress suggest the positive role of this protein.

5.3.2.5.Wound response proteins

In our study, we observed wound induced proteinase inhibitor, Proteinase inhibitor PSI-1.1, PIN-II type proteinase inhibitor family proteins. These serine proteins are demonstrated to have an important role towards enhanced defense reactions to the biotic stress. Reports had also stated that transgenic plants with PIN-II protease inhibitors with altered regulation upon dehydration stress (Tura and Lorito, 2011). This wound induced protease inhibitors in plant leaves are inducible proteins. In the present study, the up regulation of more than 50 isoforms of PIN-II type protease inhibitors suggest that it had a definite role upon drought stress at 30 days in chilli drought tolerant cultivar.

Taken together the differential pattern of drought inducible protein in treatment compare to control showed many marker proteins from the major drought responsive protein groups. Apart from this Nuclear pore Anchor (2) DNA-directed RNA polymerase subunit beta, E3 ubiquitin-protein ligase PRT6-like, vinorine synthase, uncharacterised proteins (19 Proteins) are also observed as individual proteins upon drought.

5.3.3 Drought tolerant proteins (T1 VS T2)

Comparison on T1 VS T2 found the drought induced proteins at high expressions in Arka Lohit. These induced proteins are the drought tolerance factors in this genotype. The important proteins are discussed below.

5.3.3.1. Cell wall related proteins

Drought stress directly affects the extensibility of cell wall. But the plants have a potential mechanism that control plant cell wall changes during drought. Reports suggest that the enhanced the cell wall synthesis in drought tolerant maize and brassica (Wang *et al.*, 2016) cultivar under drought stress which was associated with drought adaptation. In the present study, the proteins related to cell wall components namely Alpha-1, 4 glucan phosphorylase, Callose synthase 12, Cellulose synthase Trichohyalin isoform, Protein NETWORKED, Formin-like protein were found to be induced in Arka Lohit when compared to LCA-353. The enhanced protein expression in Arka Lohit might had enhanced the mechanical strength which minimised the water loss and cell dehydration. This suggests that in Chile the cell wall related proteins are the major players for the drought tolerance.

5.3.3.2. Histone proteins

In the present study Histones showed very high expression in T1 compare to T2. Increasing evidence showed that transcriptional reprogramming in stress-responsive gene expression, proper resource allocation to growth versus stress responses, acclimation, and long-term stress memory were at least in part attributable to changes in the chromatin organization (Chinnusamy and Zhu, 2009; Mirouze and Paszkowski, 2011 and Gutzat and Mittelsten Scheid, 2012). Histones are the major proteins of chromatin, and the dynamic association of histones and their variants can regulate gene expression (Trivedi *et al.*, 2012). In proteomic studies, several histones (e.g., H1 and H2B) appeared to cause diverse abundance changes in different plant species in response to drought stress. H2Bs were decreased in *C. albidus* (Brossa *et al.*, 2015) and *Brassica napus* (Koh *et al.*, 2015), while histone H1 was decreased in a drought-sensitive *Z. mays* cultivar, but increased in a drought-tolerant one (Benesova *et al.*, 2012). Similarly, the transcript and protein of histone H1 variant were all induced specifically in the tolerant genotype of *G. herbaceum* (Trivedi *et al.*, 2012). This suggested that the histones up regulation in T1 can be associated to the drought tolerance in chilli.

5.3.3.3. Photosynthesis related proteins

The photosynthesis related proteins, namely Phytochrome, ATP synthase subunit beta cytochrome P450 71A3, Chlorophyll a-b binding protein, pentatricopeptide repeat-containing protein were up regulated in the tolerant genotype. It is well known that photosynthetic inhibition is one of the primary detrimental effects of water stress due to stomatal closure (Patro, L., 2014). Thus, it is predictable that the universal decrease trends of photosynthesis related proteins would

be found in drought stressed leaves. Plants have developed many strategies to cope with drought stress, one important aspect is the recovery of photosynthesis. The drought increased proteins involved in photoreaction and Calvin cycle were observed in leaves. For example, light-harvesting chlorophyll a/b-binding proteins (LHCB) were increased in tolerant genotypes of *Z. mays* (Benesova *et al*.,2012) but decreased or stable in sensitive genotypes. The LHCBs have been predicted to be involved in ABA signaling partially by modulating ROS homeostasis (Xu, *et al.*, 2012). Similar to the above report the photosynthesis decreased in the susceptible genotype while it increased in T1. Hence, it is suggested that the photosynthesis machinery is one of the major player in drought adaptation in chilli.

5.3.3.4 Hormone related protein

ARF guanine-nucleotide exchange factor GNOM-like are the essential factors for transport of plant hormone auxin and also regulates the endosomal protein turnover pathways. The induced expression of the protein suggested that the auxin mediated response as one of the drought mitigating strategy in chilli. The induced proteins from Arka Lohit, the drought tolerant genotype, in the present study, are the marker proteins for the drought tolerance.

5.3.3.5. Other proteins

The other proteins include Auxilin-like protein, Nuclear pore complex protein, nuclear-pore anchor, Putative late blight resistance, WPP domain-associated protein, Tetratricopeptide repeat protein, uncharacterized proteins, Exocyst complex component EXO70A1-like, Late blight resistance protein R1-A-like and zinc finger CCCH domain.

5.3.4 Drought responsible proteins in susceptible cultivar (C2 Vs T2)

The proteins identified in this group are the drought stress induced proteins. The major groups of proteins are discussed below.

5.3.4.1. Chromatin modifying proteins

The profiling data on proteins showed induced expression of chromatin modifying proteins namely Structural maintenance of chromosomes protein, DExH-box ATP-dependent RNA helicase, DExH14 isoform, Condensin complex subunit 1, nardilysin-like isoform. When comparing the induced proteins under this group between Arka Lohit and LCA-353, LCA-353 showed less number of proteins responsible for the chromatin modification. This might be due to the susceptible nature of LCA-353.

5.3.4.2. R genes

The R-gene group with inducible expression was putative late blight resistance protein homolog R1A-10, putative late blight resistance protein homolog R1A-3. Here again the number of R-genes showing the basal resistance were less compare to Arka Lohit. The fold change expression in susceptible was only 3.42 whereas in Arka Lohit fold change was recorded as 9.68, this clearly indicates the lower level of fitness in LCA-353 compare to Arka Lohit.

5.3.4.3. Signalling related proteins

The signalling related proteins, namely kinase-interacting protein, Non specific serine or threoine protein kinase were found to be drought responsive with inducible expression in LCA-353. These results suggest that the positive regulation of signalling cascade. But when comparing the Arka Lohit protein profile, the signalling

pathway was much different than LCA-353. In Arka Lohit the coordinated signalling pathways were noticed which was absent in LCA-353.

5.3.4.4. Other proteins

The other proteins with inducible protein expression included protein TSS isoform, E3 ubiquitin-protein ligase listerin, phagocyte signaling-impaired protein, alkane hydroxylase, DNA ligase, ABC transporter B family member and uncharacterized protein

Taken together differential protein profile upon drought showed specific patteren of protein expression in susceptible compare to Arka Lohit.

5.4 Interaction of proteome and ionome

The presence and regulation of calcium in plants is important for the structural and signalling mechanism that modulates cell wall extensibility (Gilliham *et al.,* 2011). Cu is involved in cell wall metabolism in plants (Kabata-Pendias, 2010). In our study, the ionomics results showed high level of Ca and Cu in the tolerant cultivar when compared to susceptible upon drought stress. On the other hand, the proteomics profile showed the cell wall related proteins like Alpha-1,4 glucan phosphorylase, Callose synthase 12, Cellulose synthase, Trichohyalin isoform, Protein NETWORKED, Formin-like protein were up regulated. The up regulation of these proteins upon drought stress can be correlated with the high level of calcium and Cu which in turn contributed to the drought tolerance in Arka Lohit possibly through enhanced mechanical strength cell wall.

Mg is known to alleviate photo oxidative damage (Cakmek and Kirby, 2008), Mg element improves the water nutrient uptake and carbohydrates transfers (Waraich

et al,.2011). Mn has been demonstrated to avoid oxidative stress there by maintain the chlorophyll concentration (Hajiboland, 2012). Fe is the cofactors for haem proteins as well as non heam Fe proteins and known to play important role in photosynthesis.

In the present study, the ionomics data revealed that the presence of higher content Mg, Mn, Fe in the tolerant cultivar upon drought stress. This ionome enrichment can be directly correlated with the up regulation of Phytochrome, ATP synthase subunit beta cytochrome P450 71A3, Chlorophyll a-b binding protein, pentatricopeptide repeat-containing protein which related to photosynthetic mechanisms.

The element concentration of Nickel in plant tissues known to inhibit transpiration (Bazzaz *et al.*, 1974). There is evidence that nickel helps with disease tolerance in plants, although it is still unclear how this happens (Brown, 1987). We, in our presnt study, obtained higher concentration of nickel in ionome that can be correlated to the up regulation of R proteins (resistance proteins) from proteome in tolerant genotype than susceptible.

Iron directly correlated with nucleic acid methylation (Boardman, 1975). The involvement of iron nutrition in histone acetylation had been demonstrated in *Arabidopsis* (Xing *et al*,.2015). The histone acetylation level in turn regulates the gene expression. In the present study, we observed the high concentration of Fe in ionome profiling, which can be highly correlated to the up regulation of histone modifying proteins in the proteomics of tolerant genotype when compared to susceptible.

CHAPTER-6

SUMMARY AND CONCLUSION

The present investigation "**Ionome and proteome assisted characterisation of drought tolerance in chilli (*Capsicum annuum* L.)"** was carried out during 2015-16 and 2016-17 in plant growth and development laboratory of Department of Horticulture, Sikkim University. Present study carried out with the objectives of collecting and screening the germplasm of chilli for drought tolerance, Ionome and protein profiling of tolerant and susceptible cultivars and correlation of ions and protein profiles and their significance towards the tolerance to drought.

The analysis of variance indicated highly significant differences for all the growth parameters between treatments as well as among chilli cultivars. It was observed that during screening of chilli cultivars all morpho-physiological characters were reduced along with increased drought stress whereas biochemical parameter proline was increasing along with drought stress increase. In severe drought stress (T5) condition, cultivar LCA-353 could survive only for seven days. Based on mean performance of all growth parameters, it was observed that Arka Lohit showed better performance among the cultivars followed by LCA-334. Among all the cultivars Arka Lohit showed high proline content which was produced under stress condition to stabilize the ROS. This cultivar also exhibited one of the drought adaptation characters i.e. high root to shoot dry weight.

Based on ionome profiling of chilli cultivars, it was found that every cultivar had shown variation between treatments. From principle component analysis it was found that almost all morpho-physiological characters had more positive association with major essential elements (Fe, Mg, P, K, Ca, Cu) and with some of non essential

elements (Rb, Mo, Al, Na, Ba, Sn) in all stress conditions. Since, Arka Lohit fell under the quarter where those all characters had positive correlation in all treatments, it can be considered as drought tolerant cultivar.

Based on comparing of ionome profiling of drought tolerant and susceptible cultivars it was found that concentration of Ca, Mg, Cu, Fe, Mn, Mo, Ni, Sn increased in stress condition as compared to control in drought tolerant cultivar (Arka Lohit) whereas, in drought susceptible cultivar (LCA-353) these elements got reduced. P, Rb, Sr elements concentration increased in stress condition in both drought tolerant and susceptible cultivars as compared to control.

Comparative analysis of ionome profile of drought tolerant and susceptible cultivars as well as principle component analysis on ionome profiles at different stress levels had yielded that Ca, Mg, Cu, Fe, P, Mo, Rb, Sr were the common elements which were responsible for drought tolerance in the tolerant cultivar.

From label free protein quantification and comparative protein profiling of drought tolerant and susceptible cultivars. It was found that cell wall related proteins, histone proteins, photosynthesis related proteins, hormone related protein, other proteins like auxilin-like protein, nuclear pore complex protein, nuclear-pore anchor, putative late blight resistance, WPP domain-associated protein, tetratricopeptide repeat protein, uncharacterized proteins, Exocyst complex component EXO70A1-like, late blight resistance protein R1-A-like and zinc finger CCCH domain were responsible for tolerance in Arka Lohit.

From drought tolerant proteins and ionome interaction, possibly regulating elements to drought tolerant proteins were found. Cell wall related proteins regulated by Ca and Cu. Up regulation of **p**hytochrome, ATP synthase subunit beta, cytochrome

P450 71A3, chlorophyll a-b binding protein, pentatricopeptide repeat-containing protein which related to photosynthetic mechanisms were directly correlated with Mg, Mn, and Fe. Iron also correlated with histone modifying proteins which increased in tolerant cultivar compare to susceptible.

Based on the results of the present investigation the following conclusions could be drawn

- Based on *in vitro* screening of *Capsicum* cultivars, it was found that Arka Lohit was drought tolerant cultivar among collected cultivars and LCA-353 is a drought susceptible cultivar.
- From ionome profiling of drought tolerant and susceptible cultivars and PCA analysis it was found that Ca, Mg, Cu, Fe, P, Mo, Rb, Sr were responsible for drought tolerance of Arka Lohit and LCA-334.
- Chilli drought responsive proteins were found from comparative protein profiling of drought tolerant and susceptible cultivars *viz.* cell wall related proteins, histone proteins, photosynthesis related proteins, hormone related protein, other proteins like auxilin-like protein, nuclear pore complex protein, nuclear-pore anchor, putative late blight resistance, WPP domain-associated protein, tetratricopeptide repeat protein, uncharacterized proteins, exocyst complex component EXO70A1-like, late blight resistance protein R1-A-like and zinc finger CCCH domain.
- Based on correlation of ion and protein profiles, we could correlate drought responsive proteins with essential elements especially of Ca, Cu, Mn, Mg and Fe. However, as there was no previous study on the role of non essential elements towards drought tolerance, their role has to be proved by further research. Especially through the specific element mutant studies.

CHAPTER-7

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Research Article

In Vitro Screening of Chilli (*Capsicum Annuum* L.) Cultivars for Drought Tolerance

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Abstract

Chilli (*capsicum annuum* L.) is an important vegetable crop and its area under production is limited by water scarcity. An effort was made to screen eight cultivars of *Capsicum annuum* through hydroponics under controlled conditions using polyethylene glycol (PEG) with five concentrations (0%, 5%, 10%, 15% and 20%). Important growth parameters like shoot length, root length, No. of leaves, No. of internodes, leaf area, shoot dry weight, root dry weight, root to shoot dry weight and proline were observed. Evaluated growth parameters resulted from PEG imposed drought conditions revealed the variability in drought tolerance among *Capsicum annuum* cultivars. Among eight cultivars Arka Lohit showed significant increase in proline accumulation and root to shoot dry weight. These results suggested that Arka Lohit may consider as drought tolerate cultivar.

Keywords: *Capsicum annuum*, Drought Stress, Poly ethylene glycol, Proline

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Introduction

Chilli (*Capsicum annuum* L.), the green or the dried ripe fruits of pungent forms of Capsicum species, is one of the important members of the family Solanaceae. "This cultivated species has its unique place in the diet as a vegetable cum spice crop [1]". "Chilli is the largest spice item exported from India it occupies first position in terms of value. During 2015-16, chilli exported 24.21 per cent by value of the total exports of spices from India [2]". In global market, India has the highest share of 25%, followed by China with 24%. Though India has the substantial share in the world hectarage under chilli crop, the productivity (1.74 t/ha) is low when compared to the other hot pepper growing countries like Korea and Indonesia where it ranges from 2-3 t/ha. In India production of dried chillies is 1605000 MT from 760000 ha area and green chillies 678000 MT from 43000 ha area (NHB 2014-15). The main reason for low productivity is the majority of chilli cultivating area (~ 50%) is under rainfed conditions [3]. Drought is one of the major abiotic stresses which results in significant reduction in morphological traits such as plant height, plant spread and dry matter accumulation [3-6] affecting the physiological process, thereby causing considerable economic yield loss in peppers [7-12]. Genetic variability within a species is a valuable tool for screening and breeding for drought tolerance.

"Field experiments related to water stress have been difficult to handle due to significant environmental or drought interactions with other abiotic stresses [13]". "An alternative approach is to induce water stress through polyethylene glycol (PEG) solutions for the screening of the germplasm [14-17]". "Polyethylene glycol with the molecular mass of 6000 and above is non-ionic, water soluble polymer which is not expected to penetrate intact plant tissues. This solution interferes with the roots to absorb water due to the reduction of osmotic potential [18] and [19]". This synthetically created water-stress environment is used to provide the opportunity in selecting superior genotypes. On the basis of these facts, the present attempt was made to categorize chilli germplasm against drought stress to select suitable cultivars for drought tolerance.

Materials and Methods

This study was conducted in Growth and Development laboratory, Department of Horticulture, Sikkim University, Gangtok, India from 2015 to 2016. The Experimental material comprised of eight cultivars of *Capsicum annuum*, out of which, Five cultivars (LCA-334, LCA-353, G4, LCA-625, CA-960) were collected from Regional Agriculture Station, Lam farm, Guntur, Andhra Pradesh and two cultivars (Arka Lohit and Arka Mohini) from Indian Institute of Horticulture Research, Bangalore, India and one cultivar (Dallae Khursani) was collected from Sikkim. The seeds were sterilized with 70% ethanol for 1 min., followed by soaking in 0.1% $HgCl_2$ for 3 min. and thoroughly washed

with sterile distilled water for three times. Seeds were germinated in perlite media by using protrays and seedlings were transferred at the age of 14 days into a hydroponic system where, trays were filled with modified Hoagland's nutrient solution containing different concentrations of PEG-6000 *viz.* 0%, 5%, 10% 15% and 20% for imposing drought conditions. The roots of seedlings were directly submerged in aerated growth solution and the shoots were supported to grow above the solution. Solution was changed once in every 7 days. Plants of control treatment were maintained in Hoagland's nutrient solution for same period of time and aerated throughout the duration of the experiment. Whole hydroponic culture system was maintained under optimum culture conditions at 16 hours photoperiod (70 μ mol M^{-2} s^{-1}) at 28^{o} C temperature. After 30 days of treatment, measurements were recorded at five different stress levels for growth parameters like shoot length root length, No. of leaves, No. of internodes, leaf area, shoot dry weight, root dry weight and root to shoot dry weight. Shoot length was measured with help of meter scale and leaf area was measured using leaf area meter (model: 211, Systronics, India). For calculating fresh and dry weight, gravimetry was used. Fresh weight was measured immediately after removal from hydroponics and dry weight was recorded after plants were dried at 70°C for 72h in hot air oven. Proline was estimated spectrophotometrically following the method of [20]. The leaves weighing 250 mg were homogenized with 3 % sulphosalicyclic acid. The homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant collected. 2ml supernatant was reacted with 2 ml of freshly prepared ninhydrin (1.25 g of ninhydrin dissolved in a mixture of 30 ml glacial acetic acid and 20 ml of 6 molar orthophosphoric acid with warming and stirring) and 2 ml of glacial acetic acid in a test tube and then was kept in a boiling water bath at 1000C for 1 hour. The reaction was terminated in an ice bath and then shifted to room temperature. Thereafter, the reaction mixture was extracted with 4 ml of toluene, mixed vigorously with test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from aqueous phase and absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from the calibration curve. The experiment was designed in factorial completely randomized design with two factors. The first factor was the cultivars and the second one was the external water stress treatments. Data were analysed with ANOVA, and means were separated by least significance difference (LSD) using $P < 0.05$.

Results and discussion

Present study had revealed that all the observed growth parameters had shown highly significant variation between treatments as well as among cultivars (**Table 1**), (**Figure 10**). Drought stress affects most of the functions of plant growth, this effect depends on the level of drought stress, length of time to which plants subjected to water stress and genotypes of the plant species. In general drought stress reduced all phenotypic expressions such as shoot length, number of leaves, number of internodes, leaf area and dry matter of the plants. Similar type results were observed in the present experiment where all growth parameters were negatively affected by water deficient. Severity of drought stress was more in T5 (20%) PEG condition. In this condition LCA-353 could survive only seven days, this might be due to dehydration induced desiccation of the plant tissues lead to cellular death [21] or stomatal closer to prevent dehydration causes photosynthetic uptake of carbon to diminish and the plant starves as a result of continued metabolic demand for carbohydrates [22] leading to plant death.

Table 1 Mean squares of 8 chilli cultivars for various plant traits under control and PEG stress conditions

Characters	Cultivars (G)	treatment(T)	Interaction (GxT)	Error
D.F	7	4	28	78
Shoot length	40.311**	197.15**	2.2037**	0.6914
Root length	62.0109**	1112.8**	3.68002**	1.20352
No. of leaves	41.729**	129.28**	2.9548**	1.5536
No.of Internodes	8.2655**	49.304**	0.8994**	0.4085
Leaf area	964.51**	23383**	160.62**	60.978
Shoot dry weight	17956**	275995**	2144.7**	432.31
Root dry weight	7329.2**	59106**	634.65**	118.22
Root to shoot dry weight	0.094645**	0.139446**	0.010313**	0.002021
Proline	120162.9**	995877.1**	44490.21**	806.4331

Shoot length was significantly reduced with increased drought stress in all cultivars compared to control (**Figure 1**). The mean shoot length was varied from 4.084cm (LCA-353) to 8.94cm (Arka Lohit). In all drought stress conditions, Arka Lohit performed better and showed least reduction of shoot length. At highest concentration of PEG (20%), cultivars Arka Lohit, LCA334, G4 recorded 6.5cm, 5.4cm, and 5.1cm of shoot length respectively, which were higher than other cultivars. These results indicate that Arka Lohit, LCA334 and G4 showed better performance

under drought stress as far as shoot length was concerned. Similar results reported by [15] and they observed that drastic reduction in shoot growth in tomato with increased PEG concentration, which was considerably lower in mutant derivatives and hybrid which were resistant [23] and [24] also found similar results in pearl millet in drought induced by polyethylene glycol.

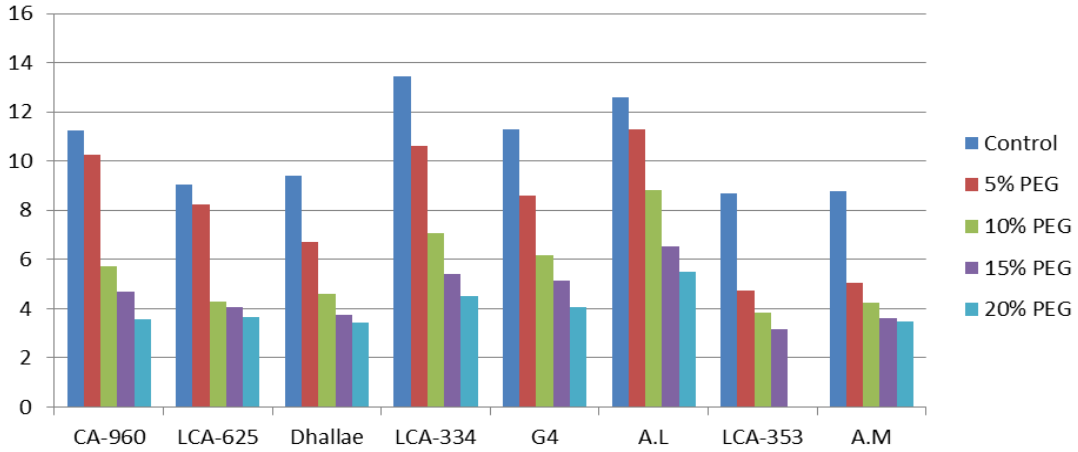

Figure 1 Shoot Length (cm) of different genotypes at different concentration of PEG

In the present experiment, compared to control root length was significantly reduced with increased drought stress in all cultivars (**Figure 2**). The mean root length was varied from 8.07 cm (LCA-353) to 14.24 cm (Arka Lohit). In all the stress condition it was observed that Arka Lohit showed higher mean root length and also shown least root length reduction at different water deficient conditions, followed by LCA 334. Comparable results found in tomato by [15] and in pearl millet by [23] and [24]. These results indicated that Arka Lohit produced more root length in drought stress condition which was most important character for drought tolerance. "Early and rapid elongation of root was important indication of drought tolerance. A root system with longer root length at deeper layer is useful in extracting water in upland conditions [25] and [26]".

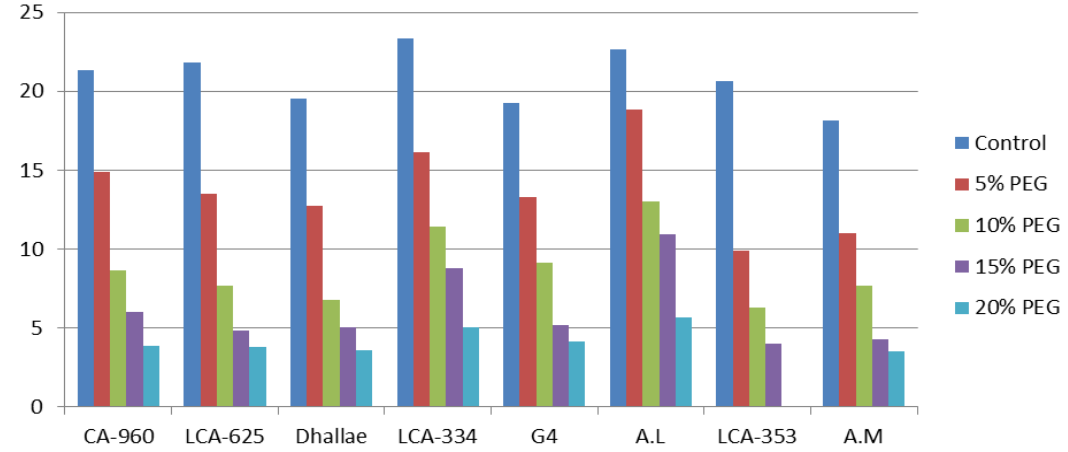

Figure 2 Root Length (cm) of different genotypes at different concentration of PEG

An increase in drought stress reduced the number of leaves (**Figure 3**). Mean value of the number of leaves varied from 4.13 (LCA-353) to 8.60 (Arka Lohit). Arka Lohit (8.60), LCA 334 (8.07), G4 (7.33) were statistically at par with each other. Number of Internodes was also reduced by increasing drought stress. The highest number of internodes was recorded in Arka Lohit (4.67) where as lowest No. of Internodes was observed in LCA-353 (2.53) (**Figure 4**). A clear difference was observed in leaf area among seven cultivars when plants were growing in control condition (0% PEG). When comparing the effects of drought stress on leaf area, the highest leaf area was found in control, followed by T2 (5% PEG), while leaf area of plants of T5 (20% PEG) had the least leaf area among survived cultivars, suggesting that severe drought stress decreased leaf area. From mean analysis, the highest leaf area was observed in Arka Lohit (47.38cm^2) and lowest was in LCA-353 (24.15 cm^2) (**Figure 5**). In the present experiment there was reduction of number of leaves, number of internodes and leaf area with increasing drought stress. Similar

results reported by other researchers during drought stress, chilli [27] and [28] cow pea [29]. Other workers had also shown that water deficit during the vegetative phase causes leaf and plant growth reductions [30]. This was due to onset of water deficient condition reduces the plant-cell's water potential and turgor, which elevate the solutes' concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases, leading to reduction of leaf development and growth inhibition, which was reflected in shoot length, leaf area, number of leaves and number of internodes and other growth parameters [31]. Reduced leaf area through the early leaf senescence profoundly reduces the photosynthetic activity of the plant. Drought-tolerant cultivars maintain reasonable photosynthetic leaf area under stress comparing to drought-avoidant cultivars [32]. Hence we conclude that Arka Lohit was tolerating drought stress.

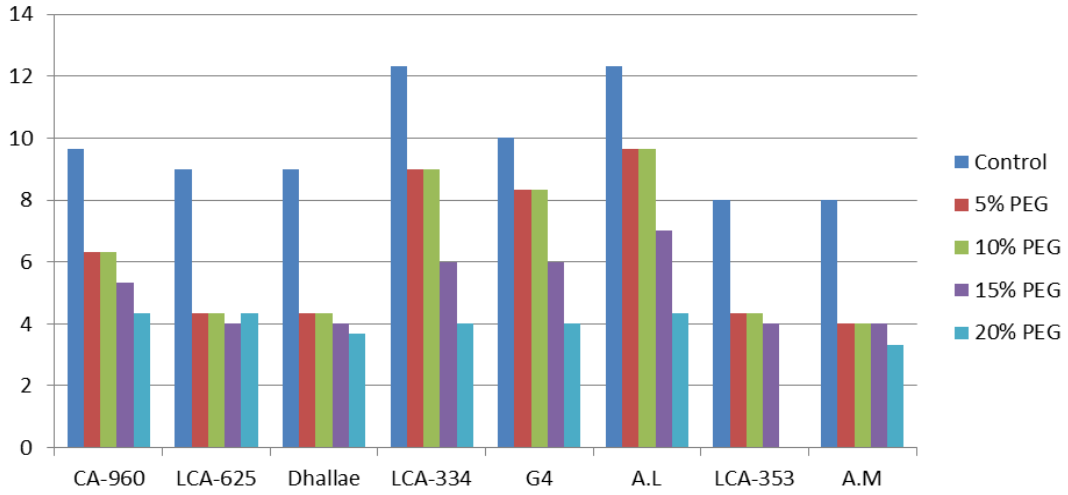

Figure 3 No. of Leaves different genotypes at different concentration of PEG

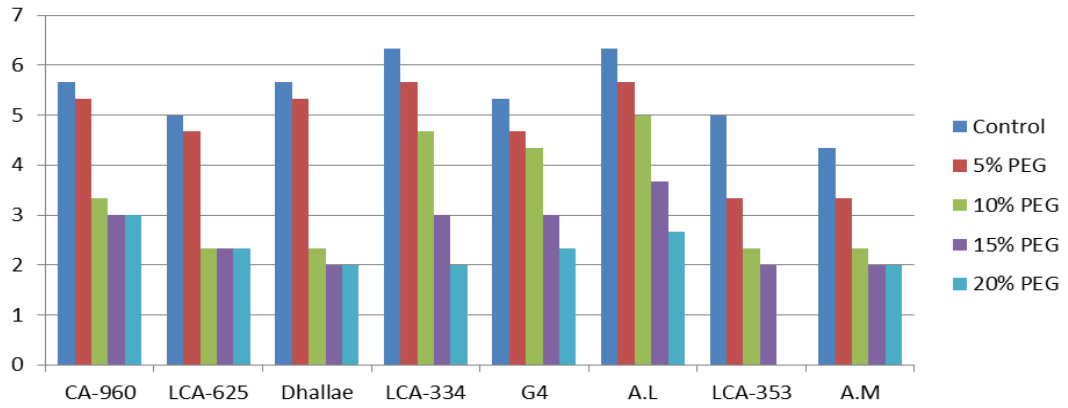

Figure 3 No. of Leaves different genotypes at different concentration of PEG

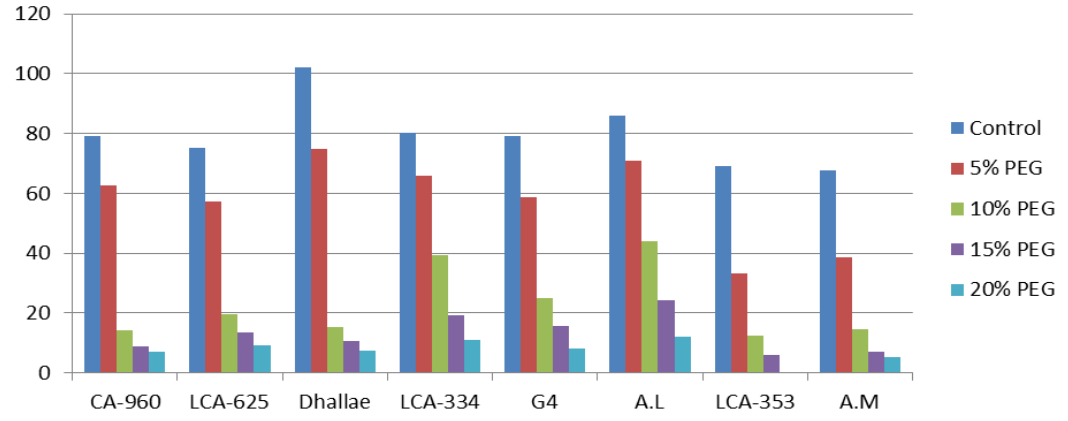

Figure 5 Leaf area (cm^2) different genotypes at different concentration of PEG

When plants were subjected to drought stress, shoot dry weight decreased significantly in all treatments in all cultivars compared to control. Highest shoot dry weight was recorded in Arka Lohit (195.83 mg) followed by LCA 334 (174.68 mg) where as lowest dry weight was recorded in LCA-353 (95.98mg) (**Figure 6**). Similarly root dry weight was also reduced along with increasing drought stress. Highest root dry weight was recorded in Arka Lohit (100.67 mg) and lowest was recorded in LCA-353 (35.69 mg) (**Figure 7**). Among all cultivars, Arka Lohit root dry weight reduction was least and even at highest drought stress condition (20% PEG), root dry weight was recorded highest among all cultivars. Reduction leaf area results in reduced transpiration surface [33] and may be a drought avoidance strategy for the plants. On the other hand, the reduction of leaf area limits photosynthesis, and further decreases biomass production, this was the reason for the reduction of shoot dry weight and root dry weight along with increasing drought stress in this experiment. Comparable results found in tomato cultivars screening under Water Stress by [34] and [15].

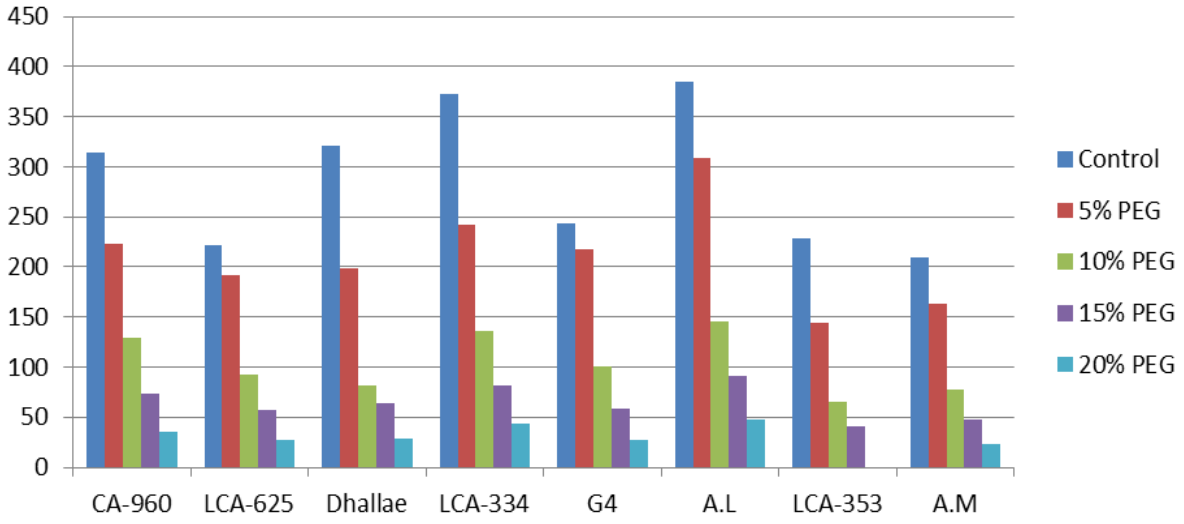

Figure 6 Shoot dry weight (mg) different genotypes at different concentration of PEG

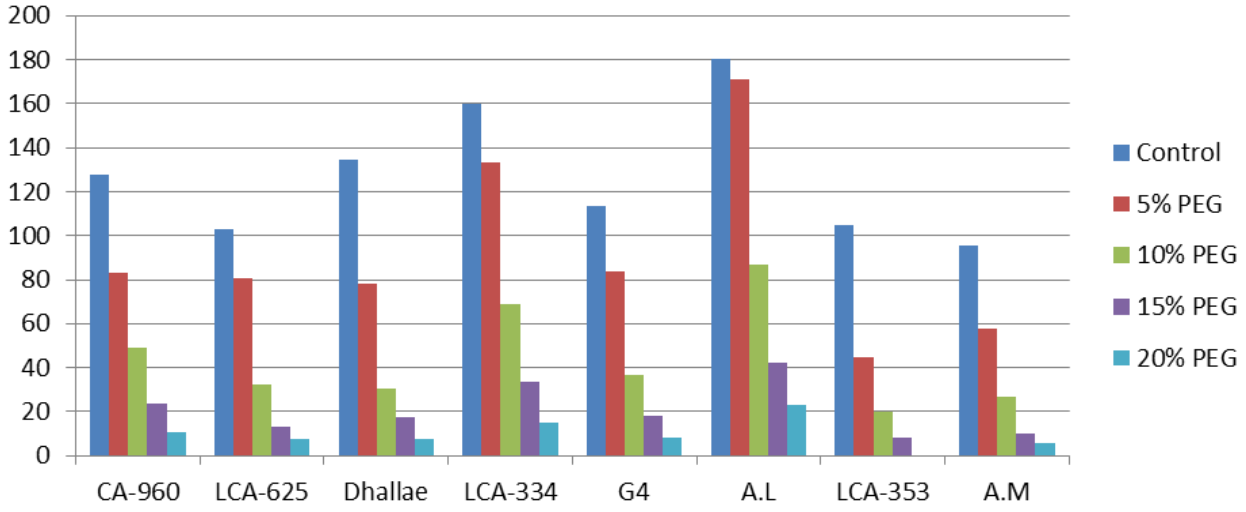

Figure 7 Root dry weight (mg) different genotypes at different concentration of PEG

Root to shoot dry weight estimates the distribution of dry matter between the root and shoot systems and it is a good indicator for effect on roots and shoot dry weight. The results (**Figure 8**) showed that root to shoot dry weight was decreased in all cultivars except LCA-334 and Arka Lohit. In LCA-334, 0% PEG, 5% PEG, 10% PEG 15% PEG and 20% PEG drought stress conditions; root to shoot dry weight ratio was measured at 0.43, 0.56, 0.51, 0.41 and 0.35 respectively, indicating that moderate drought condition increased root to shoot dry weight and in severe drought conditions it decreased. Similarly, in Arka Lohit also root to shoot dry weight showed 0.49, 0.55, 0.59, 0.50 and 0.46. This result revealed that Arka Lohit till treatment 4 (15% PEG) root to shoot dry weight was increasing and again

reduced in treatment 5 (20% PEG). It indicated that under moderate drought condition dry matter allocated to shoots was less compared to roots. Plants in dry condition often decreased biomass production and contribute more biomass to roots, maintaining a higher root to shoot ratio [35-38] as an adaptation to drought resistance.

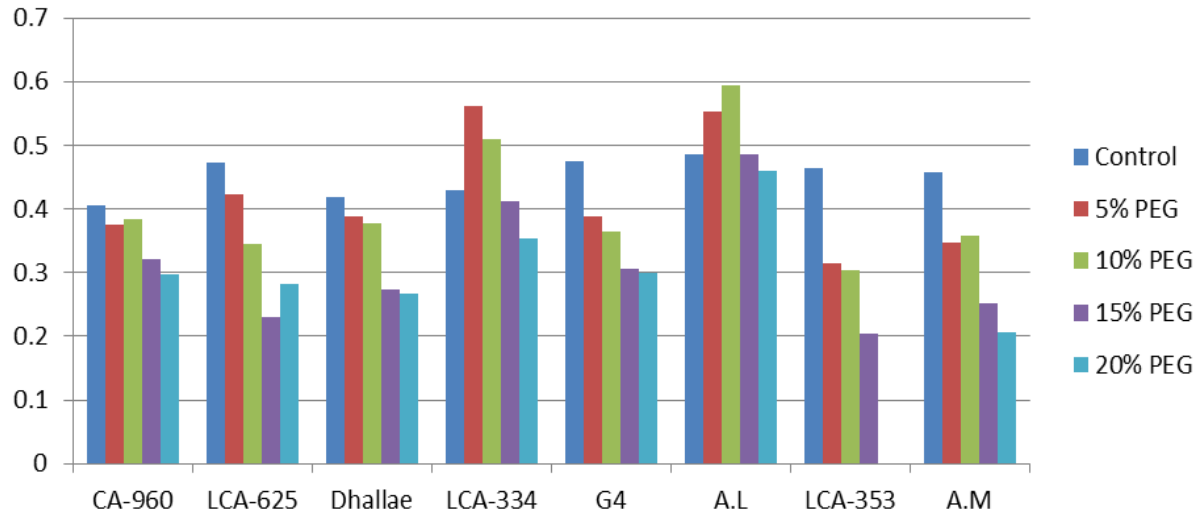

Figure 8 Root to shoot dry weight different genotypes at different concentration of PEG

As proline accumulation is a common response of plants to drought, Proline has been estimated in the present study. The present experiment revealed that increased accumulation of proline has been observed in all the cultivars with increased PEG concentration. The mean proline accumulation was varied from 229 µg g–1FW (LCA-353) to 519 µg g–1FW (Arka Lohit) (**Figure 9**). In all drought stress conditions, Arka Lohit accumulated highest proline content than other cultivars whereas lowest was LCA-353 followed by Dalle Khursani. Genotypes which accumulate high proline concentration under stress environment are generally considered to be tolerant [39-41]. Similar type results reported by [42] in chickpea genotypes which performed better under drought showed significant levels of proline than that of genotypes which were sensitive under water deficit conditions. [43] also reported increased proline content in leaves and roots than control in Capsicum annuum Solan Bharpur during PEG and NaCl induced stress. These results indicated that Arka Lohit tolerates drought stress. This may due to Proline re-establishes cellular redox balance by removing excess levels of ROS

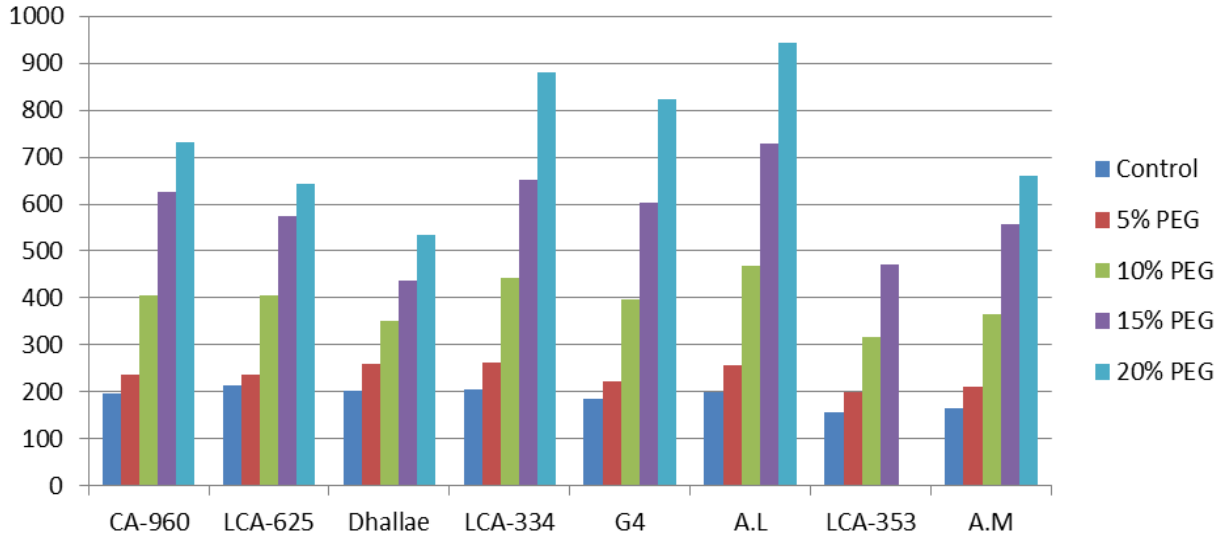

Figure 9 Proline (µg g–1FW) different genotypes at different concentration of PEG

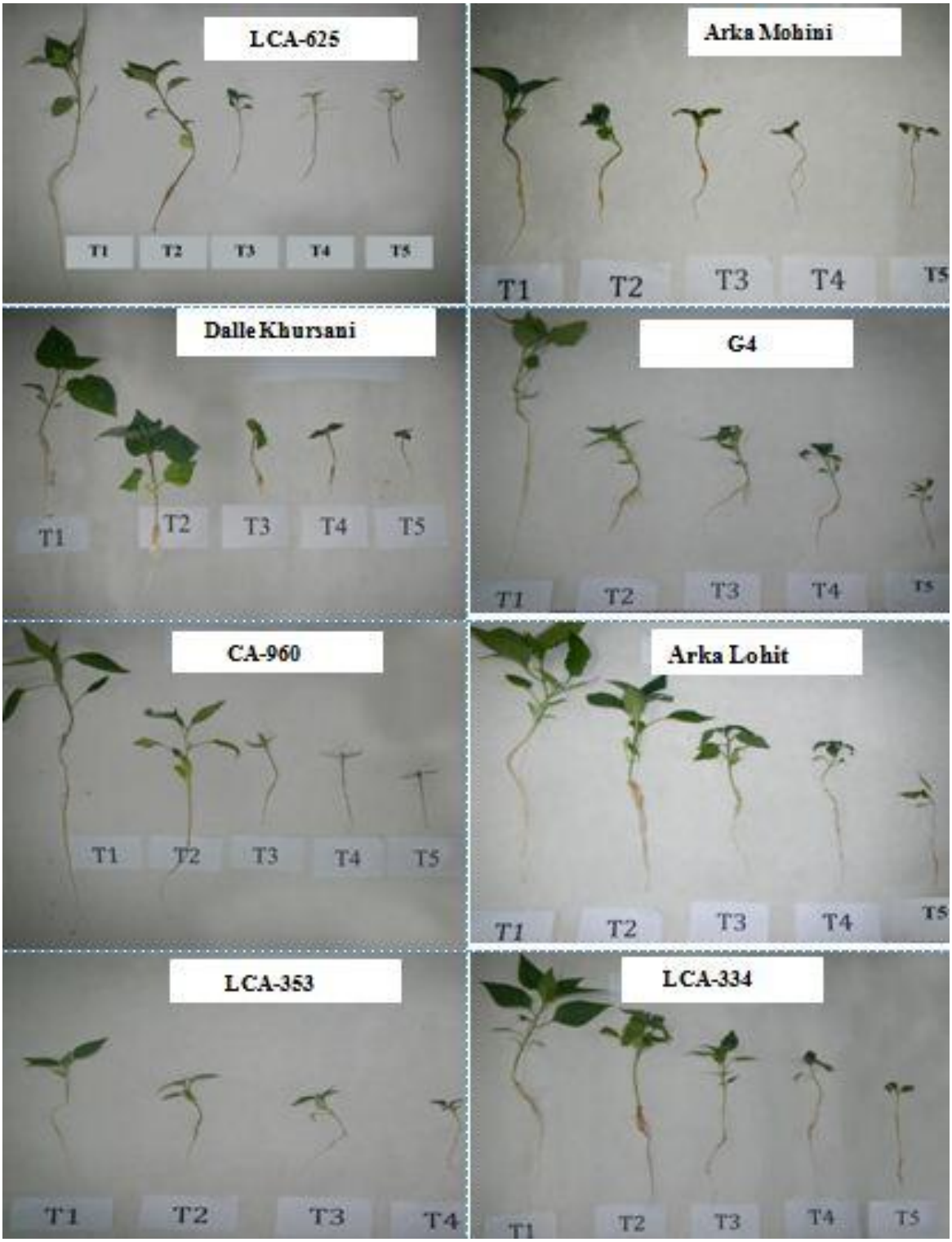

Figure 10 Samples of different chilli cultivars under different drought stress in the study (T1=control 0%PEG, T2=5%PEG, T3=10%PEG, T4=15%PEG, T5=20%PEG)

Conclusion

From the present experiment it has been concluded that performance of chilli cultivars subjected to different levels of stress showed significant differences **(Figure 10)** in all studied traits signifying the importance of the traits that are to be considered when selecting for drought tolerance. Among all the varieties studied, Arka Lohit showed high proline content, high root to shoot dry weight than other varieties, these may be considered as drought tolerant. Since LCA-353 could not survive at high concentration of PEG (20%) and remaining all treatments it showed least growth rate and low proline accumulation makes this cultivar considered as susceptible.

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