Effect of Drought Stress in Different Cultivars of Tomato

A Dissertation Submitted for the Partial Fulfillment of the Requirements of Masters of Science in Botany

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RECOMMENDATION

This is to certify that the dissertation work entitled "**Effect of Drought Stress in Different Cultivars of Tomato"** submitted by **Mr. Sagar Baral** has been carried out under our supervision and guidance. The entire work is based on the results of his research work. As to our knowledge, the results he acquired is not submitted or published for any other academic degree. So we recommend this dissertation to be accepted as a partial fulfillment of **M. Sc. Degree in Botany** at an Institute of Science and Technology.

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LETTER OF APPROVAL

The M. Sc. Dissertation entitled "*Effect of Drought Stress in Different Cultivars of Tomato*" presented by Mr. Sagar Baral for the partial fulfillment of his Master's Degree in Botany has been accepted.

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ABSTRACT

Plants are frequently exposed to many environmental stresses such as drought, cold, salt, flood, heat, heavy metal toxicity etc while growing in natural conditions. Of the different environmental stresses, drought stress is the most important stress and the main cause of significant losses in growth and productivity of many plants. Drought induces significant alterations in plant physiology and biochemistry. Severe drought stress may result in functional damage and loss of plant parts. Tomato is an important, popular and nutritious vegetable crop. This piece of work was carried out to identify the effects of drought stress in different cultivars of tomato.

Mannitol and NaCl were used to induce drought stress. For the selection of osmoticum the tomato seeds were germinated in different concentrations of mannitol and NaCl. Under controlled condition, 96% of seeds were germinated. The germination percentage was reduced upto 36 % and 57.33% in 4000 ppm of NaCl and mannitol. Further work was carried out using NaCl. Drought tolerant cultivar (NCL) and drought sensitive cultivar (BL) were selected by subjecting germination of tomato seeds in different concentration of NaCl and by the measuring different physiological attributes (RWC and ELWR). The selected cultivars were further grown and treated with four different concentrations of NaCl solutions after 30 days of germination. Shoot length, fresh weight, dry weight and chlorophyll content of the selected cultivars were measured after 60 days of germination. Shoot length, fresh weight and dry weight were found to be more in BL but chlorophyll content was found to be present in higher amount in NCL. The effects of induced stress were more pronounced in BL. Shoot length, fresh weight and dry weight were reduced upto 39.82%, 42.03% and 24.13% respectively in BL. In NCL shoot length, fresh weight and dry weight were reduced by 28.76%, 38.74% and 16.22% respectively. Chlorophyll a and Chlorophyll b were reduced upto 36.05% and 37.78% respectively in NCL and upto 27.08% and 22.86% respectively in BL.

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LIST OF ABBREVIATIONS AND ACRONYMS

µg/µl	:	microgram/micro liter
CDB, T.U.	:	Central Department of Botany, Tribhuvan University
DW	:	Dry weight
cm	:	centimeter
GDP	:	Gross domestic Product
e.g.	:	example gratia: for example
ELWR	:	Excised leaf water retention
et al.	:	*et alias*
fig., figs.	:	Figure, figures
FW	:	Fresh weight
gm	:	gram
hr	:	hour
ml	:	milliliter
mg	:	milligram
mm	:	millimeter
mM	:	milimolar
MOAC	:	Ministry of Agriculture and Cooperatives
NaCl	:	Sodium chloride
NARC	:	Nepal Agricultural Research Council
NBS	:	Nepal Biodiversity Strategy
NH_3	:	Ammonia
nm	:	nano meter
ppm	:	parts per million
rpm	:	revolutions per minute
RWC	:	Relative water content
^oC	:	Degree centigrade

Chapter One

INTRODUCTION

1.1 General Background

Stress is an altered physiological condition caused by factors that tend to disrupt the equilibrium. Strain is any physical and chemical change produced by a stress (Gaspar *et al.,* 2002). Stress contains both destructive and constructive elements and is a selection factor as well as a driving force for improving resistance and adaptive evolution (Larcher 1987).

Plants are frequently exposed to many stresses such as drought, low temperature, salt, flooding, heat, oxidative stress, heavy metal toxicity, etc, while growing in nature (Jaleel *et al.,* 2009). Due to their sedentary mode of life, plants resort to many adaptive strategies in response to different stresses such as high salt, dehydration, cold, heat and excessive osmotic pressure which ultimately affect plant growth and productivity (Epstein *et al.,* 1980; Yancey *et al.,* 1982). Plants adapt to different environmental stress via stress tolerance and stress avoidance. The ability of plants to cope with adverse environmental conditions and stresses is the stress tolerance. The degree of tolerance differs with different plant species. In stress avoidance the plant responds by somehow reducing the impact of environmental stress. Plants adapt to stresses by different mechanisms including changes in morphological and developmental patterns as well as physiological and biochemical process (Bohnert *et al.,* 1995). Tolerance to abiotic stresses is very complex due to the intricate of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Razmjoo *et al.,* 2008).

Cold, drought and salinity are those environmental stressors which affect plants in many respects and cause the greatest economic losses in agriculture due to their wide spread occurrence (Beck *et al.,* 2007). All these three forms of abiotic stresses affect the water relations of a plant at the cellular as well as whole plant level causing specific as well as unspecific reactions like damages and adaptation reaction. Water deficit and salt stress are the global issues that need to be addressed to ensure survival

of agricultural crops and sustainable food production (Jaleel *et al.*, 2007; Nakayama *et al.*, 2007).

Drought is a meteorological term and is commonly defined as a period without significant rainfall. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by evapo-transpiration. Drought stress is considered to be a moderate loss of water, which leads to stomatal closure and limitation of gas exchange (Smirnoff, 1993). Drought is the most severe stress and the main cause of significant losses in growth and productivity of crop plants (Ludlow and Muchow, 1990). Drought induces significant alterations in plant physiology and biochemistry. Some plants have a set of physiological adaptations that allow them to tolerate water stress conditions. The drought stress tolerance is seen in almost all plants but its extent varies from species to species and even within species (Save *et al.*, 1995). Plants response to water stress includes morphological and biochemical changes and later when water stress becomes more severe, functional damage and loss of plant parts take place (Sangtarash, 2010). The reactions of plants to water stress differ significantly at various organizational levels depending on plant species, intensity and duration of stress, and the growth stage of the plant (Chaves *et al.*, 2002). The most severe form of water deficit is desiccation-when most of the protoplasmic water is lost and only a very small amount of tightly bound water remains in the cell (Yordanov *et al.*, 2003). Drought stress is characterized by reduction of water content, diminished leaf water potential, loss of turgidity, closure of stomata, reduced rates of transpiration and photosynthesis, and decrease in cell enlargement and growth. Furthermore, the drought stress leads to accumulation of stress hormone abscissic acid (ABA), various kinds of osmoprotectants (like proline, mannitol, sorbitol, etc.), radical scavenging compounds (ascorbate, glutathione, alpha tocopherol etc.) and new proteins and mRNAs that help the plants in stress mitigation (Yordanov *et al.*, 2003). Besides these physiological responses plants also undergo morphological changes. One of the largest is the adaptation of plants and chloroplasts to high light (sun) and low light (shade exposure) (Lichtenthaler *et al.*, 2008). Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant (Jaleel *et al.*, 2008).

Plants can cope up with these stresses to a limited extent. Plants growing in extreme environment have evolved unique mechanisms to tolerate the extreme environment and are able to grow in these conditions. These include the presence of thick well developed cuticle, thick and fleshy stems and leaves, presence of hairs, well developed vascular system etc. The osmotic adjustment, i. e., reduction of cellular osmotic potential by net solute accumulation, has been considered an important mechanism to salt and drought tolerance in plants (Hasegawa *et al.*, 2000). The osmotic adjustment in both roots and leaves contribute to the maintenance of water uptake and cell turgor and allows occurrence of physiological processes such as stomatal opening, photosynthesis, and cell expansion (Serrai and Sinclair, 2002).

1.2 Quantification of Water stress

Relative water content, leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are some of the important parameters that influence plant water relations. Leaf water potential is considered to be a reliable parameter for quantifying plants' response to water stress (Ghobadi *et al.,* 2011) and high leaf water retention may be used as an indicator of drought tolerance (Randhawa *et al.,* 1988). Among several methods used to characterise internal plant water status, relative water content (RWC) is an integrative indicator and is used successfully to identify drought resistant cultivars (Matin *et al.,* 1989). RWC is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures (Anjum *et al.,* 2011). A decrease in RWC in response to drought has been noted in wide variety of plants wheat, tomato, rice, *Catharanthus* etc. When leaves are subjected to drought, leaves exhibit large reductions in relative water content and water potential (Nayyar and Gupta 2006).

1.3 Effects of drought stress on plants:

It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion of cells (Anjum *et al.,* 2003; Bhatt and Srinivasa Rao, 2005; Kusaka *et al.,* 2005; Shao *et al.,* 2008). Water stress inhibits cell enlargement more than cell division. It reduces plant growth by affecting various physiological and biochemical

process such as photosynthesis, respiration, translocation, ion uptake, carbohydrate nutrient metabolism and growth promoters (Jaleel *et al.,* 2008; Farooq *et al.,* 2008). The stem length reduced under water deficit conditions in soybean (Specht *et al.,* 2001) and potato (Heuer and Nadler, 1995). Similarly, the plant height in water stressed citrus seedlings reduced up to 25% with respect to untreated controls (Wu *et al.,* 2008).

Water stress greatly suppresses cell elongation and cell growth due to the low turgor pressure. Osmotic regulation can enable the maintenance of cell turgor for survival or to assist plant growth under severe drought conditions in pearl millet (Shao *et al.,* 2008). A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Farooq *et al.,* 2009). Diminished biomass due to water stress is reported in almost all genotypes of sunflower (Tahir and Mehid, 2001). Reduced biomass is also reported in water stressed soybean (Specht *et al.,* 2001)

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Chlorophyll is one of the major chloroplast components for photosynthesis and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Both the chlorophyll a and b are prone to degradation by soil dehydration (Farooq *et al.,* 2009). Decreased or unchanged chlorophyll level during drought stress has been reported in many species depending on the duration and severity of drought. A reduction in chlorophyll content has been reported in drought stressed cotton (Massacci *et al*., 2008), periwinkle (Jaleel *et al.,* 2008) and sunflower plants (Kiani *et al*., 2008). At the whole plant level the effect of stress is usually perceived as a decrease in photosynthesis and growth, and is associated with alteration in carbon and nitrogen metabolism (Cornic and Massacci 1996; Mwanamwenge *et al.,* 1999). Loss of chlorophyll content under stress is considered a main cause of inactivation of photosynthesis. Furthermore water deficit induced reduction in chlorophyll has been ascribed to loss of chloroplast membranes, excessive swelling and distortion of the lamellae vesiculation and the appearance of lipid droplets (Kaiser *et al.,* 1981).

Water stress brought about by drought and salinity is one of the most important abiotic factors limiting plant germination and early seedling stages (Almansouri *et al.,* 2001). Salts and other solutes in the medium cause inhibitory osmotic effects on the seeds' water uptake and retard and/or suppress germination (Al-Taisan, 2010). Salinity and drought affect the plants in a similar way (Katerji *et al.,* 2004) since reduced water potential is a common consequence of both salinity and drought (Legocka and Kluk, 2005).

1.4 Screening of plants for tolerance to water stress:

One of the screening techniques based on physiological traits is the use of various osmotica to induce drought stress in plant tissues. NaCl, Polyethylene glycol (PEG) and mannitol have been used to simulate osmotic stress effects for plants to maintain uniform water potential throughout (Kulkarni and Deshpande, 2007). Germination in mannitol and polyethylene glycol (PEG), measurements of root length or rooting depth and the survival or growth of seedlings subjected to osmotica have also been suggested for drought screening (Emmerich and Hardegree, 1990; Kocheva *et al.,* 2004; Farshadfar *et al.,* 2002). The water stress affects germination and seedling growth by creating an osmotic pressure in wheat (Mehmet *et al.,* 2008). The reduction in water uptake by germinating seed in stress condition resulted in decreases of seedling growth in rice (Alam, 2001).

1.5 Tomato and Water stress

Tomato is an important, popular and nutritious vegetable crop that has achieved tremendous popularity over the last century. It is grown in almost every country of the world – in the field, greenhouses and net houses. Tomato belongs to the family Solanaceae. The botanical name of tomato is *Lycopersicon esculentum* Mill. It is a diploid plant with 2*n*=24 chromosomes. Tomato by its nature is a perennial plant, but is commercially cultivated as an annual crop. The tomato crop is very versatile and is grown either for fresh market or processing (Bhatia *et al.,* 2004). Tomato production and consumption has grown quite rapidly over the past 25 years. At present, tomato is grown in an area of around 3.9 million-hectares worldwide with an annual production of 145 million metric tonnes in the year 2010 (FAO Statistical Database, 2010). Tomato is rich in vitamins A and C and fibre, and is also cholesterol free (Hobson and Davies, 1971). It plays a vital role in providing a substantial quantity of vitamin C

and A in human diet (Nahar and Gretzmacher, 2002). An average sized tomato (148 g) boasts only 35 calories. Tomato contains approximately 20–50 mg of lycopene/100 g of fruit weight (Kalloo, 1991). Lycopene is part of the family of pigments known as carotenoids which are natural compounds that create colours of fruits and vegetables. Lycopene is the most powerful antioxidant in the carotenoid family and it protects humans from free radicals that degrade many parts of the body; lycopene is also known to prevent cancer (Block *et al.*, 1992; Gerster, 1997; Rao and Agarwal, 2000). At present, tomatoes are consumed at a higher rate in the developed countries than in the developing countries and hence it may be referred to as a luxury crop.

Though Nepal is one of the smallest and poorest countries in the world, it is rich in biodiversity. Agriculture is the major sector in Nepal's economy which contributes about 42 percent of Gross Domestic Products (GDP). About 81 percent of Nepalese citizen depend on agriculture for their livelihood (NBS 2002). Agricultural resources fulfil both the immediate and long term needs of rural communities. The annual production of vegetable crops in Nepal is 3,00,3821 metric ton in 2,35,098 hectare land and Tomato is cultivated in 15609 hectare land with an annual production of 242018 metric ton in the Fiscal year 2009/2010 (MOAC 2010).

Though drought stress is an important factor that affects growth, development and yield of crops, very few studies have been conducted in Nepal. Tomato being one of the important vegetable crops the growth and yield is mostly affected by the vagaries of nature. Thus there is an immediate need to identify drought tolerant cultivar of tomato. This study tries to identify drought tolerant cultivar from some cultivars of tomato and the effect of drought on tomato.

1.6 Research questions

This study tries to seek the answer for the following questions.

What are the responses of tomato cultivars to different drought stress treatments?

Are these responses similar?

What may be underlying causes of these responses?

1.7 Objectives

In order to find the answer to the research questions, the following objectives were made.

Broad objective of this study was to assess the effect of water stress on various parameters such as shoot length, fresh weight, dry weight, and chlorophyll content, etc in different tomato cultivars grown in the country. The specific objectives of the study were

a) To screen the tomato cultivars for their tolerance against drought stress.

b) To select the most tolerant and most sensitive cultivar.

c) To compare the effects of drought stress in drought tolerant and sensitive cultivars of tomato.

Chapter Two

MATERIALS AND METHODS

2.1 Collection of seeds of different cultivars

The seeds of two different cultivars of tomato (Srijana and NCL) were collected from Horticultural Research Division of Nepal Agricultural Research Council (NARC) and three different cultivars of tomato (Dahlia, BL and CL) from the local market.

2.2 Selection of Osmoticum

Cultivar Srijana was subjected to germination in different concentrations of mannitol and NaCl. 10 gm each of mannitol and NaCl was weighed and dissolved in minimal amount of distilled water. The mixture was shaked well and the final volume was made to 1000 ml. This stock solution was used to prepare the working solutions of 1000 ppm, 2000 ppm, 3000 ppm and 4,000 ppm. Distilled water was used as control solution.

2.2.1 Viable seed selection and surface sterilization

About 75 viable seeds of all the cultivars were selected by observation. The selected seeds were first washed in mild detergent for 2-3 minutes. The seeds were then rinsed in tap water. The seeds were transferred in sterile eppendorf tube containing 0.25% sodium hypochlorite. 2 drops of tween-20 was added and the tubes were shaken mildly for 15 minute. The seeds were then washed five times with sterile water under aseptic conditions.

2.2.2 Seed germination

First of all sterile petridishes were labeled. Sterile filter paper sheets were placed on the petridishes. Each petridish was then soaked with 2 ml of working solutions (control solution, 1000 ppm, 2000 ppm, 3000 ppm and 4000 ppm NaCl and mannitol solutions). Five sterilized seeds of each cultivar were placed in each petridish. The process was repeated three times.

2.2.3 Observation

Germination was observed and data was recorded at day 10 after subjected to germination. 2mm radical protrusion was considered as germinated and data was recorded.

2.3 Drought sensitive and drought tolerant cultivar selection

Cultivar that can grow well under drought stress condition is drought tolerant and the one that cannot grow under stress condition is drought sensitive variety. Drought sensitive and drought tolerant cultivars were selected by germinating the seeds of different cultivars in different concentrations of NaCl and by measurement of physiological attributes.

2.3.1 Germination test

2.3.1.1 Preparation of stock NaCl solution and working solutions

Stock NaCl solution and working solutions were prepared by the process described in 2.2.

2.3.1.2 Viable seed selection and surface sterilization

About 30 viable seeds of all the cultivars were selected and surface sterilized by the process as described in 2.2.1

2.3.1.3 Seed germination and observation

Seed germination and observation of the selected seeds were done by the process described above in 2.2.2 and 2.2.3

2.3.2 Measurement of physiological attributes

2.3.2.1 Viable seed selection and sterilization

10 viable seeds of all the cultivars were selected by observation. The selected seeds were first washed in mild detergent for 2-3 minutes. The seeds were then rinsed in tap water. The seeds were transferred in sterile eppendorf tube containing 0.25% sodium hypochlorite. 2 drops of tween-20 were added. They were shaken mildly for 15 minutes. The seeds were then washed with sterile water for 5 times.

2.3.2.2 Soil preparation and seed germination

The soil for the seed germination was prepared in plastic tray by mixing 25% sand, 25% vermin-compost and 50% top soil. The seeds of all the cultivars were germinated in separate tray with above mentioned soil preparation. Prepared soil was also transferred to plastic bag of size 4cmx6cm.

2.3.2.3 Seedling transplantation

After 10 days of seed germination 3 identical seedlings of each cultivar were transferred to 3 separate plastic bags and were allowed to grow under similar conditions of light temperature and nutrients.

2.3.2.4 Measurement of Excised Leaf Water Retention (ELWR)

After 30 days of seed germination, the youngest leaves were collected and weighed, left for 5 hrs, at 30°C and reweighed. ELWR was calculated by using the following formula (Farshadfar *et al*., 2001)

ELWR= [1 - (weight of fresh leaves - weight of leaves after 5 hr)/weight of fresh leaves] x 100

2.3.2.5 Measurement of Relative Water Content (RWC)

A sample of 3 leaves of each genotype was taken randomly and fresh weight (FW) was measured. The sample leaves were rinsed in distilled water for 4 hr in low light density and the turgor weight (TW) was measured. Sample leaves were oven dried at 70°C for 72 hr and dry weight (DW) was measured. RWC was calculated using the formula following Eric *et al*., (2005).

RWC = (FW-DW)/(TW-DW) x 100

2.4 Effect of drought stress on drought tolerant and drought sensitive cultivars

To induce drought stress to the selected cultivars, the protocol established by Hamayun *et al*., (2010) with slight modifications was followed. The modification includes five different stress condtions viz. 50 mM, 100 mM, 150 mM and 200 mM and control instead of 70 mM, 140 mM and control.

2.4.1 Germination of selected cultivars

Seed selection, surface sterilization, soil preparation and germination of the selected seeds were done by process mentioned above in 2.2.2.1 and 2.2.2.2.

2.4.2 Transfer of seedlings to the plastic bag

After 10 days of seed germination in plastic tray, 15 identical seedlings of each variety were transferred to 15 separate plastic bags. The seedlings were then allowed to grow into fully grown plant. The plants were watered every other day.

2.4.3 Preparation of stock NaCl solution and working solutions

40 gm NaCl was weighed and mixed with 1 litre distilled water to prepare 1M NaCl solution. The mixture was shaken well. This stock solution was later used to prepare working solutions of 50mM, 100mM, 150mM and 200mM. Distilled water was used as control.

2.4.4 Induction of stress

After 30 days of germination, the plants were subjected to stress by treating them with working solutions. Each plant was treated twice with 100 ml of one working solution at a time at an interval of 1 week. So, each plant was treated with 200ml of one working solution. The process was done in triplicate.

2.4.5 Measurement of shoot length

When the plant was 60 days old, the shoot length was measured with the help of measuring tape and noted.

2.4.6 Measurement of fresh weight (shoot)

The above ground biomass of each plant was measured. The plants were cut just above the soil level and fresh weight of shoot was measured in the weighing machine and noted.

2.4.7 Measurement of dry weight

After the measurement of fresh weight the same plants were then subjected to oven dry at 70°C for 72 hour and dry weight was measured and noted.

2.4.8 Measurement of chlorophyll

1 gm leaf each from different samples were weighed and cut into small pieces. The leaves were then ground and 10 ml of extraction medium (480 ml acetone, 117 ml distilled water and 3 ml 25% Ammonia (NH_3) solution) were added and mixed well. The mixture was then transferred to the test tube. The mortar was again washed with 5 ml extraction medium and transferred to the test tube. The final volume of the mixture was made 30 ml by adding required amount of extraction medium. The tube was then closed, shaken well and kept in dark for 30 minutes. The tubes were centrifuged at 10,000 rpm for 30 minutes and the clear solution of the extract was collected.

The absorbance of the extract was measured at 645 nm and 663 nm. Chlorophyll content was then calculated by using the following formulae given by Strain and Svec (1966).

Chl. a (mg/ml) = 11.64 x A663 - 2.16 x A645

Chl. b (mg/ml) = 20.97 x A645 - 3.94 x A663

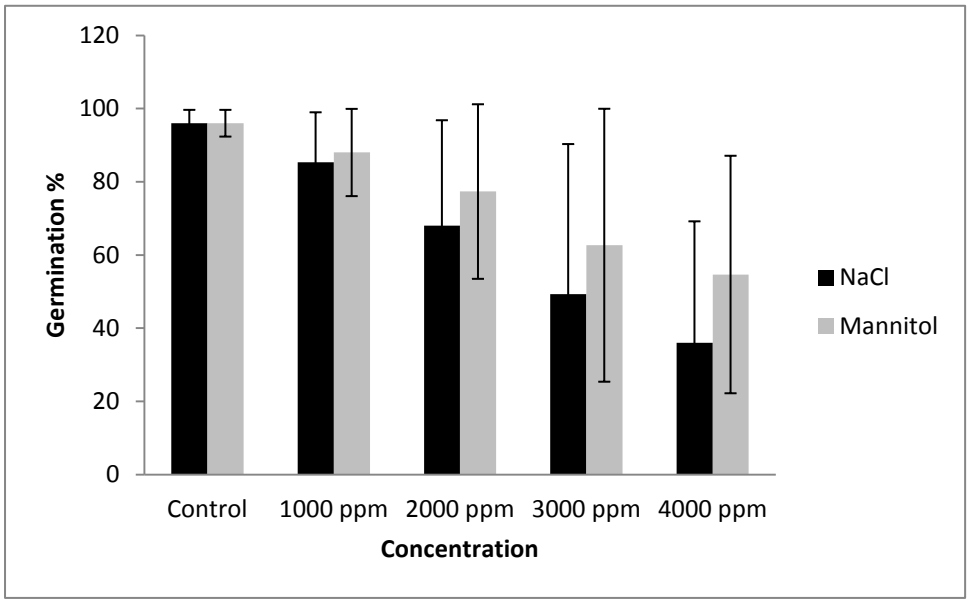
where A645 and A663 represent absorbance values read at 645 and 663 nm.

Chapter Three

RESULTS

3.1 Selection of osmoticum

Fig. 1 Germination percentage of seeds of cultivar Srijana under different osmotic treatments



The seeds of the cultivar Srijana were germinated in different concentrations of NaCl and mannitol. The germination of the cultivar was highest with germination percentage of 96% under controlled condition. Increased concentration of NaCl and mannitol caused a decrease in germination percentage. Under stressed condition germination of the cultivar was lower in NaCl than in mannitol. The germination percentage of seeds of different cultivars in NaCl in 1000 ppm was 85.33% followed by 68% in 100 mM, 49.33% in 3000 ppm and 36% in 4000 ppm. Similary the germination percentage in Mannitol in 1000 ppm was 88% followed by 77.33% in 2000 ppm, 62.66% in 3000 ppm and 57.33% in 4000 ppm. So, NaCl was found to be more effective in inducing stress in tomato cultivar (Srijana).

3.2 Selection of drought sensitive and tolerant cultivar selection

3.2.1 Germination

Fig. 2: Germination percentage of different cultivars of tomato in control, 50 mM, 100 mM, 150 mM and 200 mM NaCl

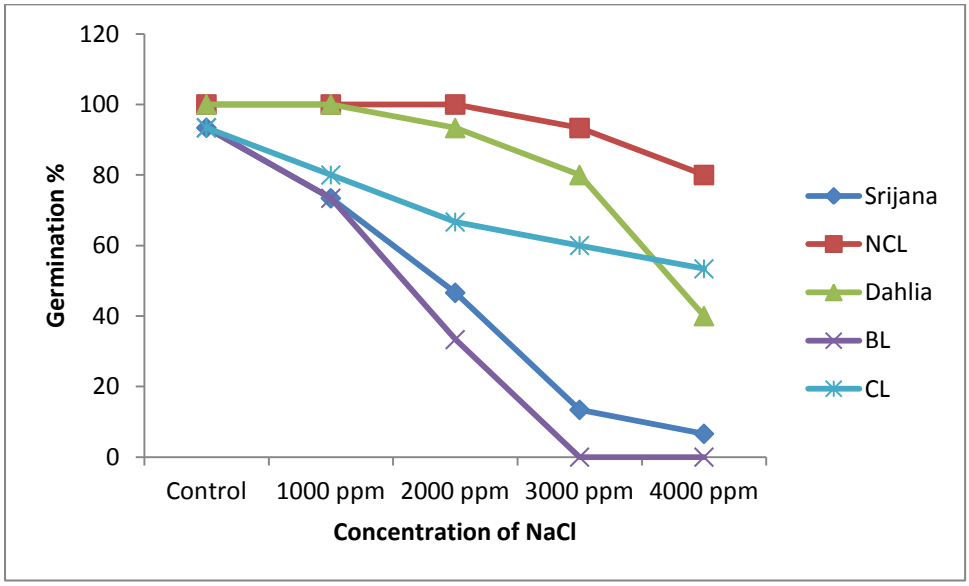
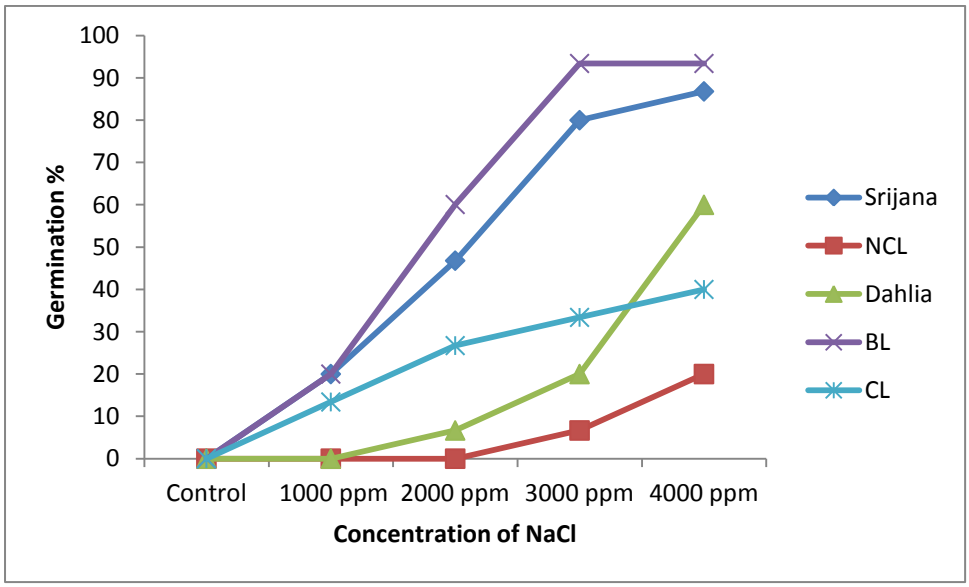


Fig. 3: Reduction in germination percentage of different cultivars as compared to control

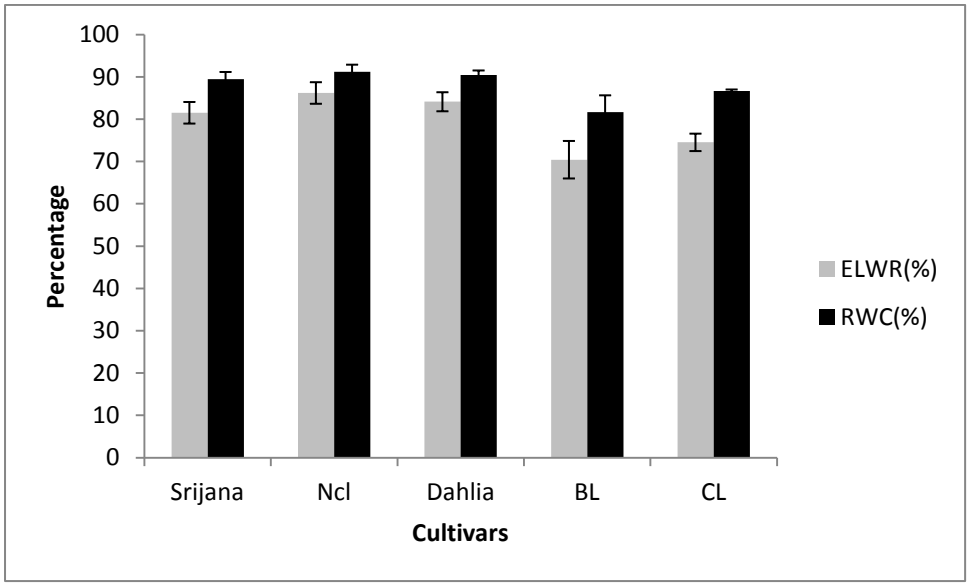


Of all the cultivars subjected to germination, NCL and BL were found to have highest and lowest germination percentages respectively in all the conditions (fig. 2). The

germination percentages of other cultivars were found to lie between these two extremes. The germination percentage of seeds in all cultivars was found to decrease with the increasing concentration of NaCl, but the extent of such changes was cultivar specific. Cultivar NCL was found drought tolerant since retained highest germination percentage in different stress conditions. Similarly, the cultivar BL was found to be most sensitive to drought stress as it showed lowest germination percentage of seeds in all the osmotic treatments tested. The reduction in germination percentage in different stress conditions was highest in BL (fig. 3)

3.2.2 Physiological attributes

Fig. 4: ELWR and RWC of different cultivars



The ELWR and RWC of all tomato cultivars is shown in Figure 4. The highest ELWR was found to be possessed by NCL (86.19±2.55%) and lowest ELWR was possessed by BL (70.42±4.43%). NCL was found to possess the highest amount of RWC (91.16±1.72%) followed by Dahlia (90.41±1.10%). BL variety was found to possess the lowest amount of RWC i.e. 81.69±3.95. Based on the values of ELWR and RWC NCL and BL were found to be the most tolerant and most sensitive cultivars to drought stress respectively. Only these two cultivars were considered for further study.

3.3 Effect of drought stress on various physiological parameters

3.3.1 Shoot Length

Fig. 5: Effect of different concentration of NaCl on shoot length of selected cultivars

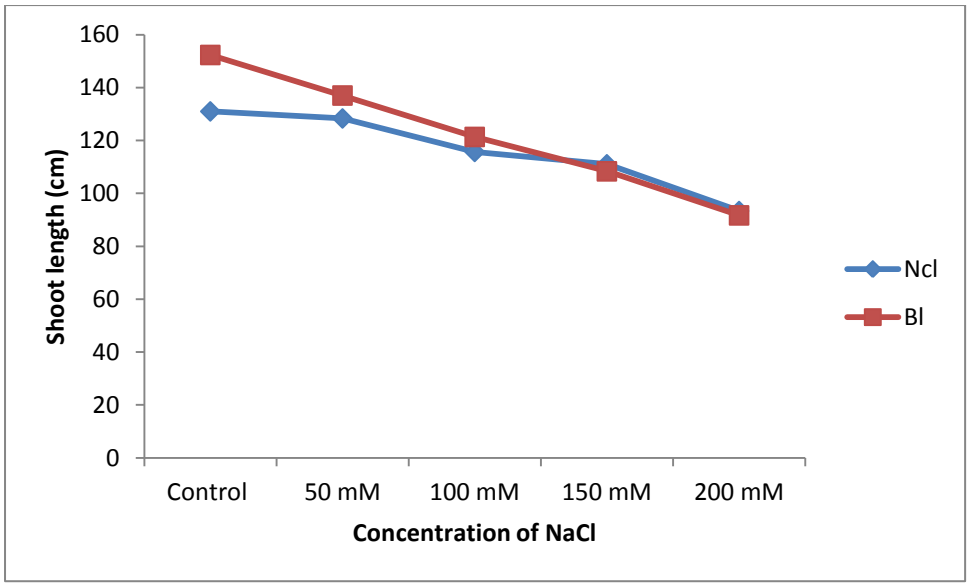
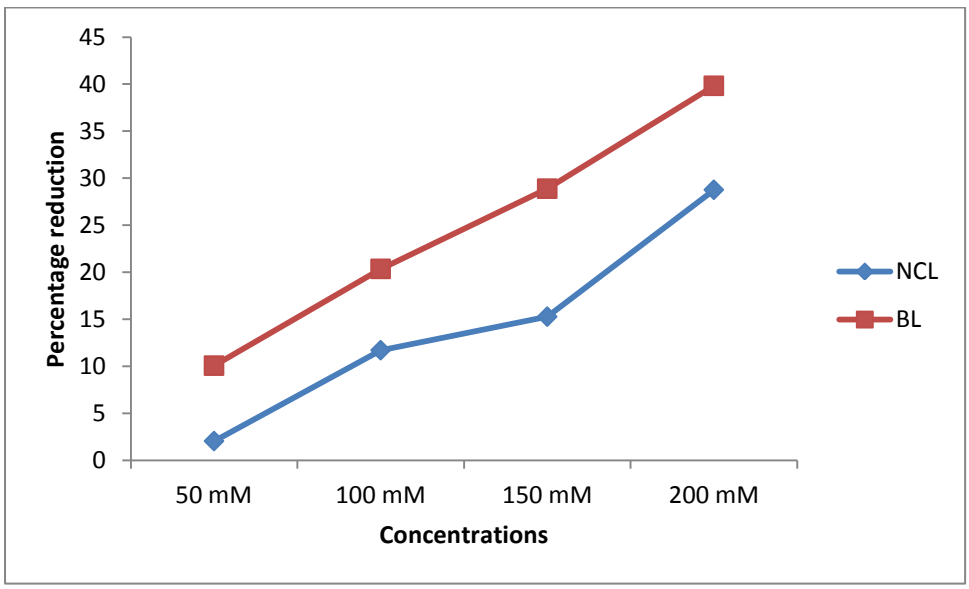


Fig. 6: Percentage reduction in shoot length of selected cultivars of tomato following treatment with NaCl.



The shoot length of BL was found to be greater than NCL under controlled condition (fig. 5). The shoot length of both the cultivars decreased as the concentration of NaCl was increased. The shoot length of NCL was 131±3.61 and 93.33±4.16 in control and

200 mM respectively. Similarly the shoot length of BL was 152.33±2.52 and 91.67±5.86 respectively. The shoot length of BL was found to be less than that of NCL at 150 mM and 200 mM. As compared to control, the percentage reduction in shoot length was more in BL than NCL (fig. 6). In 50 mM the shoot length decreased by 10.06% in BL whereas in NCL the decrease was 2.04% only. In 200 mM the shoot length in BL decreased by 39.82% whereas in NCL the decrease was 28.76%.

3.3.2 Fresh weight (Shoot)

Fig. 7: Effect of different concentrations of NaCl on fresh weight of selected cultivars

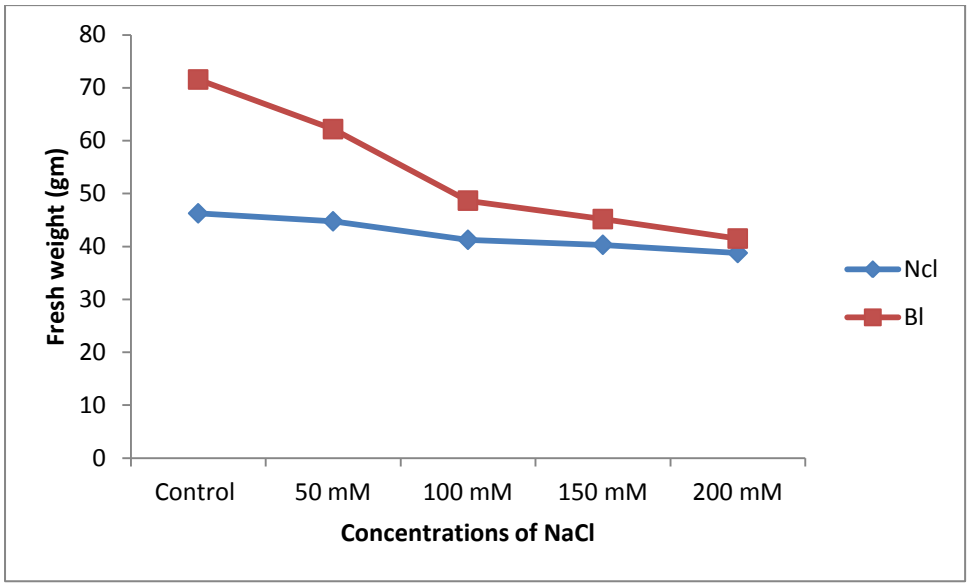
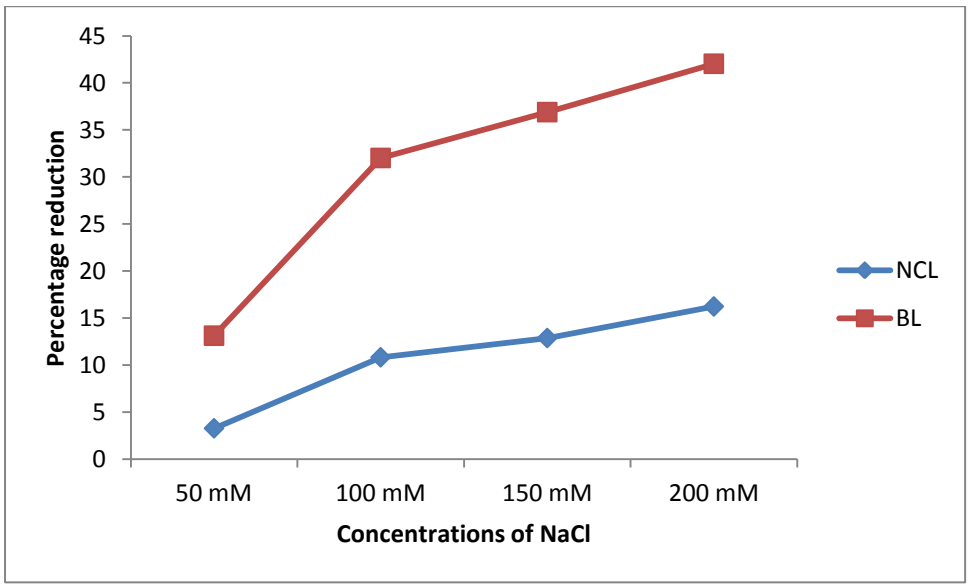


Fig. 8: Percentage reduction in fresh weight of selected cultivars of tomato following treatment with NaCl

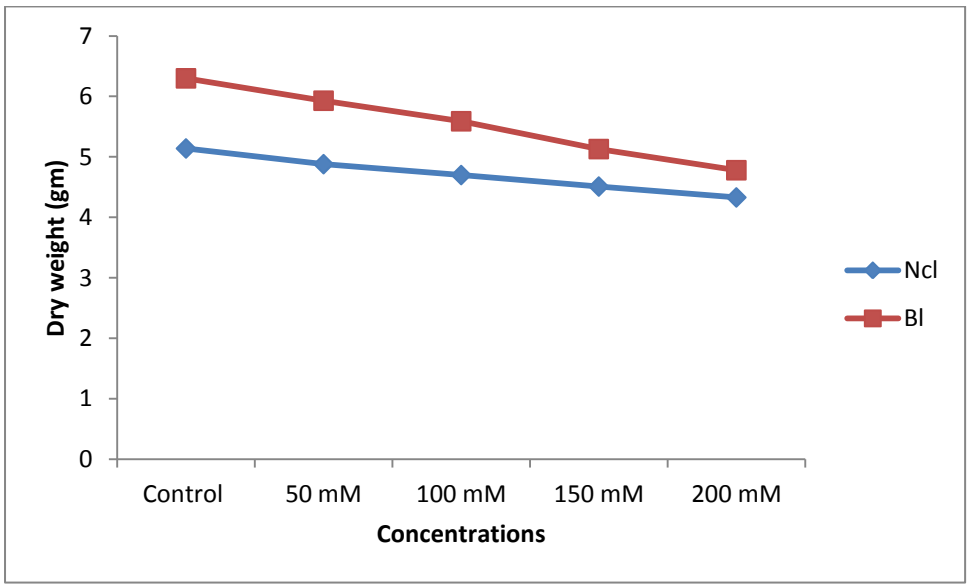


The fresh weight of BL was found to be greater than that of NCL in every stress condition. The fresh weight of both the cultivar decreased as the concentration of NaCl was increased. The fresh weight of NCL in controlled condition and 200 mM was 46.24±1.67 and 38.74±0.24 respectively. Similarly the fresh weight of BL in controlled condition and 200 mM was 71.54±1.27 and 41.47±0.52 respectively.

The decrease was more profound in BL than NCL as compared to control. In 50 mM the fresh weight of BL decreased by 13.11% and this decrement reached upto 42.03% in 200 mM. But the fresh weight of NCL decreased by 3.27% in 50 mM whereas the in 200mM, the decrease was 16.22% as compared to control.

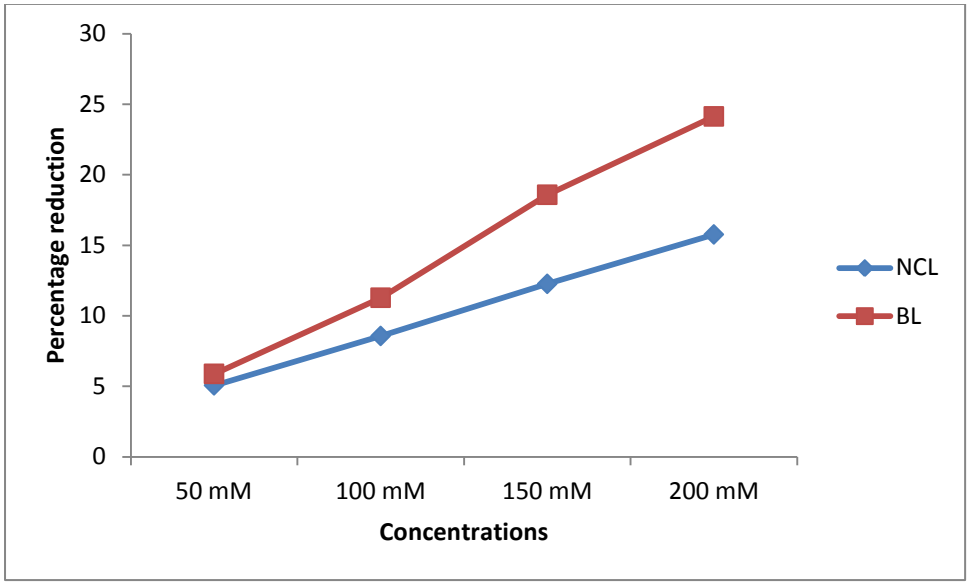
3.3.3 Dry weight (Shoot)

Fig. 9: Effect of different concentrations of NaCl on dry weight of selected cultivars



The dry weight of BL was found to be greater than that of NCL in every stress condition. The dry weight of BL under control condition and 200mM was 6.30±0.27 and 4.78±0.14, whereas the dry weight of NCL under control condition and 200mM was 4.33±0.03 respectively.

Fig. 10: Percentage reduction in dry weight of selected cultivars of tomato following treatment with NaCl



The dry weight of both the cultivars decreased as the concentration of NaCl was increased. High decrease percentage was observed in BL than NCL. In 50 mM the dry weight of BL decreased by 5.87%. The decreased percentage reached upto 24.13%. For NCL the decrease in 50mM was 5.06% and it reached upto 15.76% in 200 mM.

3.3.4 Chlorophyll content

Fig. 11: Effect of different concentration of NaCl on chlorophyll content of selected cultivars

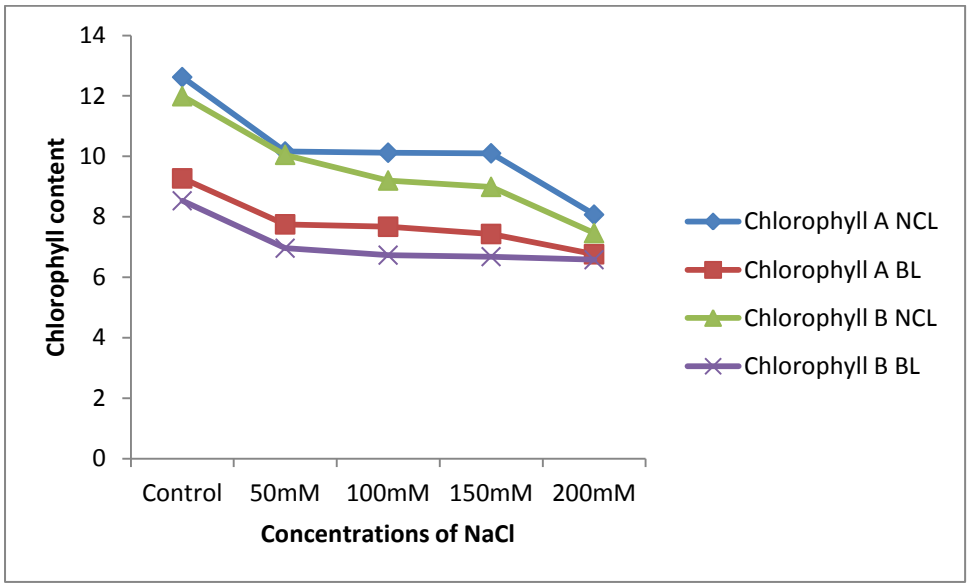
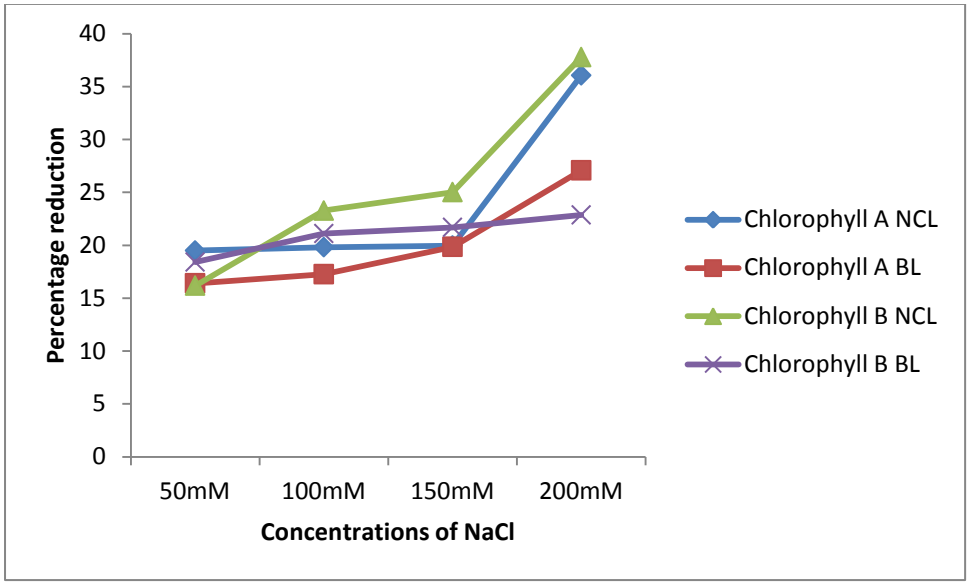


Fig. 12: Percentage reduction in chlorophyll content of selected cultivars of tomato following treatment with NaCl



NCL was found to possess more chlorophyll a, chlorophyll b and total chlorophyll content than BL in both the control and stress conditions. The amount of chlorophyll a and chlorophyll b were 12.62±0.68 and 11.99±0.31 respectively for NCL and 9.27±0.06 and 8.53±0.11 respectively for BL under controlled condition. Chlorophyll a and chlorophyll b and was found to decrease as the stress was increased. The lowest amount of chlorophyll a and chlorophyll b were found in severe stress condition. In stressed condition also chlorophyll a and chlorophyll b were found more in NCL than in BL.

Under 50 mM NaCl, chlorophyll a was reduced by 19.49% in NCL and by 16.40% in BL. Similarly chlorophyll b was reduced by 16.18% in NCL and by 18.41% in BL. There was sharp reduction in chlorophyll a and chlorophyll b in 200 mM NaCl. Under 200 mM NaCl, chlorophyll a was reduced by 36.05% in NCL and by 27.08% in BL. Similarly chlorophyll b was reduced by 37.78% in NCL and by 22.86% in BL.

Chapter Four

DISCUSSION

4.1 Selection of osmoticum

Different cultivars of tomato were subjected to germination in osmoticum NaCl and mannitol. The result showed that NaCl was more effective than mannitol in inducing drought stress. NaCl has been used to to create osmotic stress in petri dish for plants (Misra and Dwivedi, 2004). Wang *et al.,* 2005 reported that mannitol plays a role of osmolyte. The decrease in osmotic potential is considered a potential cellular mechanism of drought resistance as it enables turgor maintenance and growth continuation (Bajji *et al.,* 2000; Munns 1988). Seong *et al.,* (1988) reported that the moisture content and the seedling length decreased when the mannitol concentration increased, concluding that germination in mannitol was useful for the selection of soybean cultivar for emergency capacity under conditions of water deficit. In this study germination percentage of cultivars exposed to NaCl was found less than that of mannitol. Moreover the germination percentage of all the cultivars decreased with increasing concentrations of mannitol and NaCl. The reason for decreased germination may be attributed to the decreased osmotic potential as the concentration of mannitol and NaCl was increased. According to Mayer and Poljakoff-Mayber (1989) results like these could be attributed to absence of energy to start the germination process, as energy was obtained by increments in the respiratory pathway after the imbibition, and in low levels of water potential, water absorption proceeded slowly. Water deficit induced by mannitol affected germination and seedling development (Neto *et al.* 2004). Sodium chloride solution may have created an osmotic potential which prevented water uptake. It is also possible that solution provided the entry of the ions to the seeds that might have been toxic to the embryo or the developing seedlings (Almodares *et al.,* 2007). It affects development just by increasing the sodium concentration in the growing medium. Sodium is a small ion that can pass easily through cellular membranes, and cells must pump it out expending energy to do that, otherwise the water activity decreases and all the metabolic pathways can be disturbed or disrupted, causing some imbalance in the energy production –consumption (Neto *et al.,* 2004). So NaCl may be considered as more effective drought stress inducer than mannitol. Salts and other solutes in the

medium cause osmotic inhibitory effects on the seed's water uptake and retard and /or suppress germination.

4.2 Selection of cultivars

4.2.1 Germination in NaCl

NaCl is regularly used to induce drought stress in plants as they lower osmotic potential. Many researchers have reported germination as the criterion for the selection of drought tolerance. Cultivar having highest germination percentage under stress condition was considered to be drought tolerant and that having low germination percentage was considered drought sensitive. Of all the cultivars subjected to germination, NCL was found to have highest percentage of germination followed by Dahlia and BL was found to possess lowest percentage of germination. The germination percentage was found to decrease with increase in the concentration of NaCl and mannitol. Germination patterns could be different between species and between different varieties in the same species (McWilliam and Phillips, 1971, Therios, 1982). Percentage decrease in seed germination could be attributed to osmotic stress or to specific ion toxicity (Jamil *et al.*, 2005). Many researchers have reported low germination percentage as the concentration of osmoticum was increased (Prado *et al.*, 2000 and Patane *et al.*, 2009).

4.2.2 Physiological attributes

Certain physiological parameters which confer drought resistance in plants have been identified for screening the genotypes (Alves and Setter, 2000). There are number of plant traits like relative water content (RWC), excised leaf water retention (ELWR), stomatal frequency, stomatal size, osmotic adjustment etc., which are related to drought resistance (Lugojan and Ciulca, 2011). Clarke and McCaig (1982) evaluated leaf diffusive resistance, leaf temperature and excised leaf water retention as screening criteria for drought resistance and concluded that measurement of excised leaf water retention capability was the most promising techniques of the three. In this study NCL was found to possess high ELWR and RWC as compared to other cultivars, whereas the lowest ELWR and RWC was found to be possessed by BL. El Tayeb (2006) reported that drought caused a decrease in RWC in *Vicia faba* cultivars. Schonfeld *et al.*, (1988) observed a decline in the amount of RWC in wheat due to

drought stress and reported the highest RWC in the tolerant genotype. Drought resistant genotypes had higher osmotic adjustment, stomatal resistance, relative water content and seedling survival compared to drought susceptible genotypes (Malik and Wright 1998). Clarke and McCaig (1982) found out that drought tolerant cultivars can retain more shoot water content than drought-susceptible cultivars. Drought tolerant genotypes in other crop species are also reported to possess high RWC and ELWR (Dedio 1975; Randhawa *et al.,* 1988; Winter *et al.,* 1988; Rajeshwari 1995; Alves and Setter, 2000).

4.3 Effect of drought stress on various physiological parameters

4.3.1 Shoot length, fresh weight and dry weight

Drought resistance in wheat variety is characterized by small reduction of shoot growth in drought stressed conditions (Mehmet and Kaydan, 2008). Treatment of plants with NaCl (100 and 200 mM) resulted in significant reduction on most of vegetative growth parameters, including Number of leaves, Leaf area, Shoot and root height, and fresh and dry weight of shoots and roots. In this study, longest shoot length was observed for BL than NCL in controlled condition. However the shoot length decreased under increasing stress condition. The percentage decrease was found more in BL. Fresh weight and dry weight of the cultivars also showed the similar pattern. Fresh weight and dry weight under control conditions was found to be higher in BL than NCL. Fresh weight and dry weight relatively depended on shoot lengths. The decrease in shoot length, fresh weight and dry weight were also reported in the study of many other researchers (Akbarimoghaddam*., et al.,* 2001; Mehmet and Kaydan 2008). Reduction in fresh and dry biomass production is the common adverse effect of water stress on crop plants (Zhao *et al*., 2006). The reduction in plant height could be attributed to decline in the cell enlargement and more leaf senescence in the plant under water stress (Manivannan et al., 2007a). Drought led to substantial impairment of growth related traits of maize in terms of plant height, leaf area, number of leaves/plant, cob length, shoot fresh and dry weight/plant (Kamara *et al.,* 2003). The decreased plant growth under water stress caused by high salinity might be due to its effect in lowering in plant metabolic activities (Hossein *et al.,* 2007). The decrease in osmotic potential of NaCl reduced seedling growth such as root and shoot length, dry and fresh weight of root and shoot. Radhouane (2007) observed that

decreases in the external osmotic potential induced decreased shoot growth. The reduction in water uptake by germinating seed in stress condition resulted in decreases of seedling growth (Alam, 2001). Similar results were reported by other scientists showing reduction in seedling growth and different response of cultivars to drought in wheat (Almansouri *et al.,* 2001) and pea (Okçu *et al.,* 2005). Moreover, Soltani *et al.,* (2006) found that reduction in seedling dry weight in response to drought and salinity in wheat cultivars is a consequence of decrease in mobilized seed reserve due to low water uptake by the germinating seeds. Salt stress leads to decreased shoot length in sugar beet and amaranth. The reduction in shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings (Jamil *et al.,* 2006). Another reason for this decrease in root and shoot elongation under high salinity may be due to slow down of water uptake by the plant (Werner and Finkelstein, 1995). Shoot growth is also reduced by salinity due to the inhibitory effect of salt on cell division and enlargement in the growing point (Mccue and Hanson, 1990). Hamayun *et al*., 2010 found the shoot fresh and dry weights significantly decreased with elevated NaCl level at both pre-flowering and post flowering stage in wheat. Bradford and Hsiao (1982) and Chartzoulakis *et al.,* (1993) showed dry matter decreased at high water stress as compared to control. As NaCl concentration increased, it antagonistically affected shoot dry weight. Reduction of dry weights relatively depended on shoot or root lengths. The results are similar to those reported by researchers (Ghoulam and Fares 2001; Salim 1991) in plants like Sugar beet, wheat, rice etc.

4.3.2 Chlorophyll content

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing power. Loss of chlorophyll content under stress is considered a main course of inactivation of photosynthesis. Decreased or unchanged chlorophyll level during drought stress has been reported in many species depending on the duration and severity of drought by many researchers. A reduction in chlorophyll content was reported in drought stressed cotton (Massacci *et al.,* 2008) and periwinkle (Jaleel *et al.,* 2008a). In this study the chlorophyll content of NCL was found more than BL. However the decreases in chlorophyll pigments were observed on increasing the intensity of stress. This result is in agreement with the findings of Bradrod and

Hsiao 1982; Chartzoulakis 1993; Steinberg *et al.,* 1990; Chookhampaeng 2010; Jaleel *et al.,* 2008; Al-sobhi 2006). The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Both the chlorophyll a and b are prone to degradation due to soil dehydration (Farooq *et al.,* 2009). The chlorophyll content decreased to a significant level at higher water deficits in sunflower plants (Kiani *et al.,* 2008). At the whole plant level the effect of stress is usually perceived as a decrease in photosynthesis and growth, and is associated with alteration in carbon and nitrogen metabolism (Carnic and Massacci 1996; Mwanamwenge *et al.,* 1999). Water deficit induced reduction in chlorophyll has been ascribed to loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation and the appearance of lipid droplets (Kaiser *et al.,* 1981). Furthermore, the chlorophyll contents are sensitive to salt exposure and a reduction in chlorophyll levels due to salt stress has been reported in several plants, such as pea (Ahmad & John, 2005), wheat (Ashraf *et al*., 2002), rice (Anuradha & Rao, 2003) and tomato (Al-Aghabary *et al.,* 2004).

Chapter Five

CONCLUSION

The study concludes that NaCl induces more stress than mannitol. Germination of cultivars was subjected to different concentrations of NaCl and mannitol. The germination percentage decreased with increasing concentration of NaCl and mannitol. So, the germination of cultivar was affected by increasing concentration of mannitol and NaCl. Moreover germination percentage in NaCl was much less than that of mannitol. Hence, it was concluded NaCl induced more stress than mannitol.

Five different cultivars were subjected to germination in mannitol and NaCl. Of all the cultivars, NCL possess highest percentage of germination followed by Dahlia. The lowest germination percentage was that of BL. Moreover, ELWR and RWC of NCL were also greater than other cultivars and BL had lowest percentage of ELWR and RWC. So it can be concluded that NCL is drought tolerant and BL is drought sensitive.

Drought stress had had severe effect on different aspects of growth and development of tomato. Shoot length, fresh weight and dry weight were higher in BL than other cultivars. Under increasing concentration of stress, shoot length, fresh weight and dry weight decreased. The decrease was found to be more profound in BL than NCL. Unlike shoot length, fresh weight and dry weight, chlorophyll content was higher in NCL. Chlorophyll content was also found to decrease on increasing the concentration of stress. This study led to conclude that drought stress had effects on different aspects of tomato. As the impact of drought was more intense in BL, the yield of tomato may be low. Moreover, the chlorophyll pigments in NCL was higher than BL. This may lead to the conclusion that yield of tomato should be higher in NCL than BL.

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APPENDIX A

LIST OF TOMATO CULTIVARS USED IN THIS STUDY

1. Srijana
2. Dahlia
3. NCL
4. BL
5. CL

APPENDIX B

LISTS OF MATERIALS

A. Equipments

1. Autoclave
2. Centrifuge
3. Electric balance
4. Hot air oven
5. Spectrophotometer
6. Water distillation plant

B. Chemicals

1. Acetone
2. Alcohol
3. Ammonia solution (1%)
4. Mannitol
5. Sodium Chloride
6. Sodium Hypochlorite
7. Tween 20

C. Glasswares

1. Beakers
2. Conical flasks
3. Measuring cylinder
4. Micropippetes
5. Petriplates

D. Miscellaneous

1. Blotting paper
2. Distilled water
3. Eppendorf tube
4. Falcon tube
5. Forceps
6. Measuring tape
7. Plastic bags
8. Plastic bucket
9. Soil
10. Sticker
11. Vermi compost

PHOTO PLATE I

Different cultivars grown under similar conditions

Cultiivar Srijana

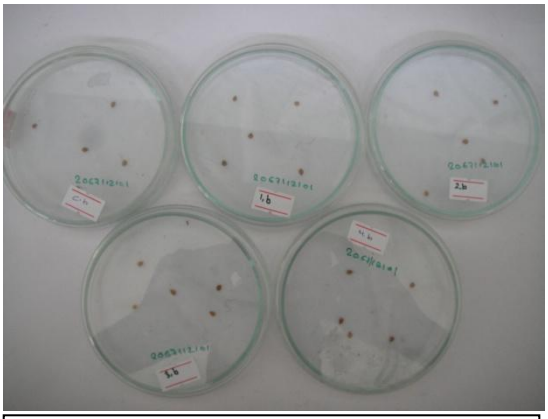
Cultiivar NCL

Cultiivar Dahlia

Cultiivar CL

Cultiivar BL

PHOTO PLATE II



Cultivar Srijana germinated in different Stress conditions

Cultivar NCL and BL grown for the measurement of effect of drought stress

Cultivar BL after treatment with different concentrations of NaCl

Cultivar BL placed for the measurement of shoot length

Cultivar NCL placed for the measurement of shoot length

Cultivar NCL and BL at 200 mM NaCl solution