IN VITRO SCREENING OF SALT TOLERANT GENOTYPES IN TOMATO (*Solanum lycopersicum* L.)

BY
MD RAIS UDDIN RASHED

REGISTRATION NO: 06-01924

A Thesis
Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfillment of the requirements for the degree of

MASTERS OF SCIENCE
IN
GENETICS AND PLANT BREEDING

SEMESTER: JANUARY-JUNE, 2013

Approved by:

Naheed Zeba

(Prof. Dr. Naheed Zeba)
Dept. of Genetics and Plant Breeding
Supervisor

(Prof. Dr. Md. Sarowar Hossain)
Dept. of Genetics and Plant Breeding
Co-Supervisor

23.3.14

(Dr. Mohammad Saiful Islam)
Chairman
Examination Committee

Prof. Dr. Naheed Zeba
Department of Genetics and Plant Breeding
Sher-e-Bangla Agricultural University
Dhaka-1207, Bangladesh

Phone: +8802-9180921-167 (Office), 8802-9140770 (Res.)
Mobile- 01913-091772
E-mail: naheed0359@hotmail.com

CERTIFICATE

This is to certify that thesis entitled, "IN VITRO SCREENING OF SALT TOLERANT GENOTYPES IN TOMATO (Solanum lycopersicum L.)" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in GENETICS AND PLANT BREEDING, embodies the result of a piece of bona fide research work carried out by MD RAIS UDDIN RASHED, Registration No: 06-01924 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly been acknowledged.

Naheed Zeba

.....

(Prof. Dr. Naheed Zeba)
Supervisor

Dated: June, 2013
Place: Dhaka, Bangladesh

DEDICATED TO
MY
BELOVED PARENTS

LIST OF ABBREVIATION

Abbreviations	Full word
°C	Degree Celsius
%	Percentage
1N	1 Normal
BBS	Bangladesh Bureau of Statistics
BARI	Bangladesh Agricultural Research Institute
Cm.	Centimeter
et al.	And others
etc.	Etcetera
FAO	Food and Agricultural Organization
G	Gram
g/L	Gram per liter
i.e	That is
IAA	Indole-3-Acetic Acid
Intl.	International
J.	Journal
Mg	Milligram(s)
mg/L	Milligram per liter
Ml	Milliliter
MS	Murashige and Skoog
PEG	Polyethelene Glycol
No.	Number
Ph	Negative logarithm of hydrogen ion concentration (-log[H+])
SAU	Sher-e-Bangla Agricultural University
Sci	Science
Univ.	University

ACKNOWLEDGEMENT

All praises gratitude and thanks are due to "Almighty Allah" who enabled the author to complete the work successfully.

The author would like to express his most sincere and deepest sense of gratitude profound respect to his supervisor, Professor Dr. Naheed Zeba Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University Dhaka-1207, Bangladesh for her scholastic guidance, valuable suggestions, constructive criticisms, helpful comments and constant encouragement and supervision throughout the research work and in preparing this thesis.

The author also expresses her gratitude, profound respect and indebtedness to his teacher and research co-supervisor, Prof. Dr. Md. Sarowar Hossain Dept. of Genetics and Plant Breeding Sher-e-Bangla Agricultural University Dhaka-1207, Bangladesh for his valuable suggestions, constructive criticisms and helpful advice during the period of research work and preparation of this thesis.

The author would like to express his sincere respect to Dr. Saiful Islam, Chairman and Associate Professor, Department of Genetics and Plant Breeding for his advices and all other logistic and academic supports.

The author is thankful to all of the teachers of the Department of Genetics and Plant Breeding specially to Professor Dr. Md. Shahidur Rashid Bhuiyan and Professor Dr. Md. Firoz Mahmood for their kind encouragement and valuable advices. All of the officer and staffs were helpful during the research period. The author is thankful to all of them.

The author is also highly indebted to his beloved parents who always helped and inspired with their blessings to complete this study. The author is thankful to his brothers, sisters, friends and relatives for their encouragement to complete this thesis.

The Author

CONTENTS

CHAPTER	TITLES	PAGE NO.
ABBREVIATIONS		i
ACKNOWLEDGEMENTS		ii
CONTENTS		iii
LIST OF TABLES		vi
LIST OF FIGURES		vii
LIST OF PLATES		ix
ABSTRACT		x
CHAPTER I	**INTRODUCTION**	01
CHAPTER II	**REVIEW OF LITERATURE**	03
2.1	Tomato status in Bangladesh compared to world aspects	03
2.2	*In vitro* investigations in tomato	04
2.3	Salt tolerance in tomato *in vitro*	05
CHAPTER III	**MATERIALS AND METHODS**	11
3.1	Experimental site	11
3.2	Plant materials	11
3.2.1	Selection of suitable genotypes	11
3.2.2	Experimental materials *in vitro*	11
3.3	Laboratory preparation	14
3.3.1	Chemicals	14
3.3.2	Instruments	14

CHAPTER	TITLES	PAGE NO.
3.4	Culture media	16
3.4.1	Preparation of the stock solutions	16
3.4.1.1	Stock solution of macronutrients (stock 1)	16
3.4.1.2	Stock solution of micronutrients (stock 2)	17
3.4.1.3	Stock solution of iron (Fe-EDTA) (stock 3)	17
3.4.1.4	Stock solution of vitamins (stock 4)	17
3.4.1.5	Preparation of 1N NaOH	18
3.4.1.6	Preparation of 70% Ethanol	18
3.4.1.7	Preparation of 50mM NaCl concentration with MS medium	18
3.4.1.8	Preparation of 50mM NaCl concentration with MS medium	18
3.4.1.9	Preparation of 50mM NaCl concentration with MS medium	18
3.4.1.10	Preparation of 50mM NaCl concentration with MS medium	18
3.4.2	MS Media preparation	19
3.4.3	pH of the medium	19
3.4.4	Agar	19
3.5	Sterilization	20
3.5.1	Sterilization of culture media	20
3.5.2	Sterilization of glassware and instruments	22
3.5.3	Sterilization of culture room and transfer area	22
3.5.4	Sterilization of seed	22
3.6	Inoculation and culture	23
3.7	Salt tolerance assay	24
3.8	Precaution of ensure aseptic conditions	24

CHAPTER	TITLES	PAGE NO.
CHAPTER IV	**RESULTS & DISCUSSION**	25
4.1	Mean performance of different tomato genotypes	25
4.2	Root Length and Plant Weight after inoculation in different NaCl concentrations	27
4.3	Root reduction and weight reduction rate	38
4.4	Genotypic distribution of Length LS-means of genotypes	53
SUMMARY AND CONCLUSION		59
REFERENCES		61

LIST OF TABLES

TABLE	TITLE	PAGE NO.
1.	List of the Tomato genotypes used in the experiment	13
2.	Root length reduction (cm) under different salt concentrations	40
3.	Fresh weight reduction (g) under different salt concentrations	44
4.	Analysis of variance for root length	47
5.	Least Squares Means for effect genotype for length	49
6.	T grouping for root length	50
7.	Analysis of Variance for weight	51
8.	Least Squares Means for effect genotype for weight	52
9.	T grouping for plant weight	55

LIST OF FIGURES

FIGURE	TITLE	PAGE NO.
1.	Average root Length after 5days from inoculation in 0mM NaCl concentration	27
2.	Average root Length after 9 days from inoculation in 0mM NaCl concentration	28
3.	Average weight after 9 days from inoculation in 0mM NaCl concentration	29
4.	Average root Length after 5days from inoculation in 50mM NaCl concentration	29
5.	Average root Length after 9 days from inoculation in 50mM NaCl concentration	30
6.	Average weight after 9 days from inoculation in 50mM NaCl concentration	30
7.	Average root Length after 5 days from inoculation in 100mM NaCl concentration	31
8.	Average root Length after 9 days from inoculation in 100mM NaCl concentration	31
9	Average weight after 9 days from inoculation in 100mM NaCl concentration	32
10.	Average root length after 5 days from inoculation in 200mM NaCl concentration	34
11.	Average root length after 9 days from inoculation in 200mM NaCl concentration	34
12.	Average weight after 9 days from inoculation in 200mM NaCl concentration	35

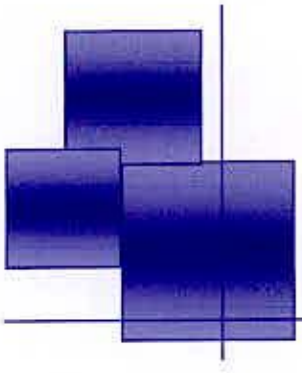
13.	Average root length after 5 days from inoculation in 250mM NaCl concentration	35
14.	Average root length after 9 days from inoculation in 250mM NaCl concentration	36
15.	Average weight after 9 days from inoculation in 250mM NaCl concentration	36
16.	Comparison of root length in different genotypes of tomato with or without salt	39
17.	Comparison of root reduction length in different genotypes of tomato under salt stress	41
18.	Comparison of root reduction length in different genotypes of tomato under salt stress.	43
19	Comparison of weight reduction in different genotypes of tomato under salt stress.	45
20.	Comparison of the Length LS-Means with different genotypes	46
21.	Root length comparison for genotypes	48
22.	Genotypic distribution of Weight LS- means of genotypes	53
23.	Weight Comparison for different genotypes	54

LIST OF PLATES

PLATE	TITLE	PAGE NO.
1.	Selection of vigorous genotypes for experimental materials *in vitro*.	12
2.	Aliquote of culture media under laminar hood	21
3.	Inoculation and incubation for germination and salt tolerance assay	23
4.	*In vitro* germination of seeds and inhibition of root elongation by NaCl	26

ABSTRACT

Soil salinity is one of the most important abiotic stress that limit crop production. Tomato (*Solanum lycopersicum* L.) is moderately tolerant to salinity and is typically cultivated in regions that are exposed to soil salinization. The aim of the study was to characterize phenotype response to salt stress under *in vitro* conditions of fourteen tomato genotypes BARI Tomato-2, BARI Tomato-11, BD-7260, BD-7290, BD-7295, BD-7286, BD-7269, BD-7258, BD-7289, BD-7292, BD-7291, BD-7302, BD-7301, and BD-7762.Tomato seeds were cultured with 0mM (control), 50mM, 100mM, 150mM and 250mM NaCl in nutrient solutions. The effect of the stress applied on the morphological traits was evaluated in 9 day-old seedlings. The analysis of variance showed that the highest root length was grown on 50mM NaCl solution. The shortest root length was observed enties BD-7260 at 250mM. Longer roots (11.6 cm) were developed by the plants from the solutions containing 50mM NaCl in entries BD-7302. NaCl concentration in the medium significantly affected the root length and plant weight of tomato. Genotypic distribution of weight LS means revealed that genotypes BARI-2 and the Line BD-7292 is the highest performed and the Line BD-7762 is the lowest performed. These findings indicated some salt tolerant tomato genotypes which will be promising for future hybridization program. Analysis of novel genes as well as some previously identified genes such as *PIPs*, *LTPs*, *AGPs*, *PRPs*, *GRPs* etc. which showed protective roles in different abiotic stresses to other crops is quiet necessary and the presence and expression pattern of those genes in these screened genotypes of tomato will provide powerful information for over-expression of those genes in transgenic plants those will confer salt tolerances to the cultivated tomato varieties.



Chapter 1

Introduction

CHAPTER I

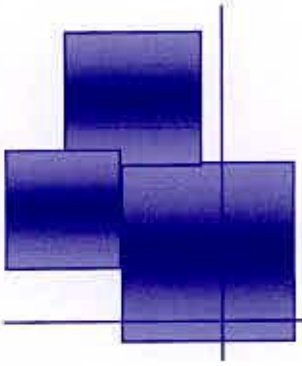
INTRODUCTION

High salinity is one of the major stress factors among the abiotic stresses. In the world, about 400 million hectares of land are affected by high salinity. In Bangladesh about 1 million hectares of land are affected by high salinity in the coastal regions. Salinity affects almost every -aspect of the physiology and biochemistry of plants and significantly reduces yield. As saline soils and saline waters are common around the world, great effort has been devoted to understand its physiological aspects of tolerance to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes. In spite of this great effort, only a small number of cultivars, partially tolerant to salinity, have been developed. Further effort is necessary if the exploitation of saline soils and saline waters that are not currently usable is to be achieved. Salinity affects yield quality and quantity, so that yield contributing characters must be taken into addressed when breeding for salinity tolerance. Not only are the yield-related characters important. As salinity affects almost every aspect of the physiology and biochemistry of the plant, the enhancement of crop salt tolerance will require the combination of several to many physiological traits (Cuartero *et al.*, 2006; Flowers and Yeo, 1995; Cuartero and Ferna´ndez-Mun˜oz, 1999), not simply those directly influencing yield. As salinity in soils is variable and plant tolerance depends on the stage of plant development, plants should be phenotyped at several salinity concentrations and at the most sensitive plant stage(s).

Tomato (*Solanum lycopersicum* L.) is one of the most important solanaceous vegetable crops in the world in terms of both production and harvested area (FAOSTAT, 2005). Though it is a self crossing annual crop, now a days, tomatoes are attempted to grow round the year. Due to increasing consumption of its products, the crop is becoming promising. Tomato is a favorable food crop for *in vitro* studies due to its low chromosome number i.e., 2n=2x=24

and due to comprehensive knowledge of tomato genetics. Plant tissue culture techniques are recognized as useful instruments in tomato improvement. Several *in vitro* investigations have been conducted on tomato in different applications. The genetics of physiological characters together with other tolerance components related to metabolic defenses against salinity have to be studied in order to advance the breeding of tomato genotypes tolerant to salinity. Despite the present limitations, it is foreseeable that our ability to design the future breeding programmes based on genetic transformation will be strengthened with the data obtained from ongoing functional genomics projects. Seedling pretreatment with NaCl are interesting strategies to be applied when tomato plants have to be grown in saline soils or soils irrigated with saline water, the stress level necessary to trigger any adaptive response seems to be related to the degree of tolerance of the genotype. Increasing humidity around tomato plants effectively alleviates the deleterious effects of salt on tomato-plant growth and on fruit yield. Grafting tomato plants onto appropriate rootstocks also increased salt tolerance. This study was conducted to explore the bioassay so as to establish a reproducible protocol for screening of different genotypes of tomato in different concentrations of NaCl. With conceiving the above scheme in mind, the present research work has been undertaken in order to fulfilling the following objectives:

- to optimize the protocol for growing tomato seedlings under control and different salt concentrations
- To screen out the better salt tolerant genotypes
- To bring the salt prone areas under the crops for potential yields
- To screen out the suitable genotypes under salt stress which are likely to provide superior segregates' on hybridization



Chapter 2
Review of literature

CHAPTER II

REVIEW OF LITERATURE

Tomato is one of the most popular and widely grown vegetable in the world ranking second in importance to potato in many countries. Tomato is used as a fresh vegetable and can be processed as paste, juice, ketch-up, sauce, powder or as a whole. Nutritionally, it is a significant dietary source of minerals, vitamin A and C, organic acid and essential amino acids. Its centre of origin is presumed to be in the present state of Mexico. It is believed that the tomato was introduced in subcontinent during the British regime. It is popular for its taste, nutritional status and various uses. The crop is adapted to a wide variety of climates ranging from the tropics to a few degree of the Arctic Circle. The present leading tomato producing countries of the world are China, United States of America, Turkey, India, Egypt, Italy, Iran, Spain, Brazil Mexico, and Russia (FAO, 2010).

2.1 Tomato status in Bangladesh compared to world aspects

Bangladesh is producing a good amount of tomatoes. In Bangladesh tomato has great demand throughout the year but it is available and cheaper during the winter season. In Bangladesh it is cultivated as winter vegetable, which occupies an area of 58854 acres in 2009-10 (BBS, 2010). The total production of tomato was 339 lac tons in China, 137 lac tons in USA, 109 lac tons in Turkey, 103 lac tons in India and 92 lac tons in Egypt in 2008 (FAO, 2010). The total production of tomato was 190 thousand metric tons in Bangladesh in the year of 2009-2010 (BBS, 2010). The average tomato production in Bangladesh is 50-90 tons/ha (BARI, 2010). The best tomato growing areas in Bangladesh are Dinajpur, Rajshahi, Dhaka, Comilla and Chittagong

2.2 *In vitro* investigations in tomato

Several *in vitro* investigations have been conducted on tomato in different applications i.e., production of virus free plants (Moghaieb *et al.*, 2004), genetic transformation (Park *et al.*, 2003) and studies about the effect of variety and plant growth regulators on callus proliferation and regeneration (Chaudhry *et al.*, 2007). Most of the reports about adventitious regeneration in tomato deal with induction of regeneration in hypocotyls or cotyledon explants (Moghaieb *et al.*, 2004, Brichkova *et al.*, 2002, Raiziuddin *et al.*, 2004). Shoot formation from different explants as apical meristem, cotyledons, stems internodes, leaves, anthers and inflorescences has been reported in tomato (Afroz *et al.*, 2010; Jatoi *et al.*, 1999, 2001; Young *et al.*, 1987; Branca *et al.*, 1990; Compton and Veilleux 1991). *In vitro* anther culture stands out and is an increasingly powerful tool when integrated into breeding programs (Jose, 2007; Hu and Zeng, 1984). This technique allows the acceleration of plant breeding by providing homozygous doubled haploids within a comparatively short time (Nurhidayah *et al.*, 1996). In addition, obtaining haploid plants from segregant generations facilitates genetic analysis, eliminating the complexity of the heterozygous state (Moraes-F, 1990). Improving the quality of *in vitro* cultured shoots of tomato by using activated charcoal and ascorbic acid is evaluated by Bhatia and Ashwath (2008). *In vitro* culture of immature seed for rapid generation advancement in tomato studied by Bhattarai *et al.* (2009). This offers an opportunity for rapid generation advancement aimed towards population development when coupled with marker assisted selection in tomato breeding for biotic and abiotic stress tolerance. Intra and interspecific variability of *in vitro* culture response in tomatoes were performed by Pratta *et al.* (1997). Embryogenesis induction, callogenesis, and plant regeneration by *in vitro culture* of tomato isolated microspores and whole anthers discussed their application to the production of doubled-haploid plants in tomato (Simarro and Nuez, 2007). Isotopically labeled tomato carotenoids,

phytoene, phytofluene, and lycopene, are needed for mammalian bioavailability and metabolism research but are currently commercially unavailable. Engelmann *et. al.* (2010) established and screened multiple *in vitro* tomato cell lines for carotenoid production, test the best producers, and to use the greatest carotenoid accumulator for *in vitro* 13C-labeling. *In vitro* selection and screening of abiotic stress tolerant cell lines for genetic variability analysis have been reported in tomato (Buiatti *et al.*, 1984; Cano *et al.*, 1996; Yusuf *et al.*, 1994; Cano *et al.*, 1998; Mercado *et al.*, 2000; Martinez *et al.*, 1996)

2.3 Salt tolerance in tomato *in vitro*

Soil salinity is one of the most important abiotic stress that limit crop production (Osman *et al.*, 2011; Debez *et al.*, 2006; Koyro, 2006). Up to 20% of the irrigated arable land in arid and semiarid regions is already salt affected and is still expanding (Muhling and Lauchli, 2003).

Tomato (*Solanum lycopersicum* L.) is moderately tolerant to salinity and is typically cultivated in regions that are exposed to soil salinization (Cuartero *et al.*, 1999). In Bangladesh, tomato is important vegetable crop ranks second to potato among vegetable crops based on cultivated area. It is grown throughout the country where irrigation water and arable land are available. Its production is affected by various stresses such as disease, high temperature, draught, salinity and its vulnerability to frequent insect and pest attacks. Hence, there is a need to select salt tolerance cultivar of tomato using modern biotechnological approaches.

Salt tolerance in plants depends primarily on the genotype which determines the alteration on processes such as, exclusion of the salt, uptake and transport of salt by roots, together with metabolic and physiological events occurring at cellular level (Silva, *et al.*, 2001). The selection of salt tolerant lines continues to challenge plant scientists, especially those working in physiology and genetics.

Most crop plants, including the cultivated tomato, are sensitive to salinity, although differences between tomato cultivars have been reported (Rus-Alvarez, *et al.*, 1994; Cano *et al.*, 1996). One strategy to reduce the deleterious effects of soil salinity on tomato production is by development of Salt -tolerant cultivars (Nabors *et al.*, 1980). The screening of a large number of genotypes for salinity tolerance under *ex vitro* conditions is rather difficult since it requires a large amount of resources and space and complex interactions between the plant and different soil components. *In vitro* culture, on the other hand, is an ideal system for screening salt-tolerance in plants, since it can be carried out under controlled conditions with limited space and time (Ghoshal and Bajaj, 1984). Therefore, many attempts have been made to screen genotypes *in vitro* using shoot apices. Chandler *et al.*, (1988) screened genotypes of sugar beet, tobacco, Chinese cabbage and canola on media with different salt concentrations. *In vitro* culture of tomato has been successfully exploited for selection of tolerant cell lines for various abiotic stresses under laboratory conditions, by exploiting the genetic variability arising during *in vitro* culture conditions (Buiatti *et al.*, 1984). It requires comparatively less effort and fewer resources than selection under field conditions. Selection for salinity tolerance can be carried out *in vitro*, by culturing explants, callus, cell suspension, protoplasts, embryos or microspores in the presence of screening agent, e.g. NaCl (Cano *et al.*, 1996). *In vitro* selection and screening for salinity tolerance have been reported in tomato by (Yusuf *et al.*, 1994; Cano *et al.*, 1998; Mercado *et al.*, 2000) An *in vitro* shoot apex culture could be a better system for testing and selecting for salt tolerance (Martinez *et al.*, 1996).

Significant differences were also found among genotypes in several other published reports. Genotypic variation was found when seeds of fourteen tomato (*Lycopersicon esculentum* Mill.) cultivars were germinated under 0, 25, 50, 75, 100 mM NaCl (Mohammad *et al.*, 2006). According to their germination

response, the cultivars were selected as, salt-tolerant and moderately salt-tolerant and salt sensitive. Differences were also found from callus of 0.2 g in callus relative growth rate (RGR), fresh and dry weights, proline, Na^+ and K^+ contents from 0.2 g callus of hypocotyls in tomato grown under previous salt levels for four weeks. Rooting parameters are the most useful traits for rapid evaluation and screening of tomato species and segregating populations through *in vitro* shoot apex culture (Cano *et al.*, 1998). He studied the possibility of using *in vitro* shoot apex culture to evaluate salt tolerance of cultivated (*Lycopersicon esculentum* Mill.) and wild (*Lycopersicon pennellii* (Correll) D'Arcy) tomato species and related to the response obtained by callus culture. Both apices and calluses were grown on media supplemented with 0, 35, 70, 105, 140, 175 and 210 mM NaCl, and growth and physiological traits were determined. Most apices of *L. esculentum* did not develop roots from low NaCl levels, whereas the apices of *L. pennellii* were able to develop roots at the different salt levels. This different degree of salt tolerance between *L. esculentum* and *L. pennellii* was not, however, clearly shown on the basis of the shoot growth of the plantlets. The callus response was similar to that shown by the rooting parameters, as callus growth in response to increased salinity was much greater in *L. pennellii* than in the tomato cultivar. K^+ decreased more and proline accumulated less with salinity in shoots of *L. esculentum* compared to *L. pennellii*, whereas the opposite response was obtained in calluses. Liza *et al.*, also evaluated salt tolerance activity of callus in different genotypes of tomato where she used the cotyledon as explants and induced in MS medium which was supplemented with different concentrations of hormones for callus induction.

Performance of salt-tolerant selected genotypes of two processing tomato varieties (*Lycopersicon esculentum* Mill.), Riogrande and Chibli F_1 conducted by Messai *et al.*, (2007) , derived from *in vitro* regeneration under salt stress (34 mM), was evaluated under greenhouse conditions. This study was conducted with salinized

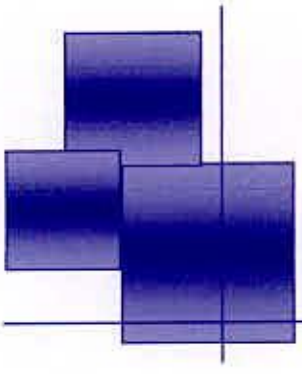
solution culture at 10 mM (control) and 44 mM NaCl. The following parameters were recorded, plant height, plant dry weight, number of tomato fruits/plant, fruit weight and size, fruit yield/plant and fruit quality. Results showed that salt-tolerant plants of both varieties produced fruits with a better quality. There was an increase of total soluble solids content (+47% Chibli F1 and +33% Riogrande, regarding control), fruit firmness (+33% Chibli F1 and +25% Riogrande) and a decrease of fruit juice pH. However, this increase in fruit quality was associated with a decrease in fruit size (-15% Chibli F1 and -20% Riogrande) and weight (-16% Chibli F1 and -19% Riogrande). Nevertheless, a significant increase in fruit yield was observed (+30% Chibli F1 and +20% Riogrande). These findings show also a better salt tolerance of Chibli F1 regenerated plants and they may be useful for exploitation of saline water (34 to 50 mM of NaCl) of Sahel regions in Tunisia.

Smolik *et al.*, (2011) carried out an experiment that characterize phenotype response to salt stress under *in vitro* conditions of four tomato genotypes:'Malinowy Ożarowski', 'Pokusa', 'Awizo' F1 and *Lycopersicon.* Tomato seeds were cultured with 0(control), 50, 75, 100 and 125 mmol·dm-3 NaCl in nutrient solutions. The effect of the stress applied on the morphological traits was evaluated in 14 day-old seedlings. The contents of proline in tomato shoots were also examined. Statistical correlations were found in shoot and root length as well as in the number of roots, with the exception of wild form *L. chmielewskii.* The analysis of variance showed that the highest shoots were grown on 50 and 100 mmol·dm-3 NaCl solution. The shortest shoots were observed in the control. Longer roots (8.6 cm) were developed by the plants from the solutions containing 100 mmol·dm-3 NaCl, the shortest (6.6 cm) – 75 mmol·dm-3 NaCl. NaCl concentration in the medium significantly affected the number of tomato roots.

Emilio *et al* (2010) conducted an experiment that the possibility of using *in vitro* shoot apex culture to evaluate salt tolerance of cultivated (*Lycopersicon esculentum* Mill.) and wild (*Lycopersicon pennellii*(Correll) D'Arcy) tomato species was determined and related to the response obtained by callus culture. Both apices and calluses were grown on media supplemented with 0, 35, 70, 105, 140, 175 and 210 mMNaCl, and growth and physiological traits were determined. Most apices of *L. esculentum*did not develop roots from low NaCl levels, whereas the apices of *L. pennellii*were able to develop roots at the different salt levels. This different degree of salt tolerance between *L. esculentum*and *L. pennellii*was not, however, clearly shown on the basis of the shoot growth of the plantlets. The callus response was similar to that shown by the rooting parameters, as callus growth in response to increased salinity was much greater in *L. pennellii*than in the tomato cultivar. KC decreased more and proline accumulated less with salinity in shoots of *L. esculentum* compared to *L. pennellii*, whereas the opposite response was obtained in calluses. The results obtained in this study suggest that rooting parameters are the most useful traits for rapid evaluation and screening of tomato species and segregating populations through *in vitro* shoot apex culture.

Aazami *et al.*, (2010) was conducted an esperiment that the response of calli of six tomato cultivars (*Lycopersicon esculentum* Mill.) to salt stress was investigated under *in vitro* conditions. Callus relative growth rate (RGR), dry matter percentage (DM), osmotic potential and proline content were evaluated. Significant differences were found among cultivars regarding above traits. 'PS-10' had the highest RGR, while 'Roma' had the lowest amount of this trait under salt levels. Any increase in salinity levels in the media led to decrease of RGR and in contrast increased DM and osmotic potential in all treatments compared to control. In all cultivars, proline levels increased in response to salinity stress. High callus formation was correlated with low

proline content. 'PS-10' and 'Imperial' had the highest callus formation and the lowest proline content. Significant differences were recorded in regeneration potential of cultivars under salt treatments. 'PS- 10' possessed the highest and 'Roma' had the lowest regeneration rate. It is concluded that the more the salt tolerant genotype the more is the reduction in osmotic potential and proline content.



Chapter 3

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

In pursuance of the stated objectives of the present thesis work experiments were conducted with the materials and the methodologies as described in the following subsections of this chapter.

3.1 Experimental site

The experiment was carried out at the Genetics and Plant Breeding Laboratory, Sher-e-Bangla Agricultural University, Dhaka period of March, 2011 to September, 2012. The place is geographically located at about $24^0$75' North latitude and $90^0$50' East longitude (Khan, 1997).

3.2 Plant materials

3.2.1 Selection of suitable genotypes

A total of 41 tomato genotypes were collected around 20 seeds of each were sown in small plastic pots and covered with swaran wrap. The pots were kept in growth chamber in controlled environment 25°C under 16 h photoperiod at 50 µmol/ m^2/s^{-1} (with white fluorescent lamp) (Plate 1A). The genotypes were selected after germination based on their vigorous appearance (Plate 1B).

3.2.2 Experimental materials *in vitro*

Out of 41 tomato collection a total of 14 genotypes were selected based on their germinability and seedling vigourness as experimental materials. Among them BARI Tomato 2, BARI Tomato -11 were recommended standard verities while the others were selected lines. Collection was made from Horticulture research centre of Bangladesh Agricultural Research Institute (BARI), Gazipur. The genotypes used in the study are listed below:

Plate.1: Selection of vigorous genotypes for experimental materials *in vitro*. **A.** Germination of seeds. **B.** Selection of genotypes based on seedling vigourness. Twenty seeds of 41 each genotype were sown in plastic pots and covered with thin polythene sheet (swaran wrap). With few holes on it for proper aeration.

Table 1: List of the Tomato genotypes used in the experiment

Serial Number	Variety name and accession number
01	BARI Tomato-2
02	BARI Tomato-11
03	BD-7260
04	BD-7290
05	BD-7295
06	BD-7286
07	BD-7269
08	BD-7258
09	BD-7289
10	BD-7292
11	BD-7291
12	BD-7302
13	BD-7301
14	BD-7762

3.3 Laboratory materials and preparation

The following chemicals, culture media preparations along with the instruments were used throughout the experimentation

Laboratory preparation was started in early January 2011 by collecting chemical and instruments.

3.3.1 Chemicals:

- MS medium
- Sterilizing chemicals
    a. Sodium hypo chloride
    b. Potassium hypo chloride
- Distiled water
- Normal water
- Sucrose
- Agar
- NaOH (10 N, 1N)
- HCl
- KCl (3M)
- NaCl (laboratory grade)
- Absolute Ethanol
- Ethanol (70%)
- Methilated spirit

3.3.2 Instruments:

- Autoclave
- Hotplate with magnetic stirrer
- Automatic drying oven
- Freezers
- Furnaces

- Incubators
- Laminar Air Flow Chamber
- Microwave oven
- Pipettors
- Plant Growth Chamber
- Safety Cabinets
- Shakers
- Shaking Incubator
- Water Purification System
- pH meter
- Course and fine electric balances
- Scalpel, forceps, scissors etc.
- Culture vials (petridishes, test tubes, culture bottles etc.)

3.4 Culture media

Success of any experiment depends on the culture media, hormone combination, tissue and employing cell. Murashige and Skoog (1962) medium (powder and prepared) were used with different NaCl concentration as culture medium for root and shoot growth study. The composition of MS medium has been presented in Appendix 1. NaCl were added to MS media as per treatment of the experiment. For the preparation of media, stock solutions were prepared at the beginning and stored in the refrigerator at 4±1°C. The respective media were prepared from stock solutions.

3.4.1 Stock solution Preparation

The first step in the preparation of the medium is the preparation of stock solutions of the various constituents of the MS medium. As different media constituents were required in different concentrations, separate stock solutions for the macronutrients, micronutrients, Fe-EDTA (Iron stock), vitamins and growth regulators were prepared separately as ready use.

3.4.1.1 Stock solution of macronutrients (stock 1)

Stock solution of macronutrients was prepared with 10 times the final strength of the medium in one liter of distilled water (DW). Ten times the weight of the salts required for one liter of medium weighted accurately. Dissolve all the macronutrient one by one except $CaCl_2$. The stock solution of $CaCl_2$ should be prepared separately in order to avoid precipitation. And in this way, dissolved all the salts thoroughly in 750 ml of distilled water and final volume was made up to one liter by further addition of DW. The stock solution was poured into a clean sterilized glass container and stored in a refrigerator at 4°C for ready use.

3.4.1.2 Stock solution of micronutrients (stock 2)

A stock solution of all the micronutrients with 100x concentration is generally prepared. Since copper and cobalt are required in very small quantities, it is preferable to first make a separate stock solution of those two salts (100*) and then an appropriate volume can be pipetted and put into the main micronutrient stock solution. This stock solution was also stored in refrigerator at 4°C.

3.4.1.3 Stock solution of iron (Fe-EDTA) (stock 3)

Iron-EDTA should be added fresh and it was made 100 times the final strength of the medium in one liter DW. Here, two constituents, $FeSO_4.7H_2O$ and Na_2EDTA, were dissolved in 750 ml of DW in a conical flask by heating in a water bath until the salts dissolved completely and final volume was made up to one liter by further addition of DW. This stock should be stored in an amber color bottle or a bottle covered with an aluminum foil and stored in refrigerator at 4°C.

3.4.1.4 Stock solution of vitamins (stock 4)

The following vitamins were used in the present study for the preparation of MS medium.

Myo-inositol (Inositol)

Nicotinic acid (Vitamin B_3)

Pyridoxin HCl (Vitamin B_6)

Thiamine HCl (Vitamin B_1)

Glycin

Each of the vitamins except myo-inositol were taken at 100 times of their final strength in measuring cylinder and dissolved in 400 ml of distilled water. The final volume was made up to 1000 ml by further addition of distilled water. This stock solution was also labeled and stored in a refrigerator at 4°C.

3.4.1.5 Preparation of 1N NaOH

40 g NaOH pellets were weighed and dissolved in 900 ml. of sterilized distilled water under stirring condition. The flask in a thermostat at 20^0C and maintain for 1 hour and volume with sterilized distilled water upto 1 L.

3.4.1.6 Preparation of 70% Ethanol

In a 100 ml measuring cylinder 70 ml 99.9% ethanol was poured. Double distilled water was poured up to the level of 100 ml. The solution was stored in a sterilized glass bottle. This solution was made fresh each time before use.

3.4.1.7 Preparation of 50mM NaCl concentration with MS medium

The molecular weight of NaCl is 58.44 gm. For preparing 1 M NaCl solution we need to add 58.44 g NaCl in I000ml distilled water. So for preparing 50mM concentration, 2.92g of NaCl was added to the MS medium.

3.4.1.8 Preparation of 100mM NaCl concentration with MS medium

For 100mM concentration, 5.84 g of NaCl was added to the 1 L of MS medium.

3.4.1.9 Preparation of 200mM NaCl concentration with MS medium

For 200mM concentration, 11.68 g of NaCl was added to the 1 L of MS medium.

3.4.1.10 Preparation of 250mM NaCl concentration with MS medium

For 250mM concentration, 14.61 g of NaCl was added to the 1 L of MS medium.

3.4.2 MS Media preparation

To prepare one liter of MS medium, the following steps were followed:

1. 500 ml double distilled water was taken into 1 liter beaker
2. 100 ml of stock solution of macro-nutrients, 10 ml of stock solution of micro nutrient, 10 ml of stock solution of Fe-EDTA and 10 ml of stock solution of vitamins and growth regulators were added in this 500 ml double distilled water
3. 30g of sucrose was dissolved in this solution with the help of magnetic stirrer
4. Different concentrations of hormonal supplements as required were added either in single or in different combination to this solution and were mixed thoroughly
5. Since each hormonal stock solution contained in 100 ml of solution, to make one liter of medium, addition of 1.0 ml/L and 1.5 ml/L NAA and 0.5 ml/L, 1.0 ml/L, 1.5 ml/L and 2.0 ml/L BAP singly was added to prepare 1 liter of medium
6. Later different combinations of these two hormones NAA and BAP respectively were used viz. (1.0+0.5), (1.0+1.0), (1.0+1.5), (1.0+2.0), (1.5+0.5), (1.5+1.0), (1.5+1.5), (1.5+2.0) mg/L
7. The whole mixture was then made up to 1 liter with further addition of double distilled water.

3.4.3 pH of the medium

pH of the medium was adjusted to 5.7±1 by pH meter with the addition of 1 N NaOH or 0.1 N HCl whichever was necessary.

3.4.4 Agar

The media was gelled with 8 g/L agar and the whole mixture was gently heated on microwave oven at 250 °C Temperature for 8-10 minutes.

3.5 Sterilization

3.5.1 Sterilization of culture media

Fixed volume of medium was dispensed into conical flasks. After dispensing the flasks were covered with aluminum foil paper and marked with different codes with the help of a permanent glass marker to indicate specific hormonal supplement. Then flasks were autoclaved at 15 psi pressure at 121°C for 20 minutes. The medium was then transfer into the culture room and cooled at 24°C temperature before used. Marking is also necessary.

Fixed volume of medium was aliquoted into petridishes under laminar hood (Plate 2). After dispensing the petridishes were covered with thin polythene (Swaran wrap) and marked with different codes with the help of a permanent glass marker to indicate specific NaCl supplements. The petridishes containing media could be store at 4^0C until use. Marking was done for identification.

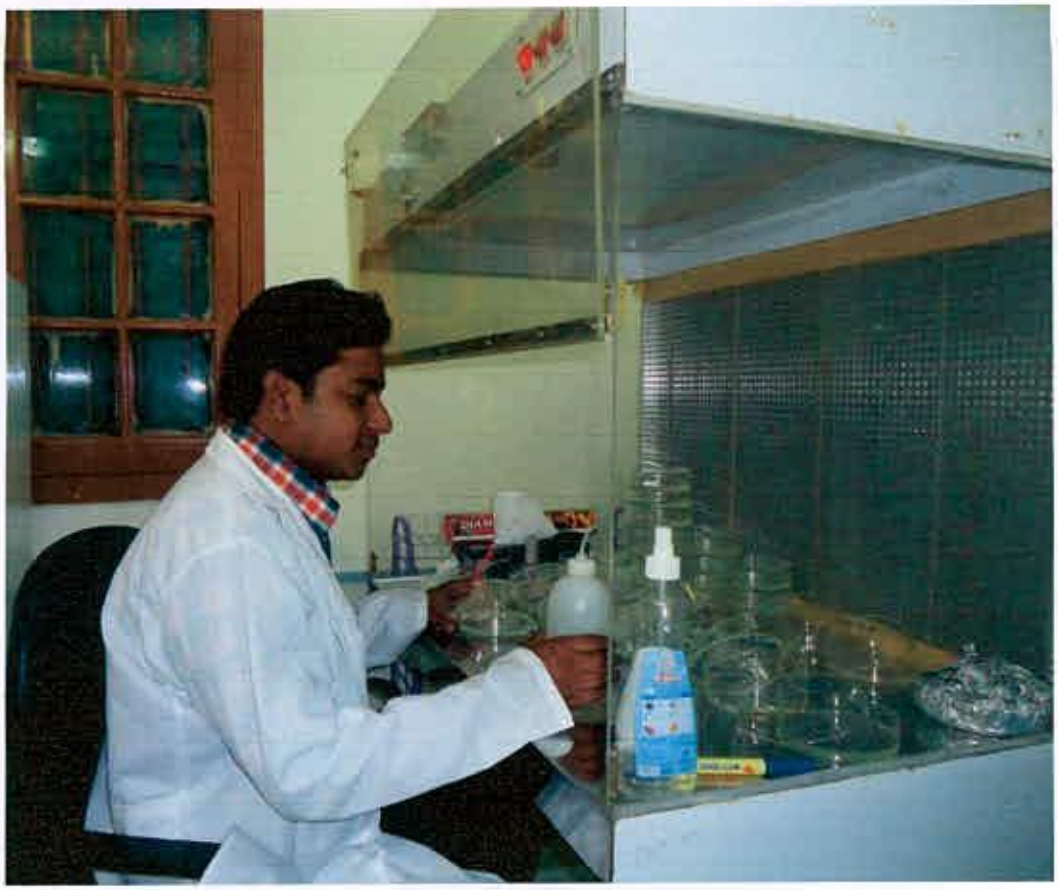


Plate 2. Aliquote of culture media under laminar hood.

3.5.2 Sterilization of glassware and instruments

Glassware, culture vessels, beakers, petridishes, pipettes, slides, plastic caps, other instruments such as forceps, needles, scissor, spatula, surgical blades, brush, cotton, instrument stand and aluminum foil were sterilized in an autoclave at a temperature of 121°C for 20 minutes at 15psi pressure. Before this, all types of glassware instrument was washed properly by liquid detergent, cleaned with running tap water and finally washed with distilled water.

3.5.3 Sterilization of culture room and transfer area

At the beginning, the culture room was spray with formaldehyde and then the room was kept closed for one day. Then the room was cleaned through gently washing the floors walls and rakes with a detergent. This is followed by careful wiping them with 70% ethanol. This process of sterilization of culture room was repeated at regular intervals. The transfer area was also cleaned with detergent and also sterilized twice in a month by 70% ethanol. Laminar air flow cabinet was usually sterilized by switching on the cabinet. The ultra violate ray kills the microbes inside the laminar airflow. It switches on 30 minutes before working in empty condition and for 20 minutes with all the instruments. The working surface was wiping with 70% ethanol, 30 minutes before starting the transfer work.

3.5.4 Sterilization of seed

Seed were treated with absolute alcohol for 1 minute. Then rinsed with distilled water for 2 times. Surface sterilization was done with NaOCl/CaOcl (20%) for 2 minute and again rins 5 times with distilled water.

3.6. Inoculation and culture

The sterilized seeds of fourteen genotypes of tomato were inoculated for germination. The petridishes were labeled properly. The culture environment was included, 25°C, 60% relative humidity, and a 16-h photoperiod from white fluorescent lamps (200µmol photons/m^2s^{-1}) (Plate 3A and B).

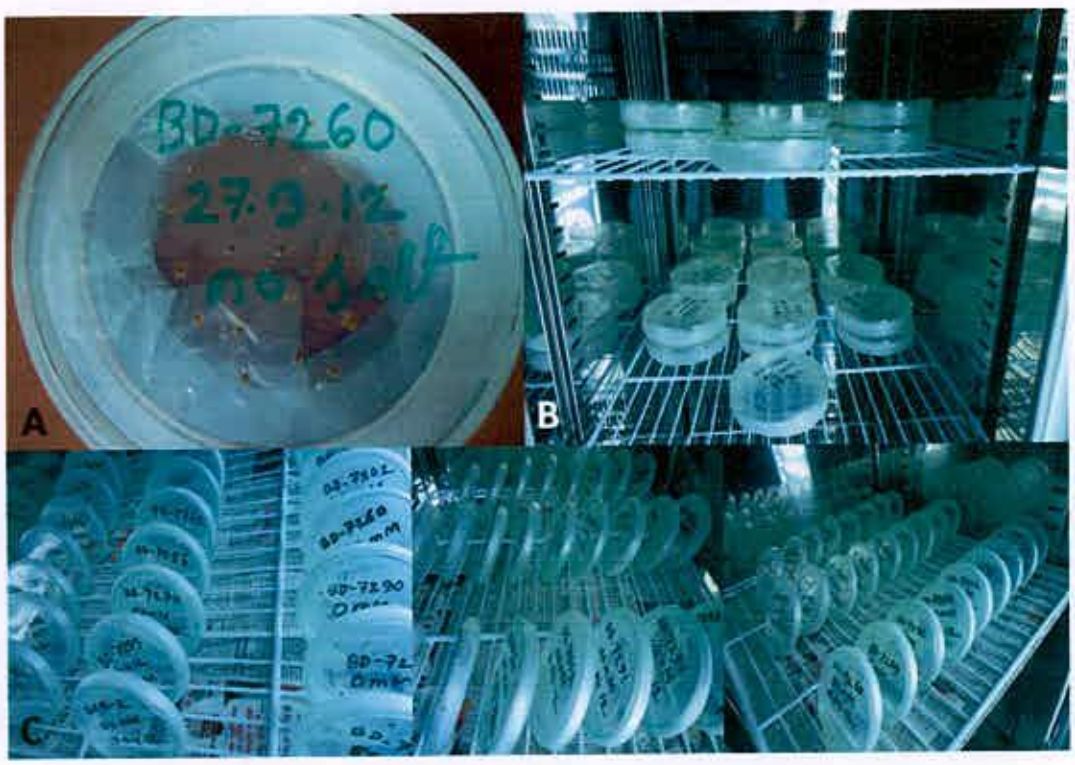


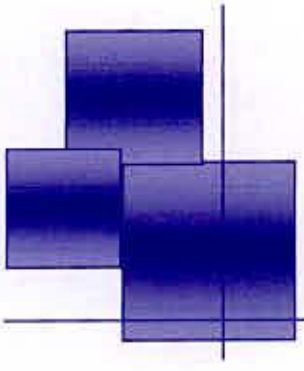
Plate 3. Inoculation and incubation for germination and salt tolerance assay. **A.** Inoculation of sterilized tomato seeds in half strength MS medium without salt. **B.** Incubation in growth chamber of sterilized tomato seeds in half strength MS medium without salt. **C.** Inoculation and incubation of four days old germinated plantlets in half strength MS medium supplemented with 0 mM, 50 mM, 100 mM, 200mM and 250 mM of NaCl. Incubation was done in growth chamber with 25 ± 1°C under 16 h photoperiod at 200 µmol/ m^2/s^{-1} (with white fluorescent lamp)

3.7 Salt tolerance assay

The salt tolerance assay was performed as Zeba,N (2009). Briefly, Four days old germinated seeds were inoculated in a linear order on MS medium supplemented with 0mM, 50mM, 100mM, 200mM and 250 mM of NaCl. Three germinated seed were inoculated per plate. The culture plates were kept in the growth chamber in vertical position (Plate 3C). The culture environment included 25°C, 60% relative humidity, and a 16-h photoperiod from white fluorescent lamps (200 µmol photons/m^2/s^{-1}). Average root length and weight was recorded on 5^{th} and 9^{th} days.

3.8 Precaution of ensure aseptic conditions

All inoculation and aseptic manipulations were carried out under laminar air flow cabinet. The cabinet was usually switched on with ultra violet light half an hour before use and wiped with 70% ethanol to reduce the chances of contamination. The instruments like scalpels, forceps, needles, surgical blades, scissor, pipettes, slides, plastic caps, spatula, brush, cotton etc. were presterilized by autoclaving and subsequent sterilization were done by dipping in 70% ethanol followed by flaming and cooling method inside the laminar flow cabinet. While not in use, the instruments were kept inside the laminar airflow cabinet into the instrument stand. Hands were also sterilized by 70% ethanol and wearing of hand gloves. It is also necessary to wear apron and mask to avoid contamination rate. Other required materials like distilled water, culture vessels, beakers, glass plates, petridishes etc. were sterilized in an autoclave following method of media sterilization. The neck of test tubes were flamed before open and also dipping with ethanol with the help of soaked cotton before closing it with the aluminum foil. Aseptic conditions were followed during each and every operation to avoid the contamination of cultures.



Chapter 4

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

4.1Performance of different genotypes under control at different salt concentration

To investigate the salt tolerance in 14 genotypes, the root growth assay was performed with the 4 days old plantlets grown on media containing 0 mM-250 mM NaCl. The trend of root growth were observed as in Plate 4 where it was highest in control condition (0mM of NaCl) and gradually decreased when the salt stress does increased to 50 mM , 100mM, 200mM and 250mM. The root length and fresh weight were measured and the results obtained from these studies have been presented and discussed separately under different heading. Each of the parameter as influenced by varieties, treatments and their combinations were discussed.

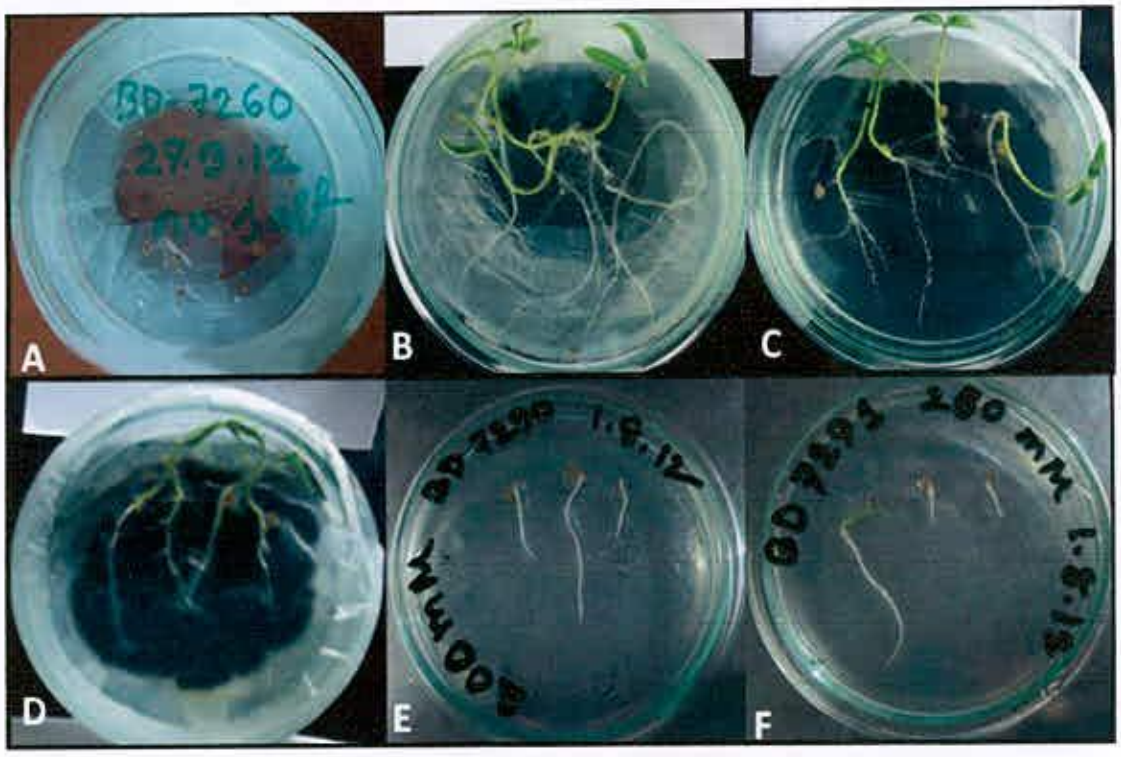


Plate. 4. *In vitro* germination of seeds and inhibition of root elongation by NaCl. **A.** Inoculation of sterilized seed in half strength MS media without salt for germination. **B-F.** Comparison of root length in tomato genotypes in half strength MS medium supplemented with 0 mM , 50mM , 100mM 200mM and 250mM of NaCl respectively. Four days old germinated plantlets plantlets were transferred to MS medium containing different concentrations of NaCl and grown in vertical position for 9 days.

4.2 Root Length and Plant Weight after inoculation in different NaCl concentrations

Length of root was recorded for different days after inoculation in different NaCl concentration and significant differences were recorded among genotypes. After 5 days of inoculation in case of 0 mM NaCl concentration highest root length was recorded in BD-7291 and the lowest root length was recorded with BD-7762 genotypes (Fig. 1). Similar trend was also observed at 9 days treatment. The variation occupied at 0mM NaCl both at 5th and 9th day's interval were due to genotypic differences among the tomato entries.

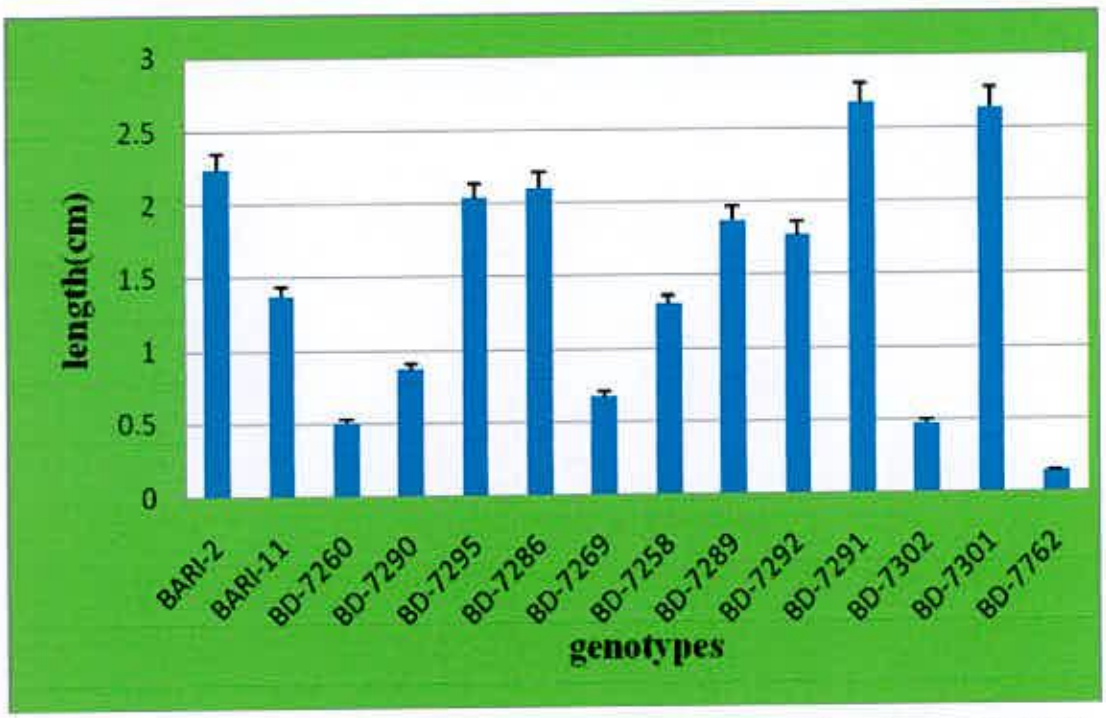


Fig. 1 Average root Length after 5days from inoculation in 0mM NaCl concentration

When the weight of regeneration plant were recorded for different days after inoculation in different NaCl concentration and significant differences also observed, In case of 0.0 mM NaCl concentration at 9 days after inoculation. The

highest weight was recorded with the entry in BD-7292 and the lowest length was recorded with BD-7762 genotypes (Fig. 3).

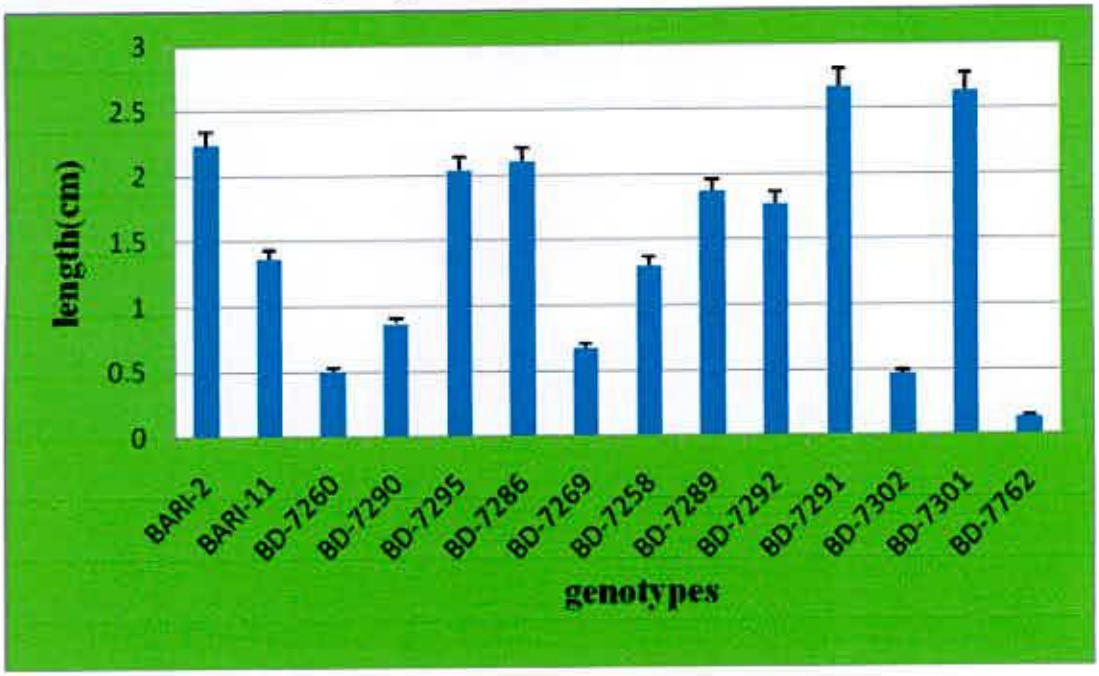


Fig. 2 Average root Length after 9 days from inoculation in 0mM NaCl concentration

After 5 days of inoculation in case of 50 mM NaCl concentration the highest root length was recorded in BD-7292 genotypes its lowest length was recorded in BD-7260 genotypes respectively (Fig. 4).

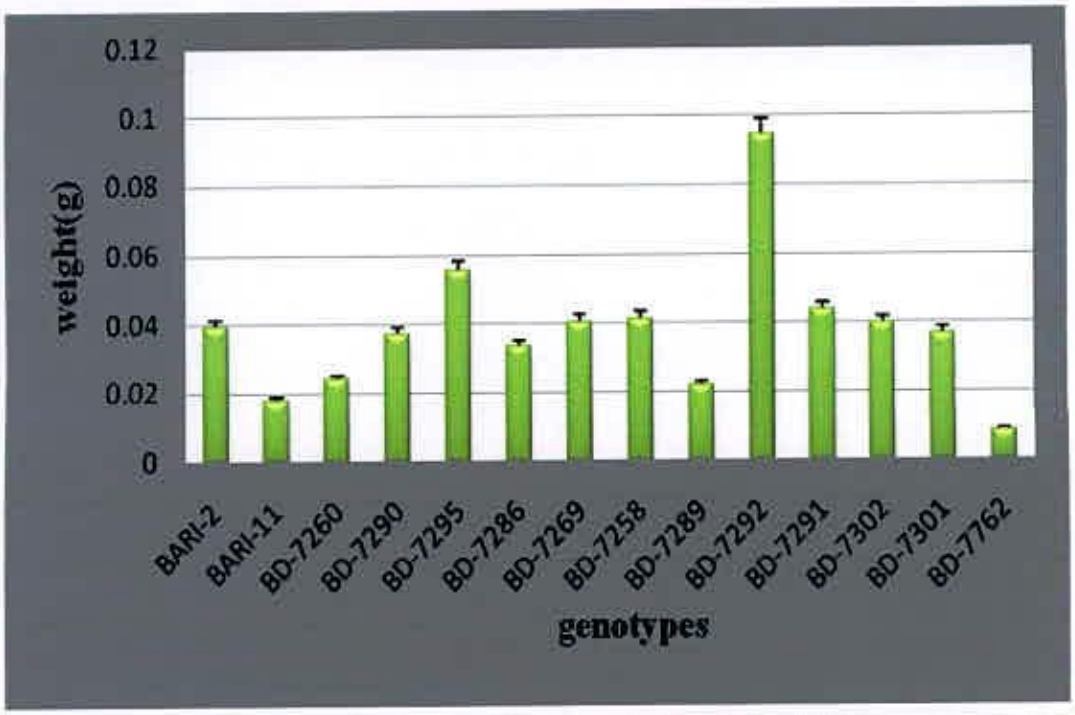


Fig. 3 Average weight after 9 days from inoculation in 0mM NaCl concentration

NaCl In case of 9 days treatment concentration at 9 days after inoculation highest weight was recorded in BARI-11 genotypes and the lowest root length was recorded in BD-7762 genotypes (Fig. 6).

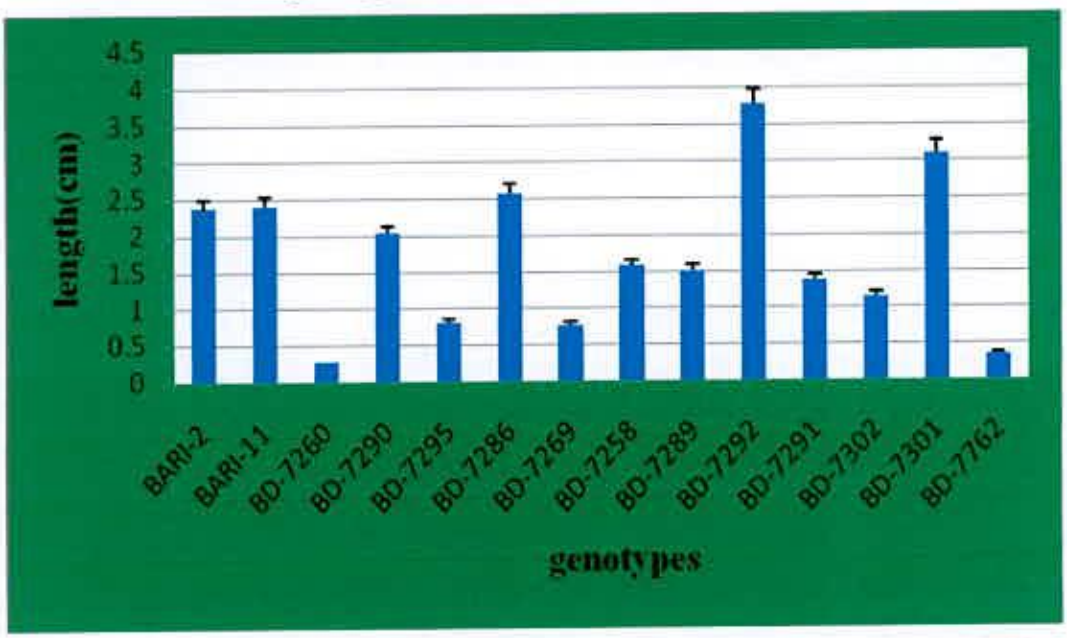


Fig. 4 Average root Length after 5days from inoculation in 50mM NaCl concentration

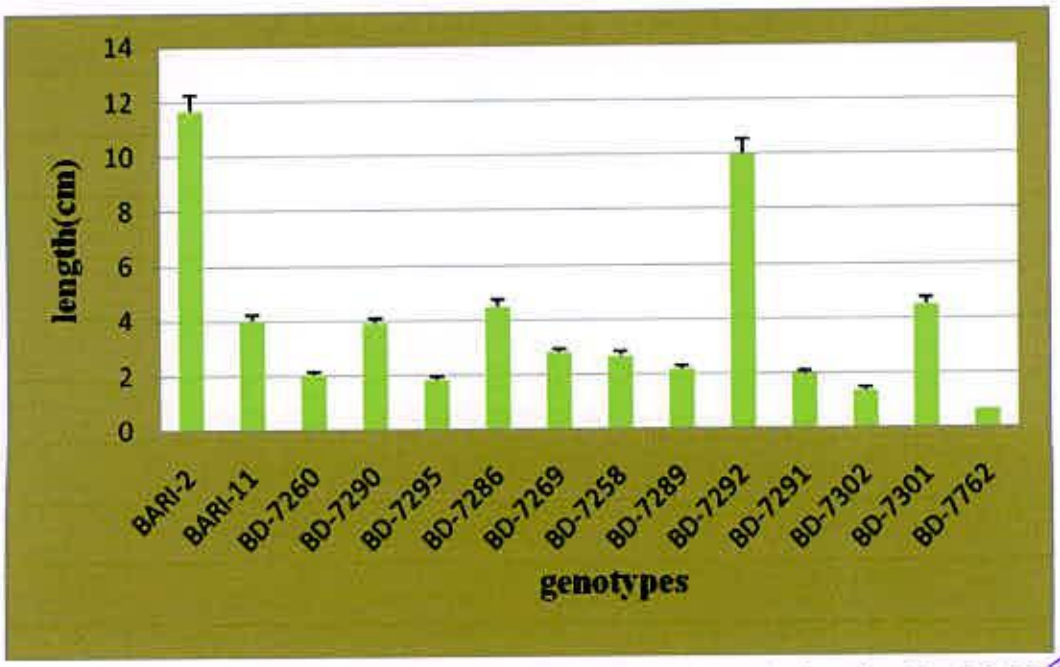


Fig. 5 Average root Length after 9 days from inoculation in 50mM NaCl concentration

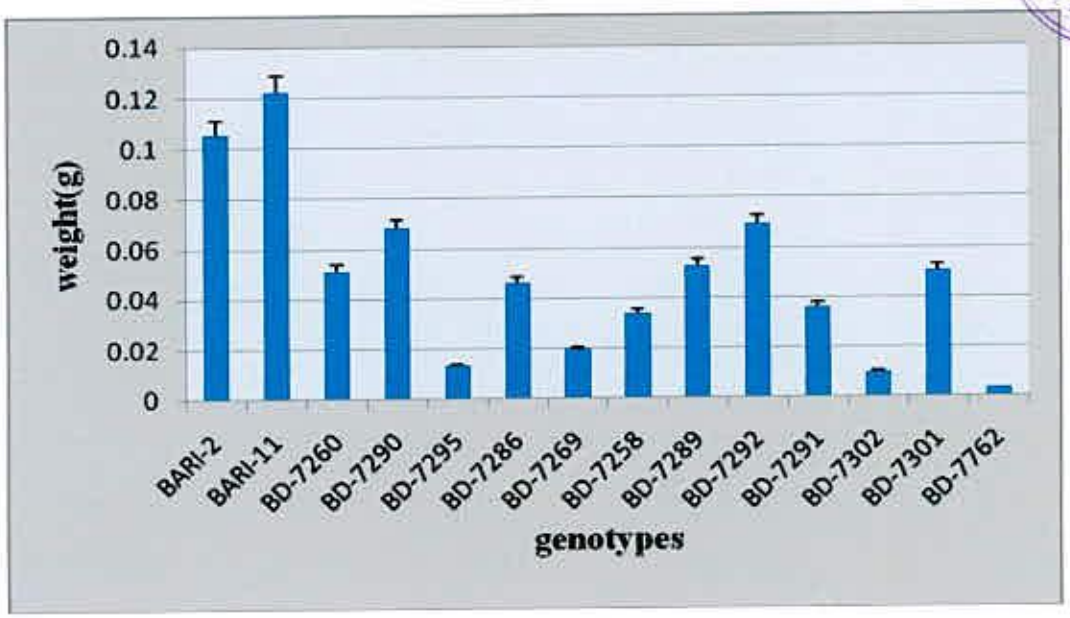


Fig. 6 Average weight after 9 days from inoculation in 50mM NaCl concentration

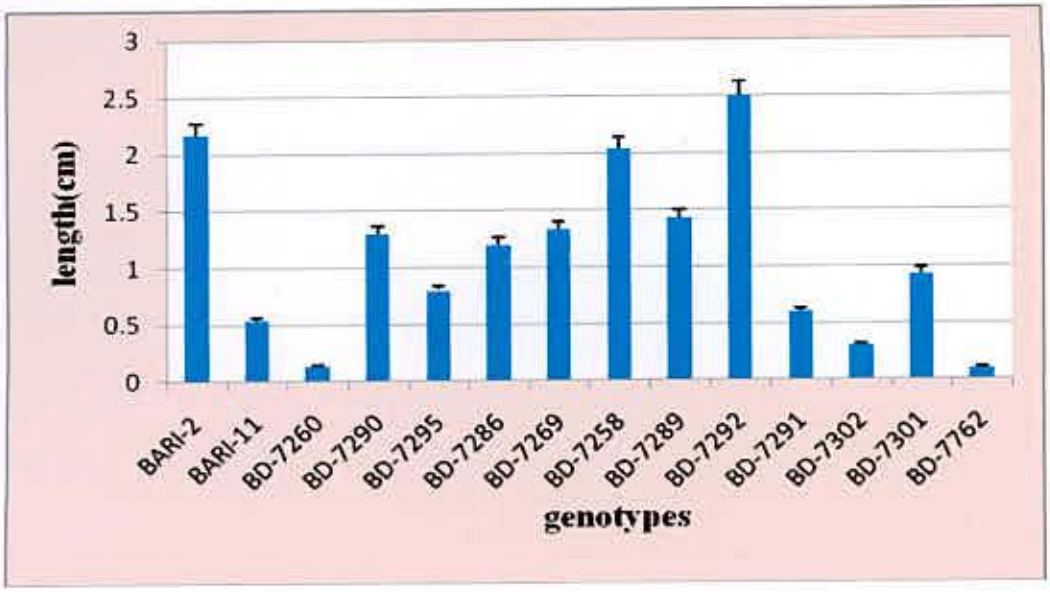


Fig. 7 Average root Length after 5 days from inoculation in 100mM NaCl concentration

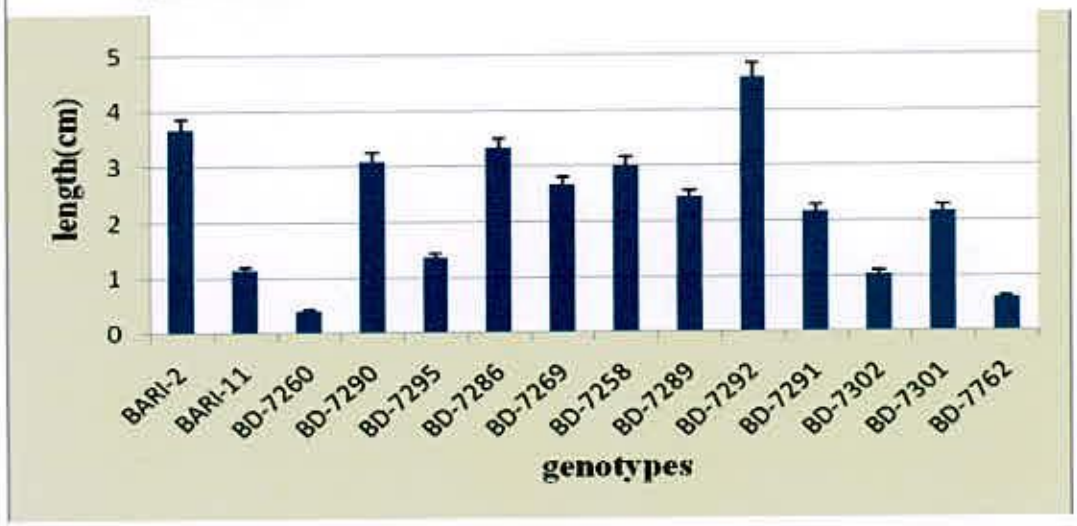


Fig. 8 Average root Length after 9 days from inoculation in 100mM NaCl concentration

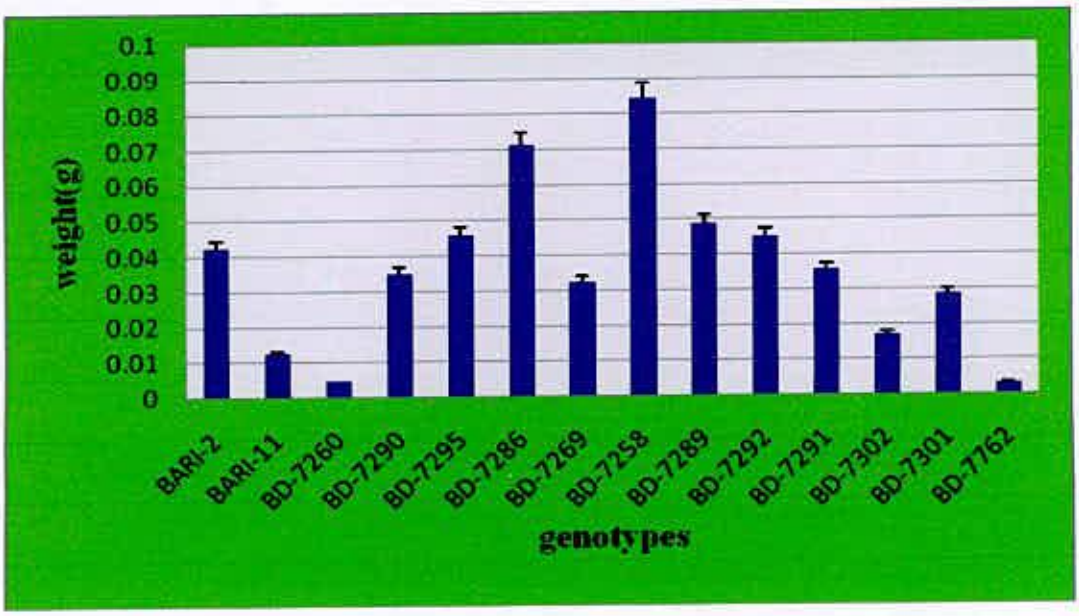


Fig. 9 Average weight after 9 days from inoculation in 100mM NaCl concentration

When the root length growth was considered at 100mM NaCl solution the entry BD-7292 was found the best consistently both at 5 days and 9 days treatment(Fig 7 and Fig 8).The average weight was found as highest with entry BD-7258and its lowest was produced by the entry BD-7762(Fig 9). At 200mM NaCl concentration the root length was highest with the entry BD-7258 both at 5 and 9 days treatment respectively. Similarly the lowest root growth was found in entry BD-7762 both at 5 and 9 days. As well as at 9 days treatment (Fig: 10) and (Fig: 11). In case of gain in weight at 9 days treatment with 200mMsolution the entry BD-7291 productivity highest weight and the entry BD-7762 was found as lowest consistently. The highest root growing entry BD-7258 produced the 3rd highest weight (Fig: 12). These differences might be due to differences in genetic makeup of the tomato entries included in the trial.

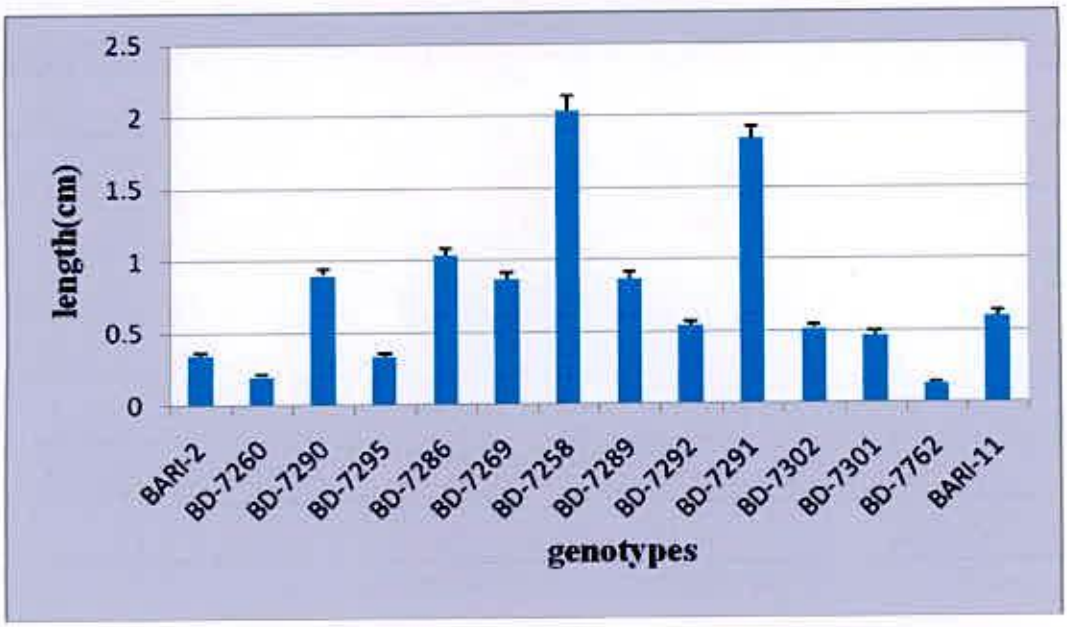


Fig. 10 Average root length after 5 days from inoculation in 200mM NaCl concentration

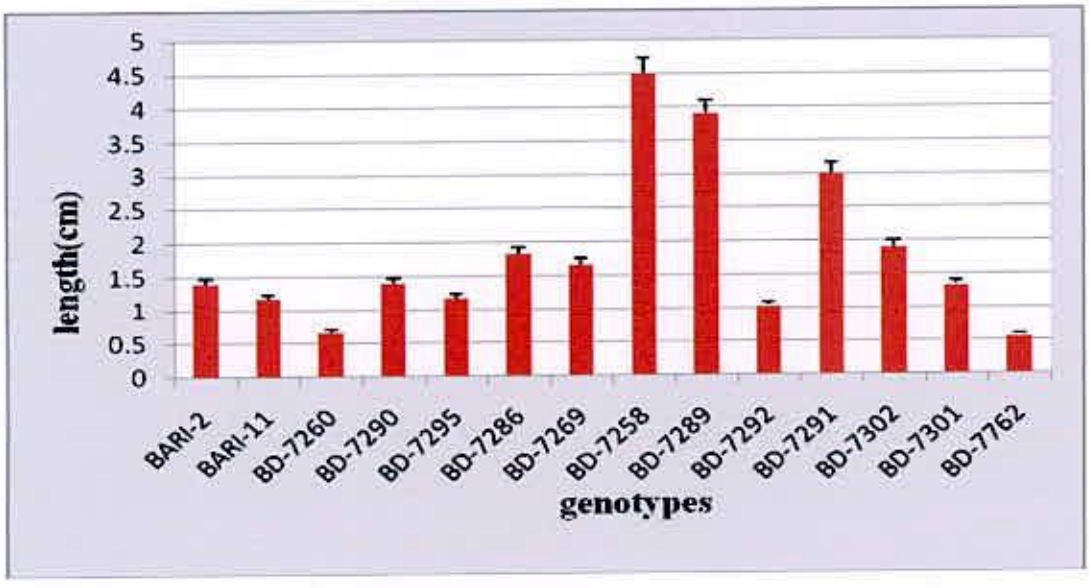


Fig. 11 Average root length after 9 days from inoculation in 200mM NaCl concentration

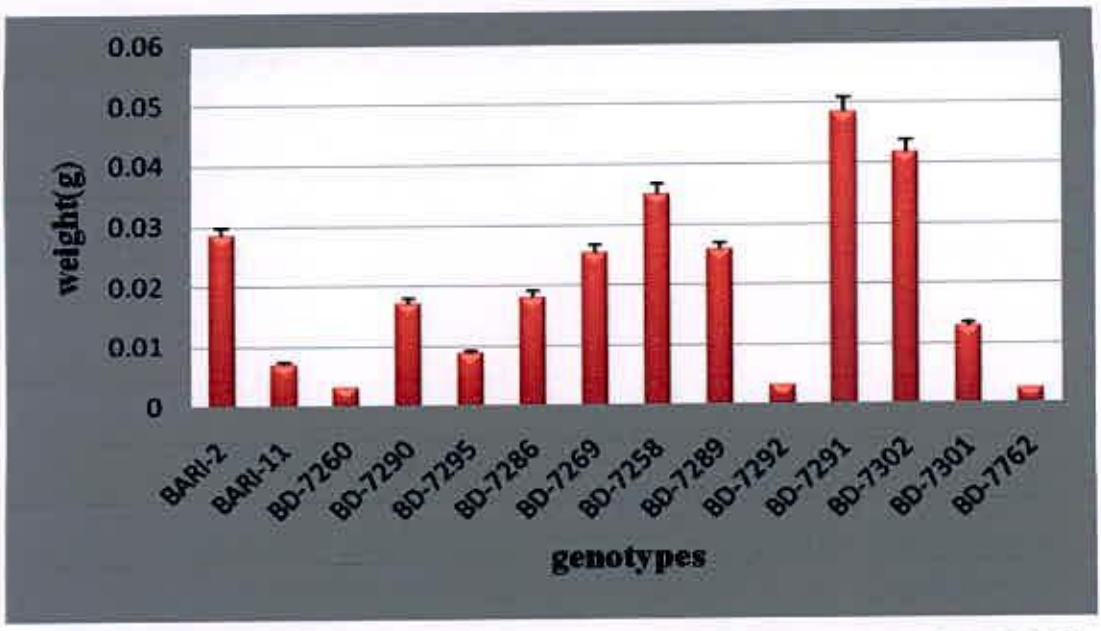


Fig. 12 Average weight after 9 days from inoculation in 200mM NaCl concentration

When we compare the root length and the plant weight with the variety to variety and variety to lines, the analysis of variance gives us significant results in Fig. 13, 14 and 15.

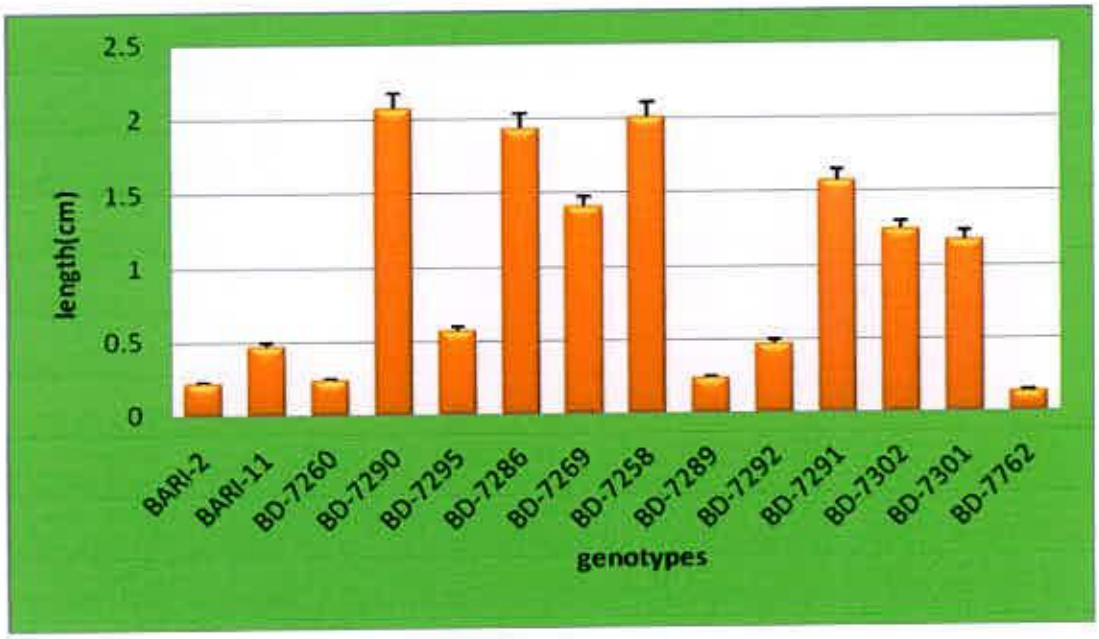


Fig. 13 Average root length after 5 days from inoculation in 250mM NaCl concentration

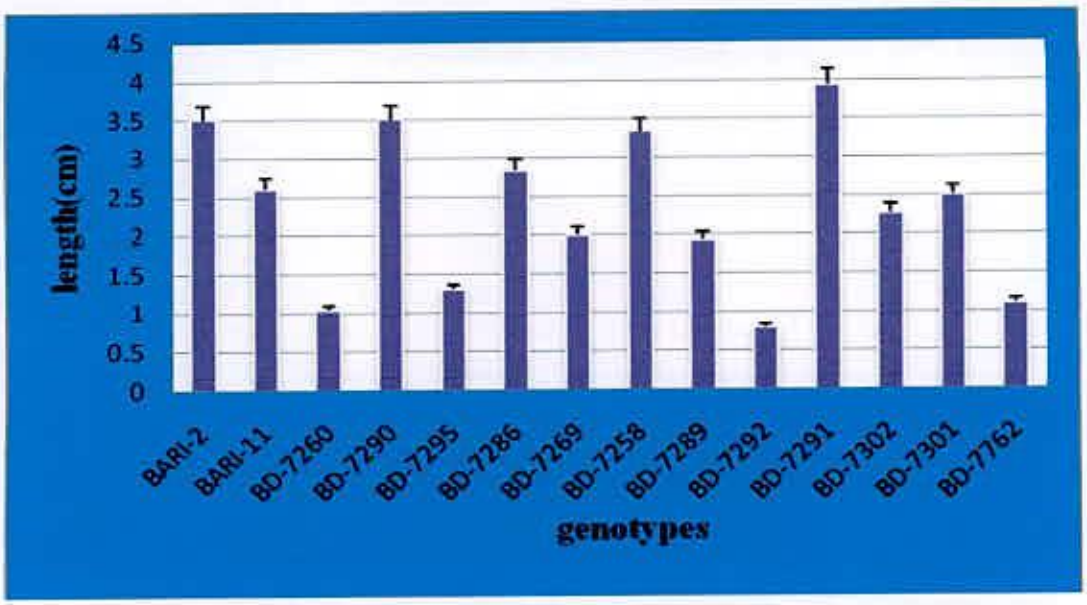


Fig. 14 Average root length after 9 days from inoculation in 250mM NaCl concentration

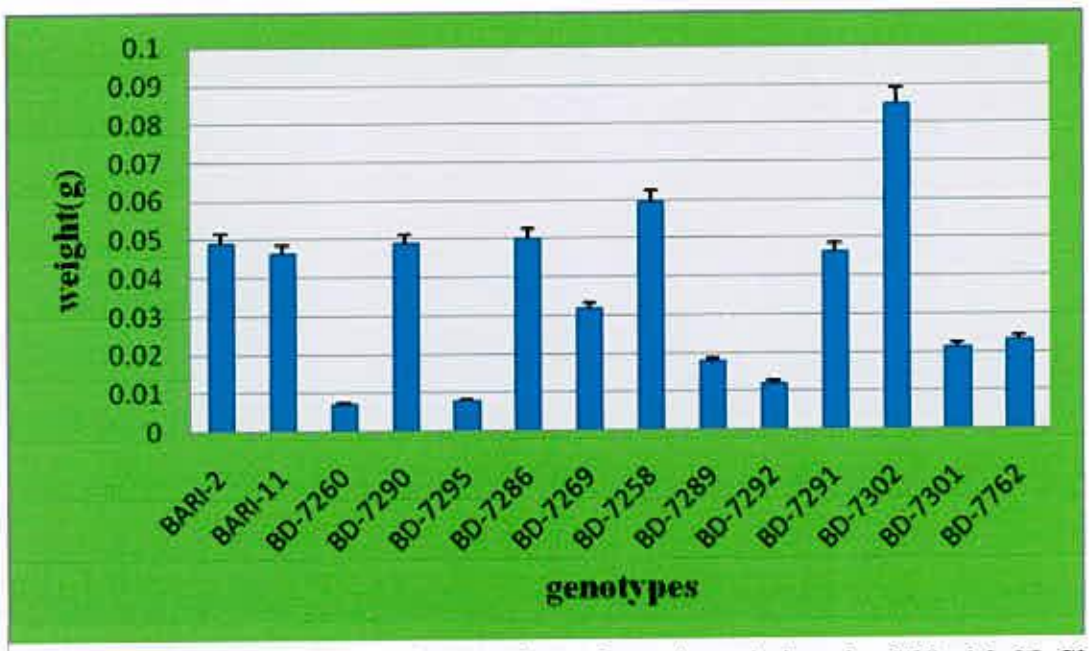


Fig. 15 Average weight after 9 days from inoculation in 250mM NaCl concentration

In case of 250mM NaCl solution the tomato entries BD -7290,BD-7286 and BD7258 produced significantly higher root growth at 5 days treatment compare with BARI-2 ,BARI11 ,BD-7289,BD7291 and BD-7762(Fig: 13). While the accession BD-7291 produced the highest root length. Among all the entries at 9 days treatment. The entries BARI-2,BD-7290 and BD-7258 produced the second highest root growth and entries BD-7292,BD-7260and BD-7762 produced lowest root length (Fig: 14). The total weight with the same salt solution at 9 days of treatment the entry BD-7302 produced the highest plant weight .The second highest weight was produced by BD-7258 and entries BARI-2, BD-7290 and BD-7286(Fig : 15)

4.3 Root Length and weight reduction rate

Genotypic variation for root length is evident in control (0 mM) and in stressed condition (50 mM, 100 mM and 250 mM) (Fig. 16). The tomato BARI-2 and BD-7292 produced the longest root at 50 mM NaCl salt concentration indicating their tolerance to salt at 50 mM, whereas BD-7762 showed the shortest root length. As the different genotypes showed varying degree of root length under unstressed condition (0 mM), it was necessary to measure the root reduction size at different salt concentrations (Table 2, Fig. 17). BARI-2, BD-7260, BD-7290, BD-7286, BD-7292, BD-7762 and BARI-11 showed negative reduction i.e., they had no effect of 50 mM salt stress on their growth and development. It was also observed that some genotypes showed no tolerance at lower salt concentration i.e., 50 mM but showed tolerance at high salt concentrations such as BD-7258, BD-7289, and BD-7289 at 100 mM salt stress and some of them even at 200 mM

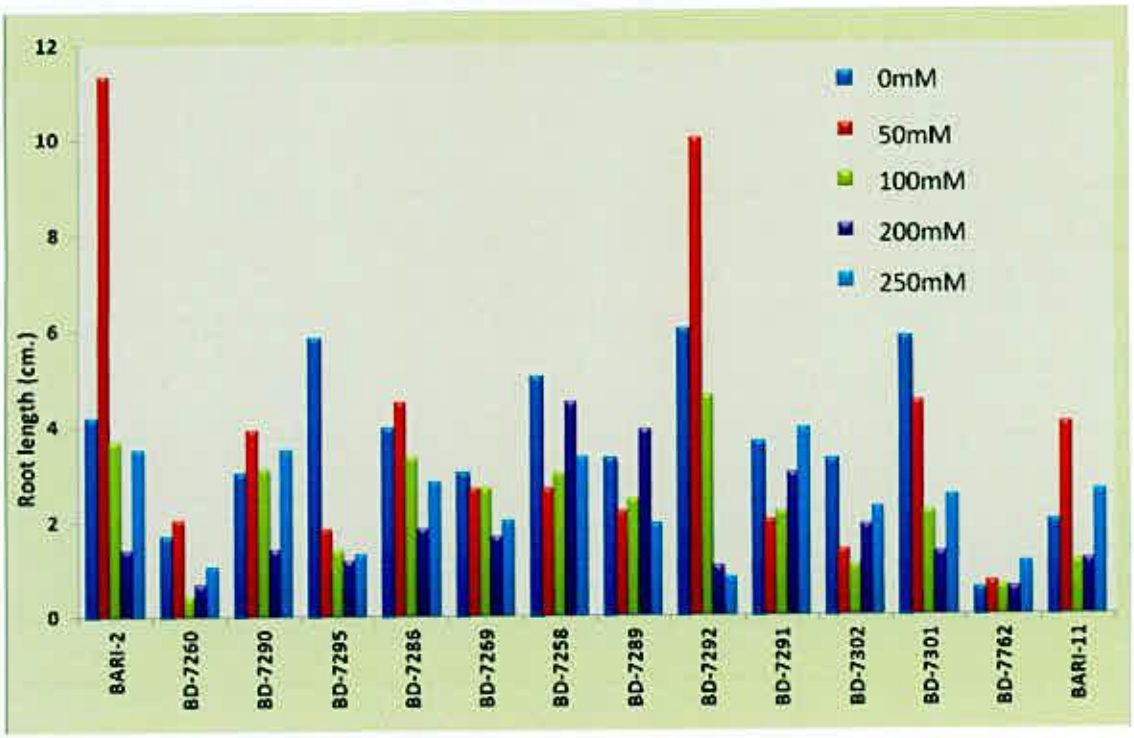


Fig. 16 Comparison of root length of different tomato genotypes with or without salt.

Table 2: Root length reduction (cm) under different salt concentrations

Genotype	Root reduction (cm.)		
	50 mM	100 mM	200 mM
BARI-2	-7.16666	7.666663	2.266667
BD-7260	-0.36667	1.633333	-0.26667
BD-7290	-0.9	0.8	1.7
BD-7295	4	0.466667	0.2
BD-7286	-0.56667	1.166667	1.5
BD-7269	0.33333	3.33E-06	1
BD-7258	2.333333	-0.33333	-1.5
BD-7289	1.1	-0.23333	-1.46667
BD-7292	-4	5.4	3.566667
BD-7291	1.633333	-0.16667	-0.83333
BD-7302	1.9	0.333333	-0.86667
BD-7301	1.333333	2.333333	0.833333
BD-7762	-0.16667	0.1	0.033333
BARI-11	-2.06667	2.9	-0.03333

salt stress (BD-7260, BD- 7289, BD-7291, BD-7302 and BARI-11 respectively). This phenomenon indicates that some genes which are responsible for salt tolerance might not be expressed or weakly expressed at low salt stress but defended strongly at high salt

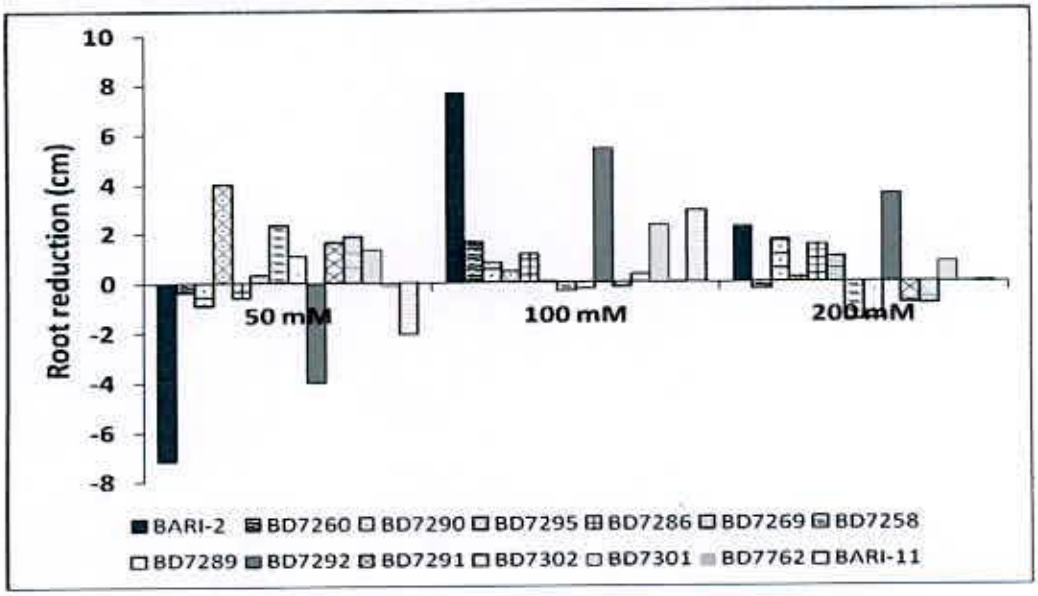


Fig. 17 Comparison of root reduction length in different genotypes of tomato under salt stress.

concentrations and produced functional protein as defense mechanisms. Buchanan *et al.*, (2000) showed similar results in case of spinach leaf tolerance to high salt but not at lower salt stress. MIP family genes showed weak expression under low salt and strong expression under high salt stress. Among the cell wall proteins most of them were previously found to play a protective role in plant cells in response to high salinity (Zeba, 2009; Garcia-Gomez *et al.*, 2000; Ueda *et al.*, 2007; Larkindale and Vierling, 2008). Therefore the gene expression pattern analysis of previously identified genes involved in protective role under salt stress is yet to be done.

Salt sensitivity and tolerances were also recorded based on the comparison of fresh weight under 0 mM (unstressed) to 200 mM salt stressed concentrations. BARI-2, BD-7260, BD-7290, BD-7286, BD-7289, BD-7301 and BARI-11 had more fresh weight at 50 mM as compared to the other genotypes (Fig. 18). They had no effect of salt and even their growth and development is increased at 50

mM of salt stress (Table 3). The fresh weight reduction is negative if observed in Fig. 19. BD-7286 showed tolerance up to 100 mM of salt stress. BD-7302 did not show tolerance response under low salt (50 mM) but showed tolerance at higher concentrations of 100 to 200 mM salt indicates the expression of some genes which have protective roles under high salinity. Gene expression data should be obtained in the control and stressed plants of the screened genotypes in support of the defense mechanism of these screened genotypes.

Analysis of some previously identified genes which showed protective roles in different abiotic stresses is quiet necessary and the presence and expression pattern of those genes in these screened genotypes of tomato will provide powerful information for over-expression of those genes in transgenic plants those will confer salt tolerances. Some of the previously reported genes which played protective role in defense mechanisms under high salinity are, Plasma membrane intrinsic protein

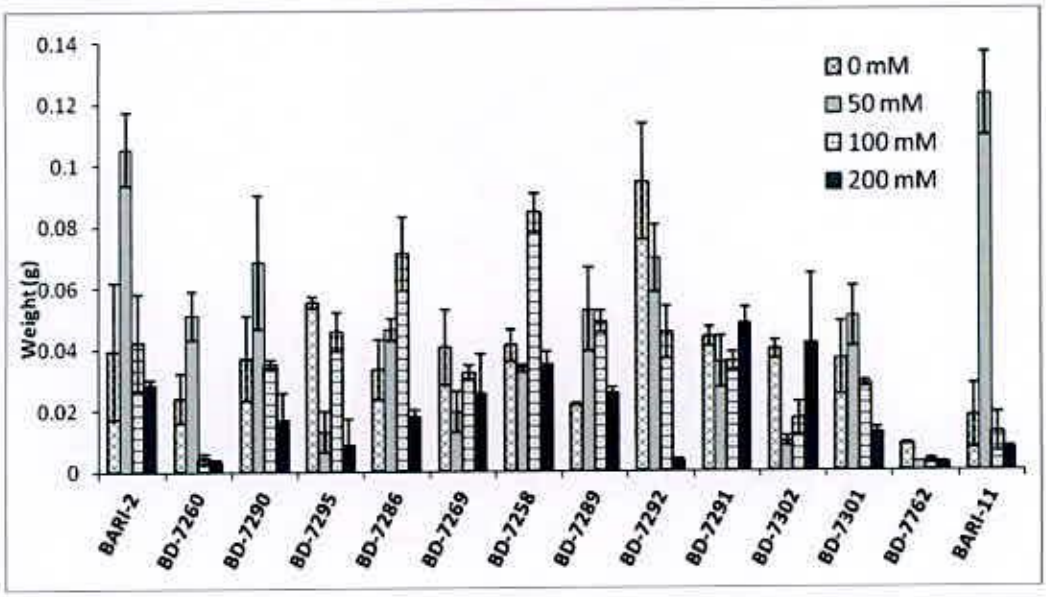


Fig. 18 Comparison of root reduction length in different genotypes of tomato under salt stress.

Table 3: Fresh weight reduction (g) under different salt concentrations

Genotypes	Weight reduction (g)		
	50 mM	100 mM	200 mM
BARI-2	-0.066	0.063	0.014
BD-7260	-0.027	0.046666667	0.001333
BD-7290	-0.03133	0.033333333	0.018
BD-7295	0.042333	-0.032666667	0.037
BD-7286	-0.013	-0.024666667	0.053
BD-7269	0.021	-0.013	0.007
BD-7258	0.007467	-0.050666667	0.049333
BD-7289	-0.031	0.004	0.023
BD-7292	0.025	0.024333333	0.042
BD-7291	0.008	0	-0.01267
BD-7302	0.03	-0.007333333	-0.02467
BD-7301	-0.01367	0.022	0.015667
BD-7762	0.005367	-3.33333E-05	0.000667

Gibberellin-Responsive Protein (GRP) (Jacobsen *et al.*, 1986; Peng *et al.*, 1999), Arabinogalactan protein (Lamport *et al.*, 2005), Prolin Rich Protein (PRP) (Ueda *et al.*, 2007), Glyceraldehyde 3-phosphate dehydrogenase (Jeong *et al.*, 2001) gene etc. Expression of these genes under unstressed and stressed condition is yet to be done.

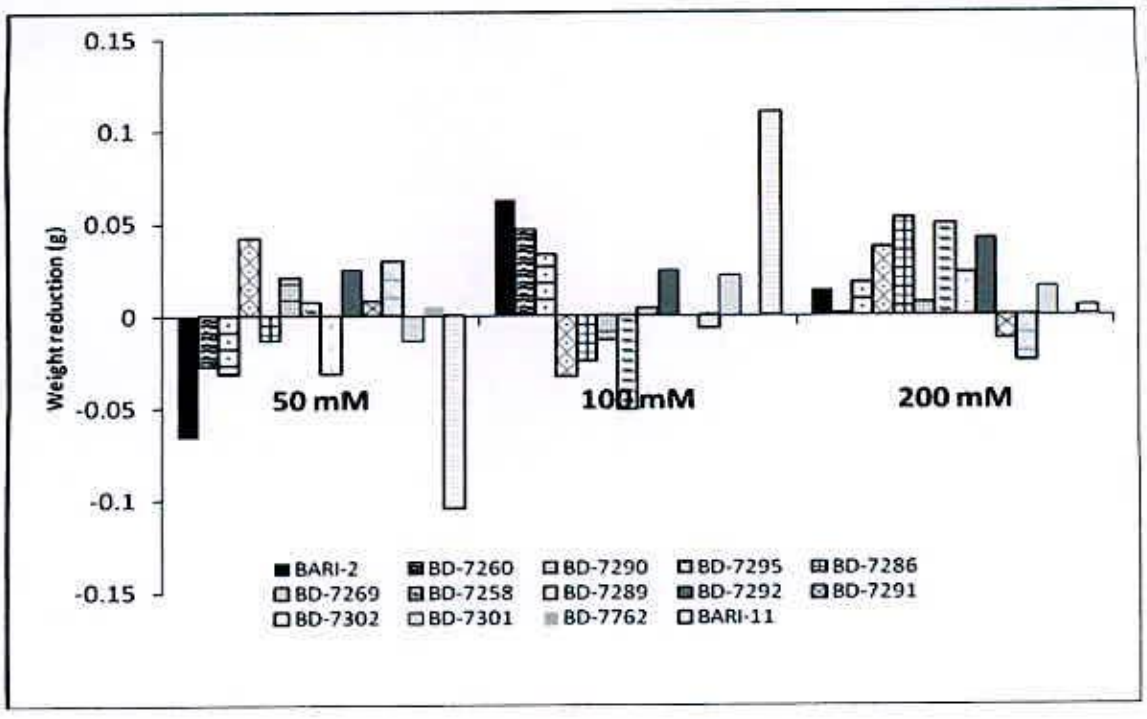


Fig. 19 Comparison of weight reduction in different genotypes of tomato under salt stress.

4.4 Genotypic distribution of Length LS-means of genotypes

Here we observed the variety BARI-2 and the Line BD-7292 is the highest performed and the Line BD-7762 is the lowest performed (Fig. 20).

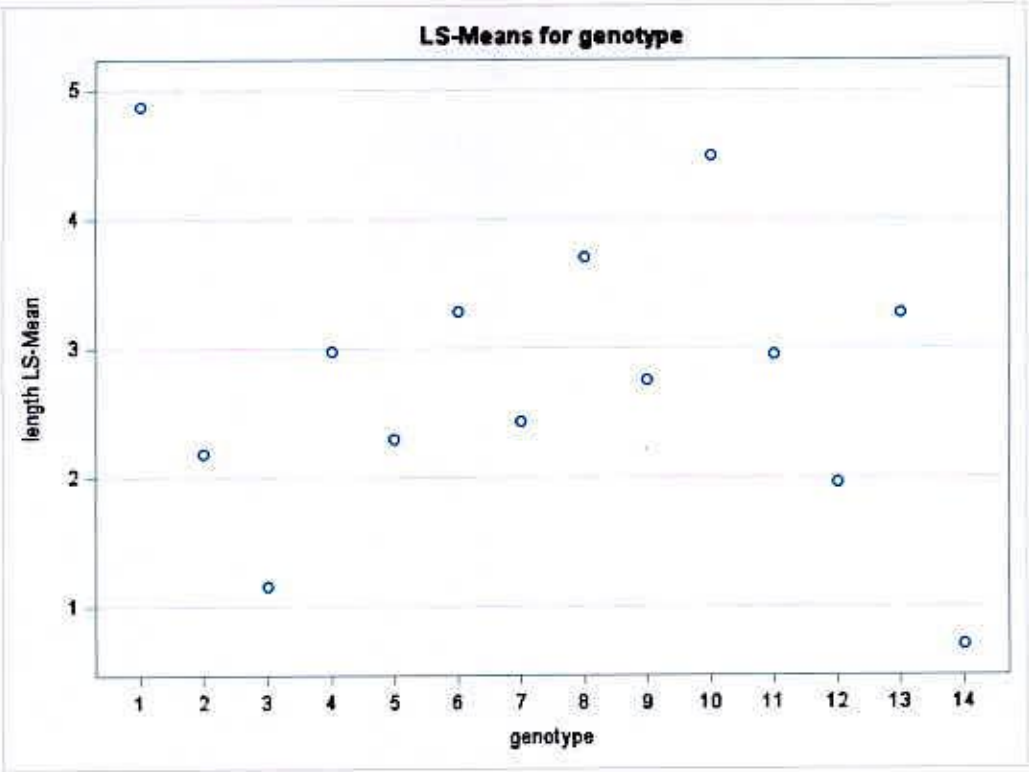


Fig. 20 Comparison of the Length LS-Means with different genotypes

According to length the analysis of variance gives us significant results for treatment and non significant results for genotypes (Table 4)

Table 4. Analysis of Variance for root length

ANOVA				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
genotype	13	52	1.49	0.1540
treatment	4	52	2.85	0.0329**

For length comparison of different genotypes most of the genotypes were not significant with the other genotypes (Fig. 21).

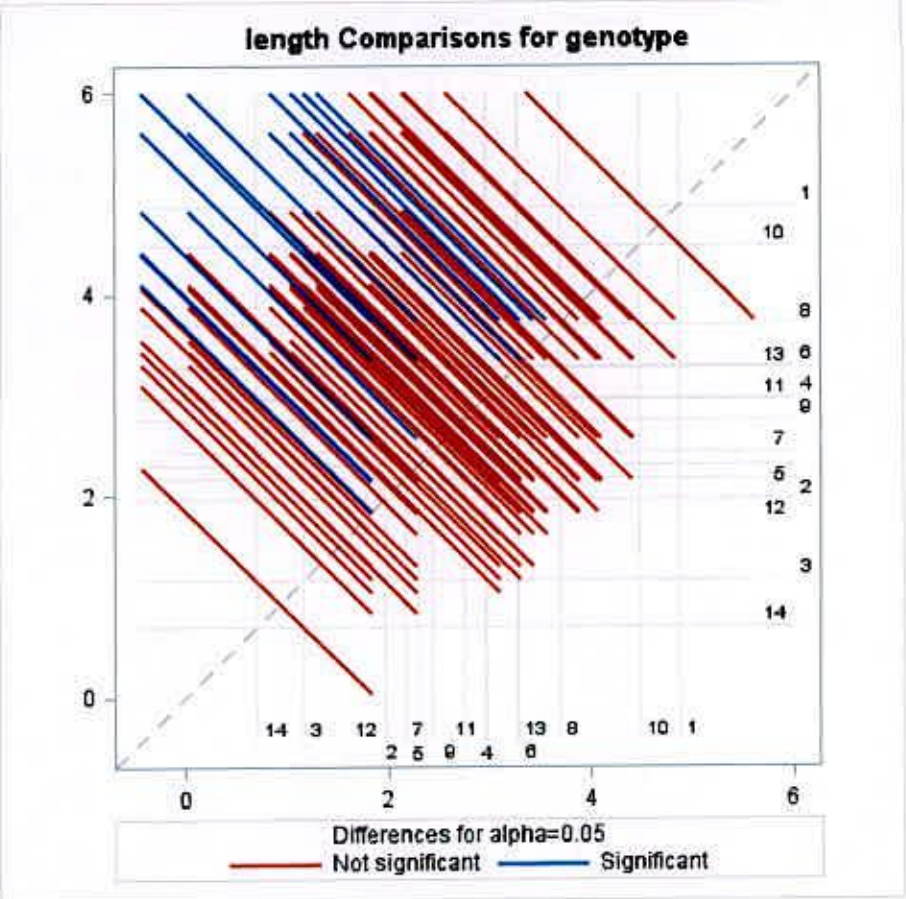


Fig. 21 Root length comparison for genotypes

When we compare only the length was compared with the variety to variety, variety to lines and line to line both significant and non significant results were observed (Table 5).

Table 5. Least Squares Means for effect genotype for root length

Least Squares Means for effect genotype Pr> |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: length														
i/j	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1		0.0206*	0.0018*	0.0996	0.0267*	0.1661	0.0348*	0.3039	0.0660	0.7337	0.0939	0.0128*	0.1609	0.0005*
2	0.0206		0.3709	0.4822	0.9159	0.3319	0.8281	0.1843	0.6141	0.0461*	0.5005	0.8510	0.3406	0.1959
3	0.0018	0.3709		0.1131	0.3177	0.0652	0.2674	0.0286*	0.1643	0.0047*	0.1197	0.4785	0.0677	0.6857
4	0.0996	0.4822	0.1131		0.5500	0.7872	0.6265	0.5269	0.8418	0.1881	0.9766	0.3740	0.8008	0.0486*
5	0.0267	0.9159	0.3177	0.5500		0.3866	0.9112	0.2208	0.6900	0.0582	0.5697	0.7692	0.3962	0.1626
6	0.1661	0.3319	0.0652	0.7872	0.3866		0.4501	0.7160	0.6390	0.2931	0.7648	0.2480	0.9860	0.0260*
7	0.0348	0.8281	0.2674	0.6265	0.9112	0.4501		0.2649	0.7737	0.0738	0.6474	0.6857	0.4606	0.1324
8	0.3039	0.1843	0.0286	0.5269	0.2208	0.7160	0.2649		0.4060	0.4895	0.5080	0.1309	0.7030	0.0104*
9	0.0660	0.6141	0.1643	0.8418	0.6900	0.6390	0.7737	0.4060		0.1309	0.8648	0.4895	0.6516	0.0747
10	0.7337	0.0461	0.0047	0.1881	0.0582	0.2931	0.0738	0.4895	0.1309		0.1786	0.0299*	0.2852	0.0015*
11	0.0939	0.5005	0.1197	0.9766	0.5697	0.7648	0.6474	0.5080	0.8648	0.1786		0.3898	0.7782	0.0518
12	0.0128	0.8510	0.4785	0.3740	0.7692	0.2480	0.6857	0.1309	0.4895	0.0299	0.3898		0.2551	0.2674
13	0.1609	0.3406	0.0677	0.8008	0.3962	0.9860	0.4606	0.7030	0.6516	0.2852	0.7782	0.2551		0.0271*
14	0.0005	0.1959	0.6857	0.0486	0.1626	0.0260	0.1324	0.0104	0.0747	0.0015	0.0518	0.2674	0.0271	

For T grouping ANOVA shows the variety BARI-2 and lineBD-7289 are the more salt tolerant genotypes (Table 6)

Table 6. T grouping for root length

T Comparison Lines for Least Squares Means of genotype								
LS-means with the same letter are not significantly different.								
						length LSMEAN	genotype	LSMEAN Number
				A		4.87328	1	1
				A				
	B			A		4.48666	10	10
	B			A				
	B			A	C	3.70000	8	8
	B			A	C			
	B	D		A	C	3.28664	6	6
	B	D		A	C			
	B	D		A	C	3.26666	13	13
	B	D		A	C			
	B	D		A	C	2.98000	4	4
	B	D		A	C			
E	B	D		A	C	2.94666	11	11
E	B	D		A	C			
E	B	D		A	C	2.75332	9	9
E	B	D			C			
E	B	D			C	2.42668	7	7
E	B	D			C			
E	B	D			C	2.30000	5	5
E		D			C			
E		D			C	2.18000	2	2
E		D			C			
E		D			C	1.96668	12	12
E		D						
E		D				1.16000	3	3
E								
E						0.70000	14	14

According to weight the analysis of variance gives significant results for both treatment and genotypes (Table 7)

Table 7. Analysis of Variance for weight

ANOVA				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
genotype	13	52	2.57	0.0080**
treatment	4	52	4.58	0.0030**

When we compare only the weight was compared with the variety to variety, variety to lines and line to line we got significant and non significant results were observed (Table 8).

Table 8. Least Squares Means for effect of genotype for weight

Least Squares Means for effect genotype Pr> |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: weight														
i/j	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1		0.4501	0.0271	0.4532	0.0880	0.5564	0.1402	0.8890	0.2101	0.6003	0.4823	0.3581	0.1415	0.0051*
2	0.4501		0.1368	0.9959	0.3333	0.8666	0.4649	0.5375	0.6138	0.8161	0.9578	0.8687	0.4680	0.0355
3	0.0271	0.1368		0.1355	0.5959	0.0989	0.4425	0.0376*	0.3207	0.0868	0.1238	0.1846	0.4395	0.5216
4	0.4532	0.9959	0.1355		0.3308	0.8707	0.4618	0.5409	0.6102	0.8202	0.9619	0.8646	0.4649	0.0351*
5	0.0880	0.3333	0.5959	0.3308		0.2572	0.8111	0.1161	0.6412	0.2315	0.3078	0.4215	0.8071	0.2436
6	0.5564	0.8666	0.0989	0.8707	0.2572		0.3696	0.6533	0.5017	0.9485	0.9085	0.7390	0.3723	0.0238*
7	0.1402	0.4649	0.4425	0.4618	0.8111	0.3696		0.1805	0.8202	0.3365	0.4334	0.5711	0.9959	0.1616
8	0.8890	0.5375	0.0376	0.5409	0.1161	0.6533	0.1805		0.2642	0.7004	0.5729	0.4349	0.1821	0.0075*
9	0.2101	0.6138	0.3207	0.6102	0.6412	0.5017	0.8202	0.2642		0.4618	0.5773	0.7341	0.8242	0.1052
10	0.6003	0.8161	0.0868	0.8202	0.2315	0.9485	0.3365	0.7004	0.4618		0.8575	0.6909	0.3391	0.0203*
11	0.4823	0.9578	0.1238	0.9619	0.3078	0.9085	0.4334	0.5729	0.5773	0.8575		0.8272	0.4364	0.0314*
12	0.3581	0.8687	0.1846	0.8646	0.4215	0.7390	0.5711	0.4349	0.7341	0.6909	0.8272		0.5746	0.0517*
13	0.1415	0.4680	0.4395	0.4649	0.8071	0.3723	0.9959	0.1821	0.8242	0.3391	0.4364	0.5746		0.1601
14	0.0051	0.0355	0.5216	0.0351	0.2436	0.0238	0.1616	0.0075	0.1052	0.0203	0.0314	0.0517	0.1601	

4.4 Genotypic distribution of Weight LS means of genotypes

The tomato variety BARI-2 and the Line BD-7292 was performed as the highest and the Line BD-7762 was as lowest

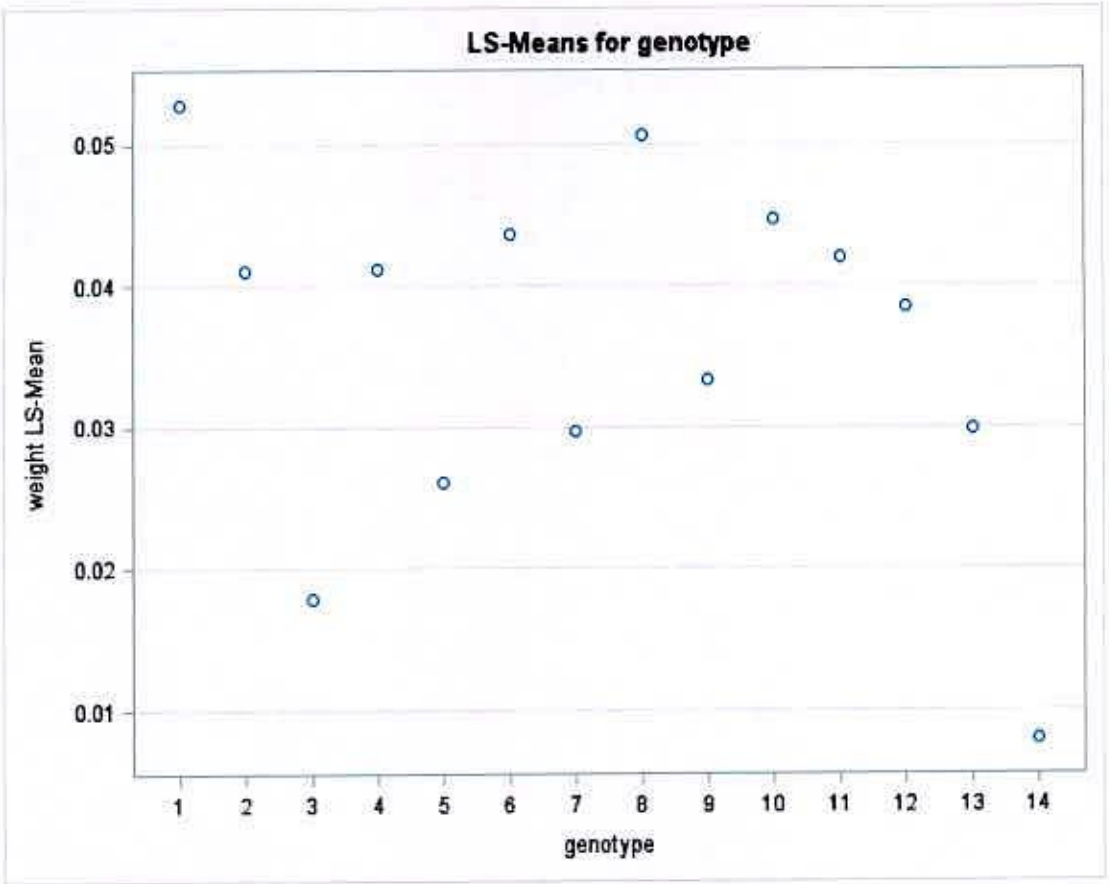


Fig. 22 Genotypic distribution of Weight LS- means of genotypes

For plant weight comparison for different genotypes we observed most of the genotypes are not significant with the other genotypes

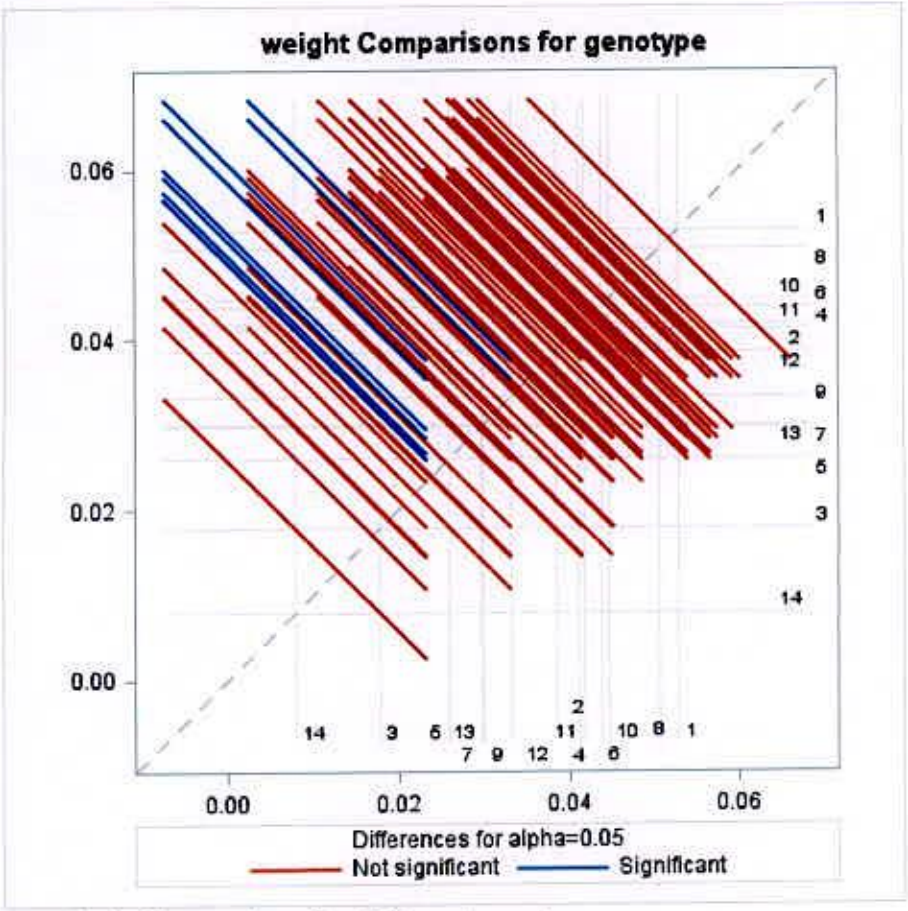


Fig. 23 Weight Comparison for different genotypes

For T grouping ANOVA shows the variety BARI-2 was as more salt tolerant genotypes

Table 9 T grouping for plant weight

T Comparison Lines for Least Squares Means of genotype					
LS-means with the same letter are not significantly different.					
			weight LSMEAN	**genotype**	**LSMEAN Number**
	A		0.05284	**1**	1
	A				
	A		0.05068	**8**	8
	A				
B	A		0.04472	**10**	10
B	A				
B	A		0.04372	**6**	6
B	A				
B	A		0.04194	**11**	11
B	A				
B	A		0.04120	**4**	4
B	A				
B	A		0.04112	**2**	2
B	A				
B	A	C	0.03856	**12**	12
B	A	C			
B	A	C	0.03330	**9**	9
B	A	C			
B	A	C	0.02986	**13**	13
B	A	C			
B	A	C	0.02978	**7**	7

T Comparison Lines for Least Squares Means of genotype					
LS-means with the same letter are not significantly different.					
			weight LSMEAN	genotype	LSMEAN Number
B	A	C			
B	A	C	0.02608	**5**	5
B		C			
B		C	0.01786	**3**	3
		C			
		C	0.00792	**14**	14

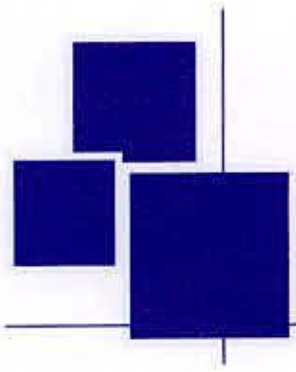
In vitro plant tissue culture is useful as quick tool to evaluate plant tolerance under salt stress. Many studies were carried out through using different tissue culture methods (Bhatia *et al.*, 2004). The result showed that shoot and root growth in these tomato genotypes were decreased with increasing NaCl concentration in the growth media in general agreement with Naseem *et al.*, (2005) study. Reduction in growth with increasing salinity in growth media may be attributed to water deficit or ion toxicity associated with excessive ion uptake particularly of [Na.sup.+] and [C.sup.l-] (Satti and Lopez, 1994). Nutrients imbalance as a result of depressed uptake, shoot transport and impaired internal distribution of minerals especially [K.sup.+] and [Ca.sup.+2] may also explained the reduction in plant growth (Munns, 1993).

At 50 mM NaCl in the growth media shoot growth was not significantly affected by the presence of salt in the growth media. However, root lengths were significantly decreased with increase in NaCl concentration. Root growth was more adversely affected by increasing NaCl concentration in the growth media than shoot growth (Mills, 1989; Bourgeais-Chaillou, and Guerrier, 1992; Sweby *et al.*, 1994). Root growth is suggested by Cano, *et al.* (1997) as better characteristic for evaluating salt tolerance of tomato species.

Cultivated tomato is generally classified as being moderately salt-sensitive. Different genotypes of tomato displayed widely different degrees of salinity tolerance (Alian *et al.*, 2000; Dasgan *et al.*, 2002; Ghoshal and Bajaj, 1984).

These results were in agreement with the previous findings concerning the physiological responses of tomato cultures to salt treatments. Marked differences in the behavior of both susceptible and tolerant tomato genotypes were evident (Cano *et al.*, 1996; Maliwal and Paliwal, 1970; Patolia, 1983;

Lynegar *et al.*, 1984; Cruz *et al.*, 1990; Rus *et al.*, 2000). Yet, an understanding of the mechanisms that plants use to cope with high salinity is necessary to select and develop tomato plants that are more tolerant to salinity. Rus *et al.* (2000) also found that adaptation capacity to salinity varies with the genotype's degree of tolerance. Perez-Alfocea *et al.* (Maliwal and Paliwal, 1970) detected different salt stress responses among several tomato cultivars, from a halophytic behavior.

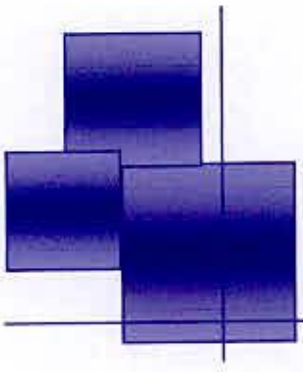


Chapter 5
Summary and conclusion

High salinity is one of the major stress factors among the abiotic stresses. Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. As saline soils and saline waters are common around the world, great effort has been devoted to understanding physiological aspects of tolerance to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes. Further effort is necessary if the exploitation of saline soils and saline waters that are not currently usable is to be achieved. Salinity affects yield quality and quantity, so that yield characters must be taken into account when breeding for salinity tolerance. But not only yield-related characters are important. As salinity affects almost every aspect of the physiology and biochemistry of the plant, the enhancement of crop salt tolerance will require the combination of several to many physiological traits not simply those directly influencing yield. As salinity in soils is variable and plant tolerance depends on the stage of plant development, plants should be phenotyped at several salinity concentrations and at the most sensitive plant stage(s). For *in vitro* screening, forty one genotype of tomato was taken for this study. Most of them were collected from Bangladesh Agricultural Research Institute (BARI). Fourteen out of forty one were selected based on their germination ability and were multiplied in the experimental farm of Sher-e-Bangla Agricultural University, Dhaka. Seeds were processed and stored at 4°C. Evaluation of response of these fourteen genotypes to normal and high salt conditions has been carried out in controlled environment with 25°C, 60% relative humidity, and a 16-h photoperiod from white fluorescent lamps (200 μmol photons/m^2/s^{-1}). Root assay and fresh weight assay were performed to compare the tolerance response of these genotypes. Five genotypes viz., BARI-2, BD-7260, BD-7290, BD-7286, and BARI-11 showed excellent performance of tolerance up to 50 mM of NaCl. BD-7302 showed better

performance under high salt concentrations i.e., at 100 mM and 200 mM but not at low salt stress.

This study dealt with the *in vitro* screening of salt tolerant genotypes in tomato and could be made the progress of gene expression analysis and thereby identify and isolate the genes involved in the process of salt tolerance for future genetic transformation. Despite the present limitations, it is foreseeable that our ability to design the future breeding programmes based on genetic transformation will be strengthened with the data obtained from ongoing functional genomics projects.



References

REFERENCES

Afroz, A., Chaudhry, Z., Rashid, U., Khan, M.R., and Ali, G.M. (2010). Enhanced regeneration in explants of tomato (*Lycopersicon esculentum* L.) with the treatment of coconut water. *African J. Biotec.* **9**: 3634-3644.

Alian, A., Altman, A., and Heuer, B. (2000). Gynotypic difference in salinity and water stress tolerance of fresh market tomato cultivars. *Plant Sci.*, **152**:5965.

Aazami, M. Torabi, M., and Shekari, F. (2010). Response of some tomato cultivars to sodium chloride stress under in vitro culture condition. *African J. Agric. Res.*, **1.5** (18): 2589 – 2592.

BBS. (2010). Year Book of Agricultural Statistics of Bangladesh. Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Govt. of the Peoples Republic of Bangladesh, Dhaka. p. 163.

Bhatia P., and Ashwath, N. (2008). Improving the quality of *in vitro* cultured shoots of tomato *(Lycopersicon esculentum* Mill. cv. Red Coat. *Biotechnology.* 7(2): 188-193.

Bhatia, P., Ashwath, N., Senaratna, T., and Midmore, D. (2004). Tissue culture studies of tomato (Lycopersicon esculentum). *Plant Cell, Tiss.Organ Cul.*,**78**: 1-21.

Bhattarai, S, de la Pena, R, Midmore, D., and Palchamy, K. (2009). *In vitro* culture of immature seed for rapid generation. *Euphytica,* **167**(1): 23-30.

Bourgeais-Chaillou, and P., Guerrier, G. (1992). Salt-responses in Lycopersicon esculentum calli and whole plants. *J. Plant Physiol.*, **140**: 494-501.

Branca, C., Bucci, G., Domiano, P., Ricci, A., and Bassi, M. (1990). Auxin: structure and activity on tomato morphogenesis *In vitro* and pea stem elongation. *Plant Cell Tiss. Org. Cult.*, **24**: 105-114.

Brichkova, G.G., Maneshina, T.V., and Kartel, N.A. (2002). Optimization of the nutrient medium for effective regeneration of tomatoes (*Lycopersicon esculentum*) *in vitro.Vestsi-Natsyyanal'nai-Akademii-Navuk-Belarusi.-Seryya-Biyalagichnykh-Navuk,* **2**: 47-52.

Buiatti, M., Tognoni, F., Lipucci, M., Marcheschi, G., Pellegrini, G., Grenci F., Scala, A. Bettini, P. P.,. Bogani, P , Betti, L., and Collina, G. (1984). Genetic variability in tomato (Lycopersicon esculentum) plants regenerated from in vitro cultured cotyledons. *Genetica Agraria* **38**: 325-331.

Buchanan, B., Gruissem, W., and Jones, R.L. (2000) Biochemistry and Molecular Biology of Plants, Rockville, MD, USA: American Society of Plant Biologists.

Cano, E.A., Perez, A., Moreno, V., Caro, M., and Bolarin, M. (1998). Evaluation of salt tolerance in cultivated and wild tomato species through in vitro shoot apex culture. *Plant Cell Tiss. Organ Cul.*, **53** (1): 19-26.

Cano, E.A., Perez-Alfocea, F., Moreno, V., and Bolarin, M. (1996). Responses of NaCl stress of cultivated and salt-tolerant breeding lines tomato species and their hybrids in callus cultures. *Plant Cell Rep.*, **15**: 791-794.

Chandler, S.F Paek, K.Y., Pua, E.C., Ragolsky, E. Mandal, B.B, and Thrope, T.A. (1988). The effectiveness of selection for salinity tolerance using in vitro shoot culture. *Bot Gaz.*, **149**:166-172.

Chaudhary, Z., Afroz, A., and Rashid, H. (2007). Effect of variety and plant growth regulators on callus proliferation and regeneration response of three tomato cultivars (*Lycopersicon esculentum*). *Pak. J. Bot.*, **39**: 857-869.

Compton, M.E., and Veillux, R.E. (1991). Shoot root and flower morphogenesis on tomato inflorescence explants. *Plant Cell Tiss. Org. Cult.*, **24**: 223-231.

Cruz, V., Cuartero, J., Bolarin, M., and Romero, M. (1990). Evaluation of characters for ascertaining salt stress responses in *Lycopersicon* species. *J. American Soc. Hort. Sci.*, **115:1000**-1004.

Cuartero, J., and Ferna´ndez-Mun˜oz, R. (1999). Tomato and salinity. *Scientia Horticulturae*, **78**: 83–125.

Cuartero, J., Boları´n, M.C., Ası´ns, M.J., and Moreno, V. (2006). Increasing salt tolerance in the tomato. *J. Exp. Bot.*, **57**(5): 1045–1058.

Dasgan, H.Y., Aktas, H., Abak, K., and Cakmak, I. (2002). Determination of screening techniques to salt tolerance in tomatoes and investigation of genotype responses. *Plant Sci.* **163**: 695-703.

Debez, A., Saadaoui, D., Ramani, B., Ouerghi, Z., Koyro, H.W., Huchzermeyer, B., and Abdelly, C. (2006) Leaf H - ATPase activity and photosynthetic capacity of Cakile maritima under increasing salinity. *Environ. Exp. Bot.*, **57**: 285-290.

Engelmann, N.J., Campbell, J.K., Rogers, R.B., and Indumathie, S. (2010). Screening and Selection of High Carotenoid Producing *in vitro* Tomato Cell Culture Lines for [13C]-Carotenoid Production. *J. Agric. Food. Chem.*, **58**(18): 9979–9987.

Emilio A. C., Francisco, P´erez-Alfocea1, Vicente, M., Manuel, C., and Maria, C. (2010) Departamento de Riego y Salinidad, CEBAS-CSIC.Apdo. 4195, E-30080-Murcia, Spain; Instituto de Biolog´ia Molecular y Celular de Plantas, UPV-CSIC, Camino de Vera s/n, E-46022-Valencia, Spain (requests for offprints)

FAO. (2010). The State of Food Insecurity in the World: Addressing food insecurity in protracted crises. Food and Agriculture Organization of the United Nations. Rome.
http://www.fao.org/docrep/013/i1683e/i1683e.pdf

FAOSTAT. (2005). Food and Agricultural Organization of the United Nations, Statistics. Available from http://faostat.fao.org/site/408/default.aspx.

Flowers, T.J., and Yeo, A.R. (1995). Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol.*, **22**: 875–884.

Garcia-Gomez, B.I., Campos, F., Hernandez, M., and Covarrubias, A.A. (2000) Two bean cell wall proteins more abundant during water deficit are high in proline and interact with a plasma membrane protein. *Plant J.* **22**: 277-288.

Ghosal, S.S., and Bajaj, Y.P.S. (1984). Isolation of sodium chloride resistant cell lines in some grain legumes. *Indian J. Exp. Biol.*, **22**:209-214.

Hu, H., and Zeng, J.Z. (1984). Development of new varieties via anther culture. *In:* Ammirato, P.V., Evans, D.A., Sharp,W.R., (eds). Handbook of Plant Cell Culture. New York: Macmillan, **3**: 65–90.

Jatoi, S.A., Ahmad, M., and Suppal, H.U. (1999). Manipulation of internodal segments of F1 tomato hybrids raised *In vitro* under different regimes of plant growth regulators. *Pak. J. Bot.*, **31**: 37-40.

Jose,´ M., Segui´-Simarro, and Nuez, F. (2007).Embryogenesis induction, callogenesis, and plant regeneration by in vitro culture of tomato isolated microspores and whole anthers. *J.Exp.Bot.*, **58**: 1119–1132.

Kirubakaran, S.I., Begum, S.M., Ulaganathan, K., and Sakthivel, N. (2008) Characterization of a new antifungal lipid transfer protein from wheat. *Plant Physiol. Biochem.*, **46**: 918-927.

Koyro, H.W. (2006). Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte Plantago coronopus (L-). *Environ. Exp. Bot.*, **56**: 136-146

Larkindale, J., and Vierling, E. (2008) Core genome responses involved in acclimation to high temperature. *Plant Physiol.* **146**: 748-761.

Lynegar, E.R.R., Chikara, J., and Sutaria, P. (1984). Relative salinity tolerance of barley varieties under semi-arid climates. *Trans. Indian Soc. Desert Technol.*, **20:** 123-8.

Maliwal, G.L., and Paliwal, K. (1970). Salt tolerance studies of some bajar varieties at germination stage. *J. Indian Soc.*, **21**:335-56.

Martinez, C.A., Maestri, M., and Lani, E.G. (1996). In vitro salt tolerance and proline accumulation in andean potato (Solanum spp.) differing in frost resistance. *Plant Sci.*, **116**:177-184.

Mercado, J.A., Sancho, Jimenez, B.S., Peran, U.R., Pliego, A.F., and Quesada, M.A. (2000). assessment of *invitro* growth of apical stem sections and adventitious organogenesis to evaluate salinity tolerance in cultivated tomato. *Plant Cell Tiss. Organ Cul.*, 62: 101-106.

Messai, A., Hannachi, C., and Zid, E. (2007).Evaluation of Salt-tolerant Genotypes of two Processing Tomato Derived from in vitro Regeneration under Salt stress. *Acta Hort.* **758**:51-58

Mills, D. (1989). Differential response of various tissues of Asparagus officinalis to sodium chloride. *J. Exp. Bot.*, **40**: 485-491.

Moghaieb, R., Saneoka, H., and Fujita, K. (2004). Shoot regeneration from Gus- transformed tomato (*Lycopersicon esculentum) hairy* root. *Cell. Mol. Biol. Lett.*, **9**: 439-449.

Mohammed, A.N., Alsadon, A.A., Alharbi,A.R., Wahb-allah, M.A., and Rahman, M.H. (2006). Salinity tolerance of tomato cultivars using *in vitro* techniques. ISHS Acta Horticulturae 760: XXVII International Horticultural Congress - IHC2006: II International Symposium on Plant Genetic Resources of Horticultural Crops.

Moraes, F. (1990). Obtenção de plantas haplóides através da cultura da anteras. In: Torres AC, Caldas LS (eds). Técnicas e aplicações da cultura de tecidos de plantas. Brasília: EMBRAPA, Centro Nacional de Pesquisas de Hortaliças, 311-332.

Munns, R. (1993). Physiological Processes Limiting Plant Growth in Saline Soils: Some Dogmas and Hypotheses. *Plant Cell Environ.*, **16**: 15-24.

Muhling, K.H., and Lauchli, A. (2003) Interaction of NaCl and Ca stress on compartmentation pattern of cations, antioxidant enzymes and proteins in leaves of two wheat genotypes differing in salt tolerance. *Plant Soil*, 253: 219-231.

Murashige, T., and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Planta* 157: 385-391

Nabors, M.W., Gibs, S.E., Bernstein, C.S., and Mise, M.E. (1980). NaCl-tolerant tobacco plants from cultured cells. *Z. pflanzen-physiol.* **97**: 13-17.

Naseem, M., Abed Alrahman, Rida, A.R., Ereifej, K., and Muna, Y. (2005). Hindiyeh influence of salinity on growth and physiology of *in vitro* grown cucumber (*Cucumis* sativus L.). *Jordan J. Agril. Sci.*, **1**(1):39-105.

Nurhidayah T, Horn R, and Rocher T. 1996. High regeneration rates in anther culture of interspecific sunflower hybrids. *Plant Cell Reports*, **16**: 167–173.

Osman, M. G., Elhadi, E. A., and Khalafalla, M.M. (2011). *In vitro* screening of some tomato commercial cultivars for salinity tolerant. *Int. J. Biotec. Biochem.*, 3946.

Park, S.H., Morris, J.L, Park, J.E, Hirschi, K.D., and Smith, R.H.M. (2003). Efficient and genotype independent *Agrobacterium* mediated tomato transformation. *J. Plant. Physiol.*, **160**: 1253-1257.

Patolia, J.S. (1983). Varietal responses of wheat to seawater irrigation. Ph.D. Thesis, Saurashtra University, Gujarat, India.

Peng, J., Richards, D.E., Moritz, T., Cano-Delgado, A., and Harberd, N.P. (1999) Extragenic suppressors of the Arabidopsis gai mutation alter the dose-response relationship of diverse gibberellins responses. *Plant Physiol.* **119**: 1199-1208.

Raziuddin, S. S, Chaudhry, H.J., Mohammad, T., and Ali, S. (2004). Hormonal effect on callus induction in tomato. *Sarhad J. Agric.*, **20**: 223-225.

Rus, A.M., Rios, S., Olmos, E., Santa-Cruz, A., and Bolarin, M.C. (2000). Long term culture modifies the salt response of callus lines of salt tolerant and salt-sensitive tomato species. *J. Plant Physiol.* **157:** 413-20.

Rus-Alvarez, A., and Guerrier, G. (1994). Proline metabolic pathways in calli from *Lycopersicon esculentum* and *L. pennellii* under salt stress. *Biologia Plantarum.*, **36**:277-284.

Satti, S.M.E., and Lopez, M. (1994). Effect of increasing potassium levels for alleviating sodium chloride stress on the growth and yield of tomato. *Commun. Soil Sci. Plant Anal.* **25**:2807-2823.

Shukla, R.K., Raha, S., Tripathi, V., and Chattopadhyay, D. (2006) Expression of *CAP2*, an APETALA2-family transcription factor from chickpea, enhances growth and tolerance to dehydration and salt stress in transgenic tobacco. *Plant Physiol.* **142**: 113-123.

Silva, J.A.B., Otoni, W.C., and Martinez, C.A. (2001). Microtuberization of Andean potato species (*Solanum* spp.) as affected by salinity. *Scientia Horticulturae*, **89**: 91-101

Simarro, J.M.S., and Nuez F. (2007). Embryogenesis induction, callogenesis, and plant regeneration by in vitro culture of tomato isolated microspores and whole anthers. *J. Exp. Bot.*, **58**(5):1119–1132.

Smolik, M., Kram, P., Krupa-Małkiewicz, M., Smolik, B., and Malinowska, K. (2011). Response of tomato genotypes to salinity stress assessed at the seedlings stage. *Elec tronic j. polish agric. univ.*, 14(4) #17 (http://www.ejpau.media.p).

Sweby, D.L., Huckett, B.I., and Watt, M.P. (1994). Effects of nitrogen nutrition on salt stressed *Nicotiana tabacum* var. Samsun *in vitro* plantlets. *J. Exp. Bot.*, **45**: 995-1008.

Young, R., Kaul, V., and Williams, E.G. (1987). Clonal propagation *invitro* from immature embryos and flower buds of *Lycopersicon peruvianum* and *L. esculentum. Plant Sci.*, **52**: 237-242.

Yusuf, A., Li, S.J., and Li, S.X. (1994). *In vitro* flowering, fruiting and differentiation of callus in different genotypes of tomato in the presence of NaCl. *Sarhad J. Agric.*, **10**(1):59-62.