

**INTERACTION EFFECT OF GENOTYPE AND SALINITY  
LEVELS ON AGROMORPHOGENIC, PHYSIOLOGICAL AND  
NUTRITIONAL TRAITS OF TOMATILLO (*Physalis ixocarpa*  
Brot./*P. philadelphica* Lam.)**

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## **CERTIFICATE**

*This is to certify that thesis entitled, "Interaction Effect of Genotype and Salinity Levels on Agromorphogenic, Physiological and Nutritional Traits of Tomatillo (*Physalis ixocarpa* Brot./*P. philadelphica* Lam.)" 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 Nabila Narzis, Registration No.: 12-04926 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

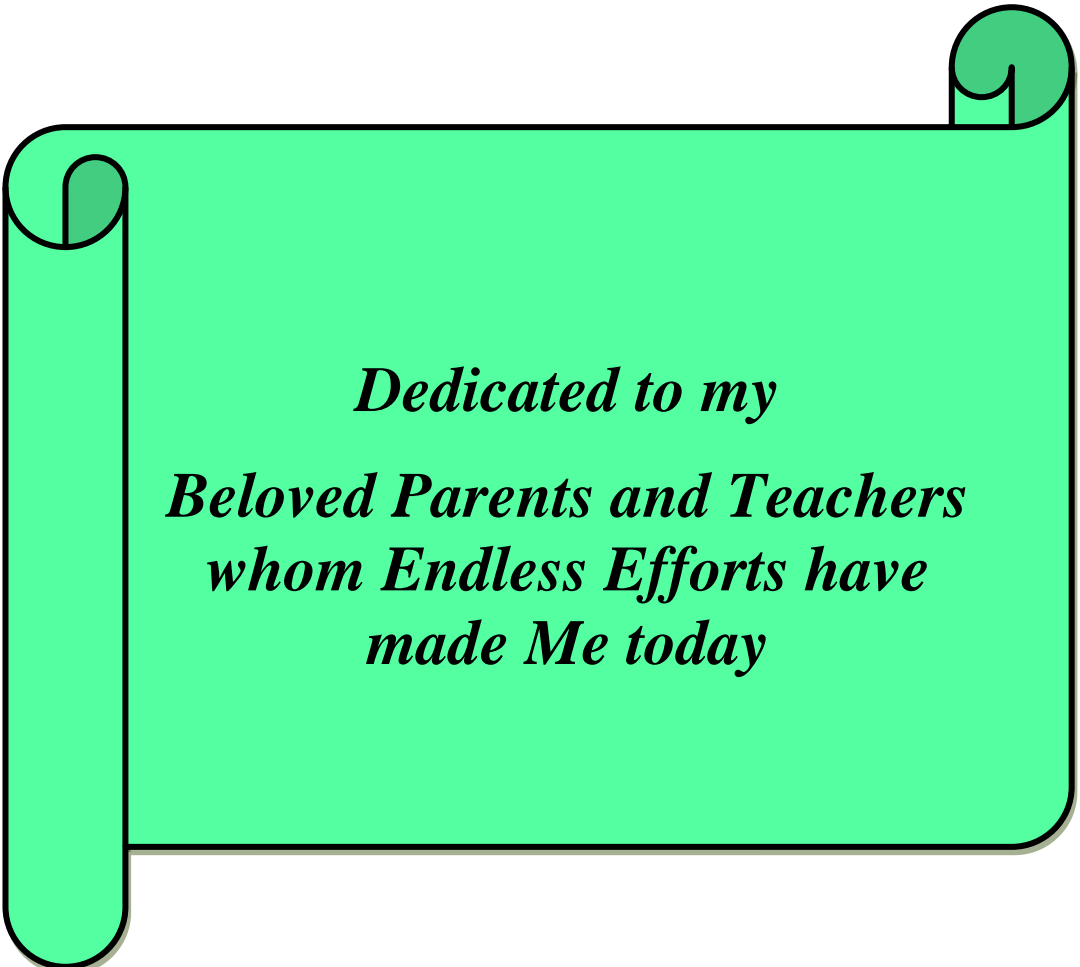
*I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.*

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**Place: Dhaka, Bangladesh**

**(Prof. Dr. Naheed Zeba)**

**Supervisor**



*Dedicated to my  
Beloved Parents and Teachers  
whom Endless Efforts have  
made Me today*

### Some commonly used abbreviations

Full word	Abbreviations	Full word	Abbreviations
Advances/Advanced	<i>Adv.</i>	Liter	L
Agriculture	<i>Agric.</i>	Milligram per liter	mg/L
Agricultural	<i>Agril.</i>	Milligram(s)	mg
Agronomy	<i>Agron.</i>	Milliliter	mL
And others	<i>et al.</i>	Millimeter	mm
Applied	<i>App.</i>	Milli mole	mM
Archives	<i>Arch.</i>	Murashige and Skoog	MS
Biochemistry	<i>Biochem.</i>	Nanometre	nm
Biology	<i>Biol.</i>	Nitric acid	HNO <sub>3</sub>
Botany	<i>Bot.</i>	Nitrate ion	NO <sub>3</sub> <sup>-</sup>
Breeding	<i>Breed.</i>	Negative logarithm of hydrogen ion concentration (-log [H <sup>+</sup> ])	pH
Calcium ion	Ca <sup>2+</sup>	Nutrient	<i>Nutri.</i>
Centimeter	cm	Nutrition	<i>Nutr.</i>
Chloride ion	Cl <sup>-</sup>	Organ	<i>Org.</i>
Critical	<i>Crit.</i>	Perchloric acid	HClO <sub>4</sub>
Culture	<i>Cult.</i>	Percentage	%
Days after transplanting	DAT	Physiology	<i>Physiol.</i>
Decisiemens per meter	dS/m	Potassium ion	K <sup>+</sup>
Degree celcius	°C	Potassium Chloride	KCl
Electrical conductivity	EC	Parts per million	ppm
Environment	<i>Environ.</i>	Review	<i>Rev.</i>
Etcetera	etc.	Research	<i>Res.</i>
Experimental	<i>Expt.</i>	Serial	Sl.
Food and Agricultural Organization	FAO	Science	<i>Sci.</i>
Genetics	<i>Genet.</i>	Sodium ion	Na <sup>+</sup>
Gram	g	Soil Resource Development Institute	SRDI
Gram per liter	g/L	Technology	<i>Technol.</i>
Hectare	ha	Technique	<i>Tech.</i>
Horticulture/ Horticultural	<i>Hort.</i>	That is	i.e.
International	<i>Intl.</i>	Tissue	<i>Tiss.</i>
Journal	<i>J.</i>	Videlicet (namely)	<i>viz.</i>
Kilogram	kg	Weight	wt.

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**INTERACTION EFFECT OF GENOTYPE AND SALINITY LEVELS  
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*philadelphica* Lam.)**

By

**NABILA NARZIS**

**ABSTRACT**

The present study was conducted as a pot experiment in order to observe the performance of tomatillo (*Physalis ixocarpa* Brot./*P. philadelphica* Lam.) genotypes under different levels of salinity treatment. The experiment was conducted beside the area of the net house of Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh, during the period of November, 2017 to March, 2018. A two factorial Completely Randomized Design (CRD) experiment was conducted which included four tomatillo genotypes (Factor A) viz. G<sub>1</sub> (SAU tomatillo 1), G<sub>2</sub> (SAU tomatillo 2), G<sub>3</sub> (PI003), G<sub>4</sub> (PI004) and three salinity treatments (Factor B) viz. T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m), T<sub>3</sub> (12 dS/m) and was outlined with three replications. Seedlings of 21 days old were transplanted into main plastic pots and two salinity treatments, 8 dS/m and 12 dS/m were started to apply after 7 days of transplanting. The observed results showed that both of four tomatillo genotypes and three salinity treatments had their independent significant influence and also had significant influence in their interaction effect between different agromorphogenic, physiological and nutritional traits. Almost all agromorphogenic and physiological traits responded negatively (%Reduction), except endogenous Na<sup>+</sup> ion and proline content whereas nutritional traits like, %Brix, titratable acid and vitamin-C content, except fruit pH responded positively (%Increase) under the increased level of salinity treatments. From the observed results of the accomplished study, considering yield and its contributing characters like, number of fruits per plant, average fruit length, diameter, fruit weight as well as yield per plant, the best salt tolerant genotypes of tomatillo were genotype G<sub>1</sub> and G<sub>3</sub> under both slightly and moderately salinity stresses. These two also showed minimum endogenous Na<sup>+</sup> and maximum K<sup>+</sup> ion content along with minimum reduction in leaf area index. The maximum amount of proline content was observed in genotype G<sub>4</sub> while G<sub>1</sub> showed the maximum increase percentage. The maximum reduction in days to maturity was found in genotype G<sub>1</sub> and G<sub>4</sub>. Considering the increased percentage of nutritional traits like, %Brix, titratable acid and vitamin-C content, the best salt tolerant genotypes of tomatillo were genotype G<sub>3</sub> along with G<sub>4</sub> and G<sub>1</sub>. Thus, genotype G<sub>3</sub> and G<sub>1</sub> could be recommended for further trial in Southern region of Bangladesh. And, genotype G<sub>2</sub> and G<sub>4</sub> could be served as parent materials for further hybridization or genetic transformation program along with G<sub>3</sub> and G<sub>1</sub>.

# CHAPTER I

## INTRODUCTION

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The tomatillo, *Physalis ixocarpa* Brot./*P. philadelphica* Lam. (2n=2x=24), is an important crop in Mexico, and now-a-days both cultivated and weedy annuals have been introduced and appreciated worldwide. It is an allogamous, annual plant of the nightshade (Solanaceae) family, along with tomatoes and peppers under the angiosperm genus *Physalis*. Tomatillos were originated and domesticated in Mexico before the coming of Europeans, and played an important part in the culture of Maya and the Aztecs (Wilf *et al.*, 2017; Small, 2011). Tomatillo plants bear small, spherical and bright green (*Physalis philadelphica* Lam.) or green-purple (*Physalis ixocarpa* Brot.) fruits surrounded by an inedible, paper-like husk formed from the calyx (Morton, 1987). Thus, it is also known as the “Mexican husk tomato”. Tomatillo is also being referred to as “tomate verde” (green tomato).

Tomatillo plants are weedy or cultivated annual of humid tropics and subtropics. They grow well in drained, fertile soil with a pH between 5.5 and 7.3 (Masabni, 2016). They grow best at 25 to 32 °C. Tomatillo plants are of about 1 meter in height, with less dense canopy containing few hairs on the stem. The tomatillo fruits are slightly acidic true berries with many tiny seeds and are typically green, yellow, or purple in color when mature. The interior texture of the fruit is denser and less watery. Fruits are harvested when the fruits fill the calyx (Diaz-Perez *et al.*, 2005). As the fruit matures, it fills the husk and may or may not split it open, but turns brown and leathery in texture. After removing the husk, the fruit seems a little sticky as it contains a pectin-like substance. Tomatillo plants show gametophytic self-incompatibility (Mulato-Brito *et al.*, 2007).

Tomatillo is a highly nutritious fruit with a combination of vitamins and minerals. 100 g of edible tomatillo fruit contains high dietary fiber, vitamin-A, vitamin-C, calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), phosphorous (P) and potassium (K). It also contains vitamin-K, niacin,

riboflavin, thiamin,  $\beta$ -carotenes (zeaxanthin and lutein) and copper (Cu) (Yamaguchi, 1983). Tomatillos have a high pectin content. Fruits are rich in antioxidants, like withanolides (ixocarpalactone A, ixocarpalactone B, philadelphicalactone B, and withaphysacarpin). The research findings revealed that withanolides (e.g. IxoA) present in tomatillo fruits were potent inducers of quinone reductase, which is more powerful in preventing colon cancer than chemotherapy (Choi *et al.*, 2006). Tomatillo fruits have anti-bacterial properties. It is rich in flavonoids that can help to protect from lung and oral cavity cancers (Quiros, 1984).

Tomatillos, as staple of Mexican cuisine (Escobar *et al.*, 2014; Waterfall, 1958) are the key ingredient in fresh and cooked Mexican and Central-American green sauces, particularly salsa verde due to their unusual flavor, bright green color and tart fruit flavor (Small, 2011; Waterfall, 1967). It can be suitable as a substitute of tomato. Fruits are often used in jams, preserves, stews, soups, salads, curries, stir-fries, baking, cooking with meats, marmalade, and desserts (Morton, 1987). Though they are native to Mexico and Central America, and they are presently one of the most important crops in Mexico (Cantwell *et al.*, 1992), being the fourth vegetable in production surface with an area of 47,473 ha in 2009 (Borja-Bravo *et al.*, 2013). Nowadays it is also cultivated in India, Australia, South Africa, as well as in the United States of America.

Tomatillo is recently introduced in our country as a vegetable crop. It has been introduced by the Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka in 2013. Even two varieties of tomatillo have been released named SAU tomatillo 1 and SAU tomatillo 2 in 2016 (Reza, 2016). As a newly introduced crop, tomatillo needs many further research in terms of its yield and yield contributing characters and other antioxidant or nutritional aspects as well as whether it shows any particular resistance or tolerance for biotic and abiotic stresses in respect of our country's atmosphere.

Salinity problem is one of the major problem of agriculture in our country. The coastal area covers about 20% of Bangladesh and over 30% of the net cultivable

area. The cultivable areas in coastal districts are affected with varying degrees of soil salinity. The coastal area of the Ganges delta in Bangladesh is characterized by tides and salinity from the Bay of Bengal. The higher salinity levels have adverse impacts on agriculture of coastal belt as well as southern part of our country (Anonymous, 2007). The effects of salinity stress on the growth and yield of crops vary with the stage of crop growth during which the stress occurs (Sionit and Kramer, 1977). To overcome the salinity problem, saline soils can be used to grow salt tolerant crop plants. Thus development of salinity stress tolerant crops is a key to global agricultural goal.

Previous several researches exhibited that tomatillo is a high yielding crop in our country's aspect than its origin, Mexico (Karim, 2016). Our Rabi season atmosphere has found to be highly favorable for growing tomatillo. Now, further efforts are obligatory to observe the performance of tomatillo under saline soil as well as to achieve the exploitation of saline soils and waters that are not currently usable. This will also provide inducement to find out new, suitable tomatillo genotypes that can ensure higher yield and also suitable for cultivating in the salinity affected southern region and coastal belt of Bangladesh. These issues were taken into account while conducting the current experiment.

This study was conducted to explore the bioassay so as to establish a reproducible protocol for selecting different salt tolerant tomatillo genotypes growing in different concentrations of salinity stress (NaCl) by analyzing their agromorphogenic, physiological and nutritional traits. With conceiving the above scheme in mind, the present research work has been undertaken in order to fulfill the following objectives:

- To observe the growth and yield of tomatillo genotypes under different salinity stress condition to identify the best recommendable genotype.
- To determine the response of genotype × treatment interaction based on their agromorphogenic, physiological and nutritional traits and
- To assess the magnitude of genotypic variation under control and stress condition.

## CHAPTER II

### REVIEW OF LITERATURE

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The tomatillo or husk tomato, a good source of functional food and medicinal compounds, has attracted renewed interest for production worldwide. It is an important crop in Mexico, and now-a-days due to its wide range of adaptability and variation, it is becoming appreciated in other countries as well. Tomatillo is a member of the Solanaceae family, and it is also referred as green tomato. It produces fruits that constitute an important component of the Mesoamerican cuisine, and is employed in a similar manner to tomatoes (*Solanum lycopersicum* L.), but it has a slight acidic flavor. It is a good source of vitamin A and C, and also has been suggested that chemicals present in tomatillos, e.g. ixocarpalactone A have cancer chemo-preventive properties (Wilf *et al.*, 2017; Choi *et al.*, 2006).

Although tomatillo has unique medicinal properties and has attracted huge attention of researchers, but in terms of breeding, especially for stress breeding, it is quite far behind of the attention of researchers. Very limited research findings are available in case of *Physalis* breeding against different abiotic stresses. As salinity is an increasingly important environmental constraint to crop production worldwide, it is highly necessary to practice breeding strategies in this aspect (Ghassemi *et al.*, 1995). In the world, about 400 million hectares of land are affected by high levels of salinity. In Bangladesh, about 1 million hectares of land are affected by salinity in the coastal regions and it is increasing day by day. The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salinity stress) or a combination of these factors (Marschner, 1995; Ashraf, 1994). All of these cause adverse pleiotropic effects on plant growth and development at physiological and biochemical levels (Munns, 2002; Gorham, *et al.*, 1985; Levitt, 1980). Thus, screening and development of new salt tolerant genotypes are key solution of this problem. Some of the important previous research findings has been briefly described in this chapter.

## 2.1 Tomatillo

The tomatillo is an important native crop to Mexico and Central America, where it has been an important food crop for millennia. Tomatillo means “little tomato” in Spanish. It is an annual, cross pollinated crop of Solanaceae family under the genus *Physalis*. *Physalis* is a genus of angiosperms (flowering plants), which grows in warm temperate and subtropical regions of the world. The Plant List (2010) includes 298 scientific plant names of species under the genus *Physalis* and among them 71 (23.8%) are accepted species names. Most of the species, of which there may be 75 to 90, are indigenous to America and at least 46 species are the endemic to Mexico (Vargas *et al.*, 2001). *Physalis* plants can be either annual or perennial. The specific name *philadelphica* dates from the 18th century (Small, 2011). Cultivated species and weedy annuals have been introduced worldwide. Tomatillos have been adopted by American farmers due to their resistance to diseases. Being a solanaceous crop, tomatillo is a distant relative of tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum spp.*). Many other closely related species of tomatillo under *Physalis* are ground cherries (*Physalis crassifolia*), inca berry (*Physalis peruviana*), cape gooseberry (*Physalis peruviana*), poha berries and golden berries (Vikram, 2013).

### 2.1.1 Nomenclature, origin and distribution

The name tomatillo has come from the “Nahuatl” word “tomatl”. It is a member of the genus *Physalis*, which was erected by Carl Linnaeus in 1753 and it contains about 463 species. Jean-Baptiste de Lamarck described the tomatillo under the name *Physalis philadelphica* in 1786. The tomatillo is also often classified as *P. ixocarpa* Brot. (Bukun *et al.*, 2002). However, *P. philadelphica* Lam. is the most economically important species (Simpson *et al.*, 1995). The nomenclature for *Physalis* changed since the 1950s. *P. philadelphica* Lam. was at one time classified as a variety of *P. ixocarpa* Brot. Later, the classification of *P. ixocarpa* Brot. was revised under the species of *P. philadelphica* Lam. Today, the name *P. ixocarpa* Brot. is commonly used for the domestic plant and *P. philadelphica* Lam. for the wild one. The tomatillo is also known as husk tomato (Valladolid, 2010), tomatillo (in Mexico; means

“little tomato”), Mexican groundcherry, Large-flowered tomatillo, Mexican green tomato, miltomate (in Guatemala, Mexico), tomate verde (in Spanish; means “green tomato”), farolito, or simply tomate (when tomato is called “jitomate”) (Morton, 1987).

The wild tomatillo and its related plants are native to Central America and Mexico with the highest diversity in Mexico. The plant is grown mostly in the Mexican states of Hidalgo and Morelos, and in the highlands of Guatemala. The tomatillo is thought to have been first domesticated by the Aztecs in central Mexico around 800 BCE before the coming of Europeans and was an important food crop to a number of pre-Columbian peoples in Mesoamerica, including the Mayans. With the Spanish conquests of Mexico and Central America in the 1500s and 1600s, the plant was taken back to Spain. In the United States, tomatillos have been cultivated since 1863. Further distribution occurred in the Bahamas, Puerto Rico, Jamaica, and Florida. By the mid-20<sup>th</sup> century, the plant was further introduced to India, Australia, South Africa, and Kenya (Morton, 1987).

Originating in Mexico and Central America, this citrusy plant has been an important food crop for millennia, though the plant has been around for even longer. In early 2017, scientists writing for the journal “Science” reported on their discovery and analysis of a fossil tomatillo found in the Patagonian region of Argentina, dated to 52 million years BP. This finding even has been pushed back the earliest appearance of the plant family, Solanaceae (Wilf *et al.*, 2017).

### **2.1.2 Morphology of tomatillo**

The tomatillo plant can be erect or prostrate and typically does not reach more than 1 meter (3.3 feet) in height, similar to the common tomato, but usually with a stiffer, more upright and a bit hairy stem. The leaves have acute and irregularly separated dents on both sides. The leaves are typically serrated and can either be smooth or pubescent (Montes and Aguirre, 1994). The flowers are borne in the axils of the leaves and feature five fused petals that are typically yellow with dark spots towards the base and may also be white, light green, bright yellow

and sometimes purple in color. The anthers are typically dark purple to pale blue. All other parts of the plant including the husk, leaves, and stem are poisonous. The tomatillo plants grow well in warm climates with full sunshine. Some species are sensitive to frost, but others, such as the Chinese lantern, *P. alkekengi* can tolerate severe cold when dormant in winter.

A notable feature of tomatillo fruit is the formation of a large papery husk derived from the calyx, which partly or fully encloses the fruit (Whitson and Manos, 2005), hence it is called husk tomato. After pollination, the calyx of the flower surrounds the ovary and grows with the developing fruit to protect it. The fruits are true berries with tiny seeds and are typically green, yellow, or purple when mature. The fruit is small and similar in size, shape and structure to a small tomato.

A single plant can produce 60 to 200 fruits within a single growing season, with an average yield of about 9 tons per acre (Masabni, 2016). The average yield of tomatillo (*P. ixocarpa* Brot.) in Mexico is 13.933 tons/ha, a low quantity considering its potential estimated yield of 40 tons/ha (Santiaguillo *et al.*, 1994). The fruits can be eaten as raw or cooked and are sometimes made into soups, jams, or chutneys . In Mexico and Guatemala, tomatillos and spicy peppers are commonly roasted and then ground together to form salsa verde, a green sauce used as a condiment on meats and other foods. Tomatillos are a good source of dietary fibre, vitamin C, vitamin K, and niacin.

Tomatillo plants show gametophytic self-incompatibility, meaning they require pollen from a neighboring plant to produce fruit. The fertile hermaphrodites fails to produce zygotes after self-pollination and this incompatibility is controlled by a dominant gene (Mulato-Brito *et al.*, 2007). Thus to increase the breeding potential of tomatillo, polyploidy (autotetraploid) development was practiced through colchicine-based induction and its success rate was found above 65 % (Torres *et al.*, 2011). Autotetraploid plants were fertile, productive and showed higher values for length of life cycle, plant height,



fruit weight and equatorial diameter, fruits per plant, and soluble solid concentration.

### **2.1.3 Nutritional and medicinal value of tomatillo**

The tomatillo fruits have received warm appreciation worldwide these days not only for its unique taste, but also for its high nutritional and medicinal properties. With a combination of vitamins and minerals that include fiber, potassium, vitamins A, C, and K, niacin, manganese,  $\beta$ -carotenes (zeaxanthin and lutein), iron, magnesium, phosphorus, and copper; the tomatillo fruits definitely have its excellent share of nutrients. Compared with tomatoes, tomatillos provide a few more calories, fat, and protein per ounce, extra fiber, minerals, antioxidants as well as vitamins.

It is quite necessary to know the per unit nutritional value of raw tomatillo fruits. 100 g (3.5 ounces) of edible tomatillo (raw) fruit contains energy 32 Kcal, carbohydrates 5.84 g, protein 0.96 g, total fat 1.02 g, dietary fiber 1.9 g, vitamins (Folates 7  $\mu$ g, Niacin 1.85 mg, Pyridoxine 0.056 mg, Thiamin 0.044 mg, Riboflavin 0.035 mg, vitamin-A 114 IU, vitamin-C 11.7 mg, Vitamin E 0.38 mg, vitamin K 10.1  $\mu$ g), Sodium (Na) 1 mg, Potassium (K) 268 mg, Calcium (Ca) 7 mg, Copper (Cu) 0.079 mg, Iron (Fe) 0.62 mg, Magnesium (Mg) 20 mg, Manganese (Mn) 0.153 mg, Phosphorus (P) 39 mg, Selenium (Se) 0.5  $\mu$ g, Zinc (Zn) 0.22 mg,  $\beta$ -carotene 63  $\mu$ g,  $\alpha$ -carotene 10  $\mu$ g and Lutein-zeaxanthin 467  $\mu$ g (Yamaguchi, 1983). Vitamin-A helps maintain healthy mucus membranes and skin as well as the flavonoids do their part in inhibiting lung and mouth cancers.  $\beta$ -carotenes, zeaxanthin and lutein impart extraordinarily potent antioxidant properties that work with vitamin-A to protect vision and helps to prevent macular degeneration (Quiros, 1984).

Tomatillo is a good source of antioxidant known as Withanolides. Plant secondary metabolites, like withanolids are produced in response to environmental stress as the response of defense strategies to successfully complete their life cycle. Three researchers at the University of Kansas

discovered 14 withanolide compounds in the wild tomatillo (*Physalis longifolia*) showing significant anti-cancer properties in preclinical testing (Monaco, 2012). The research findings revealed that withanolides (e.g. IxoA) present in tomatillo fruits are potent inducers of quinone reductase, which is more powerful in discouraging cancer growth and neutralizing colon cancer cells than chemotherapy (Choi *et al.*, 2006). These compounds are already showing promise in combating a number of different cancers and tumors like melanomas, thyroid cancer, breast cancer, cancer of the esophagus and pancreas, and even some brain tumors and leukemias without any noticeable side effects or toxicity (Choi *et al.*, 2006). Ixocarpalactone A has also anti-bacterial properties.

The genus *Physalis* contains two major groups of chemical compounds; the “tropane” alkaloids (mainly tropine and tigoidine) and the “physalins” (steroid compounds), which are responsible for various medicinal properties. Tropanes are responsible for anti-muscarinic activity and can block the activity of neurotransmitter acetylcholine by binding to muscarinic receptors of the parasympathetic nervous system. These chemical compounds are important in treatment of gastrointestinal and muscular spasms and Parkinson’s disease (Choi *et al.*, 2006). Physalins are under attention because of the anti-tumour and cytotoxic activity (Zaki *et al.*, 1987; Chiang *et al.*, 1992). *Physalis* has biological activities such as antibacterial, antiseptic, abortifacient, molluscicidal, antiprotozoal, anticancer, cytotoxic and immune modulatory activities (Bastos *et al.*, 2005; Vessal and Kooshesh, 1996).

Tomatillo fruits are rich in flavonoids that help to protect from lung and oral cavity cancers (Quiros, 1984). Traditional healers in India have been known to prescribe foods containing withanolid compounds as a tonic for arthritis and other musculoskeletal conditions (Kindscher *et al.*, 2012). According to the Native Medicinal Research Program at the University of Kansas, historical records showed that numerous North American native tribes used wild *Physalis longifolia* fruits to treat headache and stomachache (Monaco, 2012). *Physalis*

*angulata* L. (Ciplukan) has been widely consumed by people with asthma, hepatitis, malaria, rheumatism and dermatitis (Alves *et al.*, 2008).

## **2.2 Effect of salinity in soil and plant**

Salinity is a measure of dissolved salts in sea water. It is calculated as the amount of salt (in gram) dissolved in 1 kg of seawater. Soil salinity is the salt content in the soil and the process of increasing the salt content is known as salinization. Salinization can result from high levels of salt present in water, landscape features allowing salts to become mobile (movement of water table), climatic trends favoring salt accumulation, human activities like deforestation, irrigation using saline water and the use of potassium as fertilizer, which can form sylvite, a naturally occurring salt. Salinity is one of the most brutal environmental factors limiting the productivity of crop plants (Srivastava *et al.*, 2012). Most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil. The area of land affected by it is increasing day by day. Soil salinity causes due to the excess accumulation of salts, typically most pronounced at the soil surface, can result in salinity affected soil. Salts may rise to the soil surface by capillary transport from a salt-laden water table and then accumulate due to evaporation. As soil salinity increases, it can result in degradation of soil and vegetation. Salinity has detrimental effects on plant growth and yield (Vidal *et al.*, 2009; Moya *et al.*, 2003).

Worldwide, more than 60 million ha of irrigated land (representing some 25% of the total irrigated land in the world) have been damaged by salt (Mekhaldi *et al.*, 2008; Cuartero and Fernandez-Munoz, 1999). Salt stress is a polymorphous stress that affects plant growth and reduces yield through three direct ways. First, the presence of salt reduces the ability of the plant to take up water which leads to reductions in the growth rate. This is referred to as the osmotic effect of salt stress, which starts immediately after the salt concentration around the roots increases over a threshold level. There is a second and slower response due to the accumulation of ions in leaves; this ion-specific phase of plant response to salinity starts when accumulated salt reaches toxic concentrations in the leaves

and the third one is nutritional stress (Gomez-Cadenas *et al.*, 1998). Within many species, documented genetic variation exists in the rate of accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in leaves; and in the degree to which these ions can be tolerated as well (Munns and Tester, 2008). For most species, Na<sup>+</sup> appears to reach a toxic concentration before Cl<sup>-</sup> does. However, for some Cl<sup>-</sup> is considered being more toxic ion (Lopez-Climent *et al.*, 2008).

### **2.3 Mechanism of salinity tolerance in plants**

Plants on the basis of adaptive evolution can be classified roughly into two major types: the halophytes; that can withstand salinity and the glycophytes; that cannot withstand salinity and eventually die. Majority of major crop species belong to glycophyte category. Thus, salinity is one of the most brutal environmental stresses that hamper productivity of crops worldwide. At a basic level, the response of plants to salinity can be described in two main phases: the shoot ion-independent response occurs first, within minutes to days, and is thought to be related to Na<sup>+</sup> sensing and signaling (Gilroy *et al.*, 2014; Roy *et al.*, 2014). In this first phase, effects of salinity on water relations can be important, causing stomatal closure and the inhibition of leaf expansion (Munns and Termaat, 1986). The second phase, the ion-dependent response to salinity, develops over a longer period (days to weeks) and involves the build-up of ions in the shoot to toxic concentrations, particularly in old leaves, causing premature senescence of leaves and ultimately reduced yield or even plant death (Munns and Tester, 2008).

Mechanisms of plants towards salt tolerance occur by restricting the entry of salt into the plant (especially minimizing the accumulation of salt in photosynthetic tissues and cytoplasm) (Munns, 2002). Three main salinity tolerance mechanisms have been proposed by Munns and Tester (2008); ion exclusion: the net exclusion of toxic ions from the shoot; tissue tolerance: the compartmentalization of toxic ions into specific tissues, cells and subcellular organelles; and shoot ion-independent tolerance: the maintenance of growth and water uptake independent of the extent of Na<sup>+</sup> accumulation in the shoot.

One of the most detrimental effects of salinity stress is the accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in tissues of plants exposed to soils with high NaCl concentrations. Entry of both  $\text{Na}^+$  and  $\text{Cl}^-$  into the cells causes severe ion imbalance and excess uptake might cause significant physiological disorder(s). High  $\text{Na}^+$  concentration inhibits uptake of  $\text{K}^+$  ions which is an essential element for growth and development that results into lower productivity and may even lead to death (James *et al.*, 2011). In response to salinity stress, the production of ROS, such as singlet oxygen, superoxide, hydroxyl radical, and hydrogen peroxide, is enhanced (Apel and Hirt, 2004; Mahajan and Tuteja, 2005; Ahmad, 2010; Ahmad and Umar, 2011; Ahmad and Prasad, 2012). Salinity induced ROS formation can lead to oxidative damages in various cellular components such as proteins, lipids, and DNA, interrupting vital cellular functions of plants. Other physiological components are also likely to contribute to salinity tolerance, such as the maintenance of plant water status, transpiration and transpiration use efficiency (Harris *et al.*, 2010; This *et al.*, 2010; Barbieri *et al.*, 2012); leaf area (Maggio *et al.*, 2007); seed germination (Foolad and Lin, 1997); production of antioxidants (Ashraf, 2009); early seedling growth (Kingsbury and Epstein, 1984); and harvest index (Gholizadeh *et al.*, 2014).

The plant follows two major adaptive strategies towards high environmental salinity tolerance; firstly, avoiding stress due to different physical and physiological barriers, and secondly, enhancing the adaptive mechanisms internally that will enable successful survival. Therefore, the  $\text{Na}^+$  uptake and its transport regulation across the plasma membranes and tonoplast is one of the key factors that establish the plant cell response to salinity stress (Dajic, 2006). Avoidance of salt uptake can take place by salt exclusion; it is a very efficient but complex way of reducing the permeability of massive ion in the root zone, especially  $\text{Na}^+$ . This process enables a low uptake and accumulation of salts in the upper parts, especially in the transpiring organs of the plant. Many glycophytes are known to show better skills for  $\text{Na}^+$  exclusion from the shoot and also for maintaining elevated levels of  $\text{K}^+$ . A study done by Munns *et al.* (1988) and Jeschke and Hartung (2000) have shown salt exclusion to function at

the cellular as well as at the whole plant level and to a greater extent is related to regulation of  $K^+/Na^+$  ion selection.

In mangrove *Avicennia marina* is known to have 98% degree of salt exclusion property (Ball, 1988). Whereas it was demonstrated by Munns *et al.* (1999) glycophyte or halophyte, has the property of restraining of  $Na^+$  uptake and accumulation in the shoots. In some salt tolerant species, for example wheat have the property to exclude salts is achieved by changing  $Na^+$  and  $Ca^{2+}$  ions, rather than bringing about modification in osmotic potential, as adsorption on membranes of root cells of calcium ions directs towards reduced penetration of monovalent cations (Munns *et al.*, 1999).

Salt excretion is another very efficient way of preventing excessive absorption and building up of salts in photosynthetic tissues. This mechanism is equipped with developed special features, which are mostly present in leaf epidermis, known as salt glands and salt hairs (bladders). These structures are commonly found in many halophytes such as *Spartina*, *Aeluropus* (Poaceae), *Limonium*, *Armeria* (Plumbaginaceae), *Atriplex* (Chenopodiaceae), *Glaux* (Primulaceae), *Tamarix*, *Reamuria* (Tamaricaceae) and in mangrove species, e.g. *Avicennia*, *Aegiceras* and *Acanthus* (Popp, 1995). Among all these synchronized physiological responses in plants, the plant hormone abscisic acid (ABA) plays an essential role. ABA is a stress hormone as for its rapid accretion towards the response to stress and its intervention helps plants to endure over much stress. The first requirement is that ABA production should be rapidly triggered by the stress signals so that inhibition of physiological functions is avoided, and secondly, ABA should be quickly degraded and deactivated once the stress is reassured so that normal plant growth and functions can recommence.

#### **2.4 Effect of salinity stress on different traits of tomatillo**

Genetic variability among the genotypes for different traits is very important consideration for selecting desirable genotype in case of breeding for salt tolerance. The selection of traits could be based on agromorphogenic, physiological or nutritional parameters. Salinity usually effects adversely on

plants regarding the agromorphogenic traits like, flowering, fruiting, plant height, fruit number, fruit weight, maturity time, yield etc. Physiological traits such as chlorophyll content of leaves, Na<sup>+</sup> and K<sup>+</sup> concentration in plants, proline content and similarly nutritional values (brix percentage, vitamin-C content etc.) of plants could also be affected by salinity stress.

#### **2.4.1 Effect of salinity on agromorphogenic traits**

The hazardous effect of salinity can induce disorder(s) even during germination of seeds and propagules. In many crops, seed germination and early seedling growth are the most sensitive stages to environmental stresses like salinity (Sivritepe *et al.*, 2003). Salinity can either completely inhibit germination at higher levels or induce a state of dormancy at lower levels (Khan and Ungar, 1997). Salinity can also affect germination by facilitating the intake of toxic ions, which can cause change of certain enzymatic or hormonal activities of the seed. Rapid, uniform and complete germination is a prerequisite for successful transplant production and stand establishment in vegetable crops (Demir and Ermis, 2003). Salinity has been reported to cause significant reduction in the rate and final percentage of germination and emergence of many vegetable crops, which in turn may lead to uneven stand establishment and reduced crop yields (Yildirim *et al.*, 2002; Yildirim and Guvenc, 2006).

Patel *et al.* (2010) reported that the reduction in plant growth by NaCl might be attributed to the inhibitory effects of toxic ions mainly Na<sup>+</sup> and Cl<sup>-</sup>. Diaz-Lopez, *et al.* (2013) illustrated that the negative effect of salinity were mainly due to Cl<sup>-</sup> and/or Na<sup>+</sup> toxicity and to a nutritional imbalance caused by an increase in the Na<sup>+</sup>/K<sup>+</sup> ratio. The decrease in leaf number with increased salinity might be due to tolerance of plant to the toxic effects of Cl<sup>-</sup> and/or Na<sup>+</sup>, by their accumulation in the older leaves. Then, plants may avoid ion toxicity by leaf shedding (Cuartero and Fernandez-Munoz, 1999). Salinity also reduces leaf area per plant by accelerating leaf death as indicated by the development of leaf tip burning symptoms and leaf loss. Munns (2002) mentioned that salinity may frequently accelerate leaf senescence that can reflect a decrease of fresh and dry mass.

Decrease in dry weight seems to be due to reduction in the number of leaves and to a reduction in leaf area under salinity condition (Van-Ieperen, 1996).

#### **2.4.1.1 In case of *Physalis* species**

An *in vitro* experiment was conducted by Celikli *et al.* (2017) the evaluation of salinity in golden berry (*Physalis peruviana* L.). In that study, golden berry shoot apex were cultured within the Murashige and Skoog (MS) medium with 1 mg/L indole acetic acid (IAA), 3% sucrose and 0.7% agar supplemented with NaCl (0, 25, 50, 75 and 100 mM) with five replications of each treatment. After four weeks, fresh and dry weight, leaf length and diameter, shoot length and diameter as well as root length of plantlets were measured along with their chlorophyll content. Experimental results revealed that different level of salinity treatments in *in vitro* culture had notable effect on growth parameters and they were decreased significantly with the increasing salinity levels. In root length parameter, the averages were decreased gradually with the increase of salt rate compared to the control group. It was observed that 25 mM application in both leaf measurements didn't make a statistical difference compared to control, however, a decrease was observed in especially 100 mM following the increasing of dose. Moreover, it was seen that 75 and 100 mM applications were not statistically so different from each other.

An experiment was carried out by Helaly *et al.* (2017) in Egypt to study the response of husk tomato (*Physalis pubescens* L.) cv. (local variety) to different levels of saline water. In that study, plants were irrigated with saline water with concentration of control (260 ppm), 2000, 4000, 6000 and 8000ppm. The results illustrated that increasing salinity levels from 2000 to 8000ppm caused significant decrease in some physical characteristics like, plant height, stem diameter, number of branches per plant, leaf area, average fruit weight, size, fruit diameter and ultimately yield but it caused an increase in fruit firmness in husk tomato. Yield reduction was caused by reduced enlargement rate during the exponential phase of fruit growth, which has been reported to be particularly



sensitive to ionic and osmotic damages caused by ion accumulation in the plants throughout the growth season.

A study was conducted by Manuela *et al.* (2016) aiming to evaluate the effects of priming on seed germination under salt stress and gene expression in seeds and seedlings of *P. angulata* L. After priming with saturated KCl solution for 10 days, seed germination was tested in plastic trays containing 15 ml of water (control, 0 dS/m) or 15 ml of NaCl solution (2, 4, 6, 8, 10, 12, 14 and 16 dS/m). Priming is a technique to improve seed germination. Germination of both non-primed and primed seeds decreased when seeds were submitted to imbibition under increasing salt concentrations up to 16 dS/m. Besides germination, the non-primed *P. angulata* seeds that germinated in different salt concentrations from EC of 8 dS/m or higher, showed significantly slower germination and worse uniformity. Primed seeds initially kept a higher germination rate, but then gradually became slower with increasing salt concentrations.

Khalil and Leila (2016) conducted two pot experiments at the green house of National Research Centre, Egypt to study the role of magnetic treatments (0, 2 and 4g/L) on growth, productivity, %RWC and fruit quality of *Physalis pubescens* cv. Balady under irrigated with saline water (fresh water, 2000, 4000 and 6000 ppm). Results showed that, although replications with magnetic treatments way performed better, but agromorphological traits like, plant height, number of branches per plant, number of leaves per plant, root and stem length, leaf area expansion, total biomass, dry matter accumulation as well as yield in *Physalis pubescens* plants were significantly decreased with the increase of salinity levels as compared with the control one. The reduction in plant growth exposed to salinity may be attributed to the reduction in water content and water potential of plant tissues, which resulted in internal water deficit to plants (Hishida *et al.*, 2013).

An experiment was conducted by Yildirim *et al.* (2011) to evaluate the effect of NaCl salinity on germination and emergence of *Physalis ixocarpa* as well as *Physalis peruviana*. Seeds of *P. ixocarpa* and *P. peruviana* were germinated by

using 0, 30, 60, 90, 120 and 180 mM NaCl solutions. The study result showed no emergence of *Physalis* at 90, 120 and 180 mM levels of NaCl salinity. Final observations showed that the germination percentage, fresh and dry weight of seedlings of both species were decreased with the increasing level of salinity. The findings of the experiment concluded that seedling emergence and growth is more sensitive to salt stress than seed germination in *Physalis*. In this experiment, *P. ixocarpa* showed better emergence than *P. peruviana*. *P. peruviana* was tolerant to salt stress during germination, but became more sensitive during emergence and early seedling stages. On the contrary, *P. ixocarpa* showed more tolerance to salt stress than *P. peruviana* during emergence and early seedling stages. Similarly, according to Chartzoulakis and Klapaki (2000), seedling emergence and growth of pepper was found to be more sensitive to salt stress than seed germination. Foolad and Lin (1997) suggested that salt tolerance is a developmentally regulated, stage-specific phenomenon such that tolerance at one stage of plant development may not be related with tolerance at other developmental stages.

#### **2.4.1.2 In case of *Solanum* species**

According to Islam *et al.* (2011), salt stress has adverse effect on plant growth and development. The study results revealed that in higher salinity level (10 dS/m) parameters like, plant height, primary branches, cluster per plant, fruit per cluster, number of fruits and total yield per plant, individual fruit weight were decreased gradually. Eight tomato genotypes *viz.*, J-5, 'Binatomato-5', 'BARI tomato-7', 'CLN-2026', 'CLN-2366', 'CLN-2413', 'CLN-2418' and 'CLN-2443' were used in that experiment.

A two-factor experiment was demonstrated by Al-Yahyai *et al.* (2010) to evaluate the performance of yield and quality of tomato with three different levels of saline water (3, 6 and 9 dS/m) and three types of fertilizers *viz.*, inorganic NPK, organic manure and a mixed fertilizer of both. The experimental results indicated that growing tomatoes under 3 dS/m and 6 dS/m irrigation water produced the highest yield whereas irrigating with 9 dS/m significantly reduced

the final fruit number and fruit weight. Tomatoes grown using manure produced the least amount of yield compared to those with inorganic and mixed fertilizers.

An experiment was conducted by Magan *et al.* (2008) to evaluate the effect of salinity on fruit yield, yield components and fruit quality of tomato grown in soil-less culture in plastic greenhouses in Mediterranean climatic condition. Two spring growing periods, one long season, autumn to spring growing period studies were conducted with two cultivars, 'Daniela' and 'Boludo'. Seven levels of electrical conductivity (EC) in the nutrient solution were compared in (2.5 to 8.0 dS/m) in one experiment and five levels in another two experiments (2.5 to 8.5 dS/m). The research findings showed linear decrease of total and marketable yield with increasing salinity levels above a threshold EC value. Increase of salinity levels improved fruit quality like, proportion of extra fruits, total soluble solid and titratable acidity content. But salinity caused decrease in fruit size, which is a major determinant of price.

An experiment was conducted by Agarwal *et al.* (2005) on the effect of water salinity on tomato under drip irrigation. Study findings reported that the tomato yield was drastically affected when the salt level was increased in the root zone. The results showed that the number of fruits per cluster, fruits per plant, fruit weight, fruit maturity and other yield contributing characters were also decreased with higher salinity levels. According to Ghadiri *et al.* (2005), restricted water uptake by salinity due to the high osmotic potential in soil and high concentrations of specific ions may cause physiological disorder(s) in plant tissues, fruit size and maturity and as a result ultimately reduced yields.

A book named "Tomatoes (Crop Production Science in Horticulture)" written by Ephevelink (2005) stated that salinity can reduce the fruit growth rate and final fruit size by an osmotic effect. High salinity can lower water potential in the plant that results the reduction of water flow in the fruit and therefore the rate of fruit expansion. A study done by Munns *et al.* (2002) showed that a clear stunting of plant growth was caused by salinity stress resulting a considerable decrease

in the fresh weight of leaves and stems. Increased level of salinity also conveyed significant reduction in shoot weight and plant height.

Reduction in total marketable yield and fruit size, but improved tomato fruit quality at higher higher salinity levels was found by Hao *et al.* (2000) growing tomato cv. Trust with Nutrient Film Technique (NFT). The adverse impact of high levels of salinity was observed by Cuartero and Fernandez-Munoz (1999) on number of fruits per plant of tomato. All other yield contributing characters also adversely reacted with the increase of salinity treatment levels.

#### **2.4.2 Effect of salinity on physiological traits**

Salinity can cause stress at different levels of severity depending on species, salinity kind and stress time in plants. Salt amount increasing around the root zone can cause the increase of Na<sup>+</sup> and Cl<sup>-</sup> in plant tissues and organelles. This increase causes ion stress and increase of osmotic pressure bringing instability between K<sup>+</sup> and Ca<sup>+2</sup> ions (Shannon and Grieve, 1998; Hasegawa *et al.*, 2000; Xie *et al.*, 2000 and Zhu, 2002). Water uptake and growth of plants slow down together with rising osmotic pressure. Salinity can inhibit photosynthesis by damaging protein, chlorophyll, DNA and cell membrane and even can cause death of the cells (Dasgan *et al.*, 2002; Borsani *et al.*, 2003 and Amini and Ehsanpour, 2005). The suppressive effect of salinity on yield was also consequence of marked inhibition in photosynthesis (Taha *et al.*, 2011). Salinity mainly affects leaf elongation which decreases the development of photosynthetic surface area. Salinity also reduces xylem development, and that reduction would explain the reduction in fruit weight under saline conditions (Akinici *et al.*, 2004; Cuartero and Fernandez-Munoz, 1999; Shannon and Grieve, 1998). According to Ehret and Ho (1986), the reduction of fruit yield by salinity was proportional to the reduction of plant vegetative growth.

Growth inhibition due to soil salinity is caused by low external water potentials, ion toxicity and ion imbalance (Munns, 1993). Due to growth reduction in leaves and roots, the salts in soil solution decrease stomatal conductance and, consequently, photosynthesis (Munns, 1993). The lower leaf area index causes

with the increase of higher salinity stress, which is responsible for a decrease in leaf growth (Walker and Bernal, 2008). Reduction in leaf growth rate has been related to a reduction in cell turgor, cell wall rheological properties and a reduction in photosynthetic rate (Munns, 1993). Salinization causes an abrupt fall in the leaf water potential, which is not immediately counterbalanced by the slower decrease in leaf osmotic potential (Cuartero and Fernandez-Munoz, 1999). Relatively low levels of salinity can result in a transient reduction in turgor and leaf growth rate (Yeo *et al.*, 1991). During long term exposure to salt, leaf expansion, probably, depends on the abilities of a plant to avoid excessive concentration of ions in the transpiring tissues and to produce new leaves at a faster rate than the old ones (Munns, 2002). Munns (2002) also stated that salt injury was due to Na<sup>+</sup> and/or Cl<sup>-</sup> accumulation in transpiring leaves at excessive levels, exceeding the ability of the cells to compartmentalize these ions in the vacuole.

Lycoskoufis *et al.* (2005) supposed that growth restriction predominantly was caused by a reduced stomatal conductance, while after a long-term exposure to salinity, growth may also be suppressed due to inhibition of photosynthesis at the chloroplast level. This effect was attributed by Cramer *et al.* (2001) to a decrease of leaf cellular expansion, whereas Ali-Dinar *et al.* (1999) and Ebert *et al.* (1999) stated that leaf area was reduced as a consequence of physiological disorders triggered by salt stress, accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in different plant tissues, as well as by reduction of net photosynthesis rate and pigment contents in the leaf tissue. Na<sup>+</sup> can facilitate the accumulation of nitrites in plant tissue that, in turn, causes toxic effects and slow assimilation of nitrogen (Navarro and Navarro, 2000) affecting leaf area growth.

Salt stress has severe effect(s) on plant growth and productivity and interrupt in the normal metabolic processes. Proline may alleviate the negative impact of salinity by decreasing osmotic stress and consequently maintaining the membrane integrity and its function (Mahdi and El-Katony, 2001). Proline is multifunctional amino acids and a signalling molecule acting as a plant growth

regulator by triggering cascade signaling processes (Yang *et al.*, 2009). Proline preferred as a common osmolyte in plants and get up-regulated against different stresses (Yildiz and Terz, 2013; Szabados and Savoure, 2009). Proline accumulation in plants provides protection against stress, and thus called stress protein. Plants continuously tend to enhance endogenous level of proline with increasing levels of stress, like salinity (Molazem *et al.*, 2010). Proline accumulation under stress might occurs due to an increase in pyrroline-5-carboxylate synthetase (P5CS), the rate-limiting enzyme in proline biosynthesis (Singh *et al.*, 2000) and a decrease of proline dehydrogenase (PDH) activity (Spoljarevic *et al.*, 2011). Proline synthesis initiates the generation of NADP<sup>+</sup>, which acts as the backbone for ribose 5-phosphate which is required for the purines synthesis, and proline oxidation yields the reduced electron carriers, which provide energy for the numbers of biochemical reaction like nitrogen fixation (Kim and Nam, 2013). It was reported that, the exogenous application of proline alleviates the adverse effects of salt by reducing the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in plants (Khattab and Afifi, 2009; Aggarwal *et al.*, 2011). Proline regulates the expression of a number of genes related to antioxidant enzymes under salt stress. Among them, one of the gene *1-pyrroline-5-carboxylate synthetase* is responsible for up-regulating the stress-induced proline accumulation under salinity stress (Kim and Nam, 2013).

#### **2.4.2.1 In case of *Physalis* species**

An experiment was conducted by Celikli *et al.* (2017) for *in vitro* evaluation of salinity in golden berry (*Physalis peruviana* L.). In that *in vitro* salinity study, golden berry shoot apex were cultured within the Murashige and Skoog (MS) medium with 1 mg/L indole acetic acid (IAA), 3% sucrose and 0.7% agar supplemented with NaCl (0, 25, 50, 75 and 100 mM) with five replications of each treatment. After four weeks, chlorophyll content of plantlets was measured in daylight with Spectrum Technologies Field Scout CM1000 Model Chlorophyll Meter along with their several root and shoot parameters. From the experimental result, it was observed that the salt applications at 25 and 50 mM doses were not different from control group in terms of chlorophyll content

whereas a significant difference was seen at 75 and 100 mM doses. A gradual decrement of chlorophyll content occurred with the increasing of salinity concentration which was statistically significant. It is considered that this decrement is related to the increase in the activity of chlorophyllase enzyme which causes in decomposing chlorophyll (Reddy and Vora, 1986).

Negative effects of salinity on husk tomato (*Physalis pubescens* L.) cv. (local variety) to different levels of saline water (260 ppm, 2000, 4000, 6000 and 8000ppm) have been attributed to disturbance in both protein assimilation, mineral uptake and distribution activities of growth hormones, enzymes activities and oxidative defense (Helaly *et al.*, 2017). The research findings showed significant influence of salinity from 2000 to 8000ppm on total chlorophyll content, macro and micro elements (N, P, K and Na) and proline content. Proline and Na<sup>+</sup> content in husk tomato was increased with increasing salinity. The rise of pH level in the root zone resulted from salinity led to unavailability of K<sup>+</sup> and Ca<sup>2+</sup>. The harmful effects of salinity may be attributed to the inhibited effects on the activity of Fe<sup>2+</sup> that reflects on the reduction rate of chloroplast structure and chlorophyll accumulation in plants (Sevengor *et al.*, 2011). The lowest photosynthetic ability under salt stress conditions was due to stomata closure, inhibition of chlorophyll synthesis and due to decrease in the absorption of minerals needs for chlorophyll biosynthesis, i.e. Fe<sup>2+</sup> (Sevengor *et al.*, 2011).

The effect of increasing NaCl (0, 60 and 120 mM) stress was investigated by Miranda *et al.* (2014) on growth, proline content and total antioxidant activity in leaves of cape gooseberry plants kept in 2 L pots and grown under greenhouse conditions. Research findings showed that vegetative growth, dry weight, leaf number, leaf area and plant height was significantly lower at higher salinity levels as compared to control plants. The leaf proline content was increased significantly during the evaluation period and tended to be higher with increasing level of NaCl. Total antioxidant activity was increased constantly and was significantly higher than in leaves control plants.

A greenhouse experiment was conducted to assess the effect of salt stress on the growth of the cape gooseberry by Miranda *et al.* (2010). Cape gooseberry plants were surveyed in perlite pot cultures salinized with varying concentrations of NaCl (0, 30, 60, 90 and 120 mM) for 75 days. The growth indices like leaf area index, crop growth rate, relative growth rate, unit leaf rate, leaf area ratio, leaf weight ratio and specific leaf area were calculated. Results showed that increasing levels of NaCl (60 to 120 mM) in the growth medium caused a reduction in the leaf growth parameters: leaf area index, unit leaf rate, leaf weight ratio and specific leaf area. The reduction of leaf area expansion per unit of plant biomass (leaf area ratio) was primarily caused by a decrease in the specific leaf area, which played an important role in determining the relative growth rate of salt stressed plants. In this study, salt-specific effects also included burning of leaf margins, reduced leaf area and death and abscission of older leaves.

#### **2.4.2.2 In case of *Solanum* species**

The strong adverse effect of salinity treatment on yield in cherry tomato was reported by Edris *et al.* (2012). The study findings showed that addition of supplemental Ca<sup>2+</sup> and K<sup>+</sup> can ameliorate negative impact of high salinity. Small fruit development in salinity conditions could be related to disorder in water relations and decrease in photosynthetic productions (due to leaf area reduction) as well as chlorophyll content. Negative effect of salinity on plant height, leaf area, plant growth, yield, dry matter plant, Na<sup>+</sup> and Cl<sup>-</sup> accumulation in tomato tissues were demonstrated by Siddiky *et al.* (2012). Under salt stress, all plant parameters of tomato varieties were reduced compared to control. Plant growth, fruit number, fruit size and yield were decreased gradually with the increase of salinity levels. An experiment was done by Hajiboland *et al.* (2010) where plants treated with the arbuscular mycorrhizal fungi *Glomus intraradices* (+AMF) showed beneficial effect in salt condition. In this study, tomato cultivars “Behta” and “Piazar” were cultivated in soil with EC of 0.63 dS/m, 5 dS/m and 10 dS/m. Plant growth and yield reduction was found affected by salinity. It may be the cause of variation in photosynthetic products translocation toward root, decrease



of plant top especially leaves, partial or total enclosed of stomata, chlorophyll content, direct effect of salt on photosynthesis system and ion balance.

The effect of MS and agar medium containing NaCl and sucrose on germination percentage, seedling growth, chlorophyll content, acid phosphate activity and soluble proteins in different cultivars of tomato (cv. Isfahani, Shirazy, Khozestani and Khorasani) was demonstrated by Amini and Ehsnapour (2006). In this study, seeds were germinated under different concentrations of NaCl (0, 40, 80, 120 and 160 mM). Study findings showed that, increasing salinity caused decrease in germination percentage and seedling dry weight. Chlorophyll content were decreased with increasing salinity levels. Acid phosphates activity was decreased in stem leaf while it was increased in roots. An experiment was carried out by Hajer *et al.* (2006) to assess the effect of sea water salinity (1500, 2500 and 3500 ppm) on the growth of different tomato cultivars (Trust, Grace and Plitz) in Saudi Arabia. The salinity of sea water caused the delayed seed germination and reduced germination percentage with increasing salinity. It was also observed that leaf area, total chlorophyll and  $k^+$  content, fresh weight of areal parts and %Dry weight of areal parts and yield responded negatively with the increase of salinity levels.

The gradual reduction in chlorophyll content (chlorophyll-a and chlorophyll-b) of tomato leaves was found with the increasing sea water salinity by Al-Sobhi *et al.*, 2005. The chlorophyll content of leaves of different tomato cultivars decreased by NaCl stress. An experiment was conducted by Juan *et al.* (2005) to identify the most reliable nutritional and biochemical indicators for improving salt tolerance in tomato. The study findings showed that salt-resistant tomato cultivars were characterized by reduced uptake and foliar accumulation of  $Na^+$  and  $Cl^-$ , increased  $K^+$  uptake and greater amount of sucrose, carotenoids and thiol synthesis. Dasgan *et al.* (2002) worked on 55 tomato genotypes to investigate the relationships among the salinity scale classes based on visual appearance and shoot  $Na^+$  accumulation,  $K^+/Na^+$  and  $Ca^+/Na^+$  ratios and shoot root dry weights. In that study, higher  $Na^+$  on tomato shoots indicated higher

shoot damage. Shoot  $K^+/Na^+$  and  $Ca^+/Na^+$  ratios were significantly correlated with the salinity. The higher shoot  $K^+/Na^+$  and  $Ca^+/Na^+$  ratios indicated lower shoot damage. An experiment done by Akinçi *et al.* (2004) concluded that increase level of NaCl stress cause the reduction in relative root, shoot and whole plant growth. Findings also showed that salinity increased  $Na^+$  content and decreased  $K^+$  content of tomato seedling leaves. Low stem water potentials may have an immediate and direct effect on phloem turgor, reducing the driving force for sap flow into the fruit (Johnson *et al.*, 1992). Research findings on tomato showed that fruit diameter was increased when apoplasmic water potential gradient favored the flow of solution into fruit and fruit shrinkage occurred only when the water potential gradient was inverted.

#### **2.4.3 Effect of salinity on nutritional traits**

Excessive salinity is the most important environmental stress factor that has great influence in the nutritional properties of many plant species. Excessive salt exposure reduces fruit size, total yield, and photosynthesis and increases blossom end rot (Saito *et al.*, 2008), while moderate salt stress generally improves fruit quality by increasing carotenoids and total soluble solids, which are important components of taste in ripen fruits (sugars, organic acids, and amino acids) (De Pascale *et al.*, 2001). Soluble solids and pH are determined by both the acid and sugar concentrations of the ripen fruit. High water stress leads to decreased yield, maximum accumulation of soluble solids and reduced viscosity.

The light intensity received by the plant directly affects the quantity of photo assimilates available to the fruit, it also increases their sugar-acid ratio, and influences the transpiration rate and the water uptake by the plant, which in turn, affect the EC around the root. Depending upon the composition of the saline solution, ion toxicities or nutritional deficiencies may arise because of a predominance of specific ion or competition effects among cations and anions. Several EC and fertigation management regimes could improve fruit quality (Dorai *et al.*, 2001). Increase of EC with NaCl may increase the  $Na^+$  content in

fruits. NaCl enhances the sweetness of fruit and improves the overall flavor intensity.

#### **2.4.3.1 In case of *Physalis* species**

An experiment was carried out by Helaly *et al.* (2017) to study the response of husk tomato plants (*Physalis pubescens* L.) cv. (local variety) to different levels of saline water (260 ppm, 2000, 4000, 6000 and 8000ppm). Study findings revealed that fruit quality which included total soluble solids, total titratable acidity, vitamin-C, total sugar, total carotenoids and dry matter was influenced positively under irrigation with saline water. The enhancing contents of total soluble solids and ascorbic acid may be attributed to saline concentrations effect originating from reduced fruit water content due to adaptation of husk tomato plants to salinity. Husk tomato grown under saline water showed high titratable acidity which may be attributed to the accumulation of organic acids thus maintaining fruits pH. In addition, increased total soluble solids, acidity and sugar content associated with saline irrigation may also be ascribed to concentration effects due to smaller fruit size.

Two pot experiments were conducted during at the green house of National Research Centre, Egypt by Khalil and Leila (2016) to study the role of magnetic treatments (0, 2 and 4g/L) on growth, productivity, %RWC and fruit quality of *Physalis pubescens* cv. Balady under irrigated with saline water (fresh water, 2000, 4000 and 6000 ppm). Results showed that, replications with magnetic treatments way performed better, and total soluble solids (%), acidity (% Total acid), phenolic compounds and carotenoids content in *Physalis pubescens* fruits were significantly increased with the increase of salinity treatment levels as compared with the controlled one. Reverse trend was observed in case of different morphological and yield contributing traits.

The increase in total soluble solid of husk tomato under salinity were recorded by Shakhov (1956). He mentioned that salt ions especially the Na<sup>+</sup> might increase the hydrophilous properties of plasma colloids that played a very important role to protect the bio-colloids and plasma from the effect of higher

salinity. These observations were in agreement with those obtained by Medhat, 2002; Fathy *et al.*, 2005; Khalil, 2006 and Taha *et al.*, 2011. The increase in acidity as a result of salinity treatments were also reported by Janse, 1989; Chartzoulakis, 1992; Adams, 1991; Yungfu and Dashu, 2002; Krauss *et al.*, 2006 and Al-Harbi *et al.*, 2015.

Transitional water stress although reduce crop yield but results in enhanced soluble solids along with good viscosity (May and Gonzales, 1999; May, 1993). The increase in total soluble solid (TSS) under salinity stress might be attributed to osmotic adjustment of husk tomato plant to maintain its turgidity and to overcome the increasing resistance of water uptake by the roots (Taha *et al.*, 2011). Moreover, the decrease in water content and turgidity of the plant under saline irrigation can increase total soluble solid and acidity of the fruit (Saied *et al.*, 2005). Higher values of total soluble solid and acidity in the juice of fruits from salinized plants means that the quality of the products is better than control. Antioxidants like vitamin-C and lycopene with their antagonist functions against free radicals are very useful in protection against various biotic and abiotic stresses.

#### **2.4.3.2 In case of *Solanum* species**

An experiment was carried out by Yong-Gen *et al.* (2009) to elucidate the mechanisms of the transport of carbohydrates into tomato fruits and the regulation of starch synthesis during fruit development in tomato plants. In this experiment, tomato cv. 'Micro-Tom' was exposed to high levels of salinity stress to be examined. Results showed that, growth with 160 mM NaCl doubled starch accumulation in fruits compared to control plants during the early stages of development, and soluble sugars increased as the fruit matured. Tracer analysis with <sup>13</sup>C confirmed that elevated carbohydrate accumulation in fruits exposed to salinity stress was confined to the early development stages and did not occur after ripening. Salinity stress also up-regulated sucrose transporter expression in source leaves and increased activity of ADP-glucose pyrophosphorylase (AGPase) in fruits during the early development stages. The results indicated

that salinity stress can enhance carbohydrate accumulation as starch during the early development stages and it is responsible for the increase in soluble sugars in ripe fruit.

In a separate experiment, Satio *et al.* (2008) investigated the effects of 50 mM NaCl in a hydroponic solution on the levels of various metabolites, including amino acids, soluble sugars, and organic acids, and on the expression level of salinity-responsive genes during fruit development. Results indicated that under salinity, %Brix, surface color density and flesh firmness of the fruit were significantly enhanced, whereas fruit enlargement was suppressed. Salinity stress strongly promoted glucose and amino butyric acids.

Cuartero *et al.* (2003) demonstrated an experiment on the effect of salinity on tomato and reported that salinity was seemed to increase fruit taste by increasing both sugars and ascorbic acid, but couldn't produce much acid. Flores *et al.* (2003) demonstrated an experiment with tomato plants cv. Daniela grown in a nutrient solution containing 0, 30 and 60 mM NaCl and fertilized with 14/0, 12/2 and 10/4  $\text{NO}_3^-/\text{NH}_4^+$  mM ratio to determine the effect of salinity and nitrogen source. The increase in salinity and  $\text{NH}_4^+$  concentration in the nutrient solutions showed increased fruit quality by increasing the content of sugars, organic acids and antioxidants. However, the increase in fruit quality was associated with a decrease in yield.

In an another experiment, Mizrahi (1982) found that tomato (*Lycopersicon esculentum* Mill.) plants from various cultivars growing on half-strength Hoagland solution were exposed at anthesis to 3-6 g/L NaCl. Salinity shortened the time of fruit development by 4 to 15%. Fruits of salt-treated plants were smaller and tasted better than did fruits of control plants. This result was obtained both for ripe fruits tested on the day of picking and for those picked at 100% development and allowed to ripen at room temperature for 9 days. %Dry weight, total soluble solids, and titratable acidity, reducing sugar content,  $\text{Cl}^-$ ,  $\text{Na}^+$ , and various pericarp pigments; and electrical conductivity of the juice were higher in fruits of saline-treated plants than they were in those of control plants, while the

pH was lower. Ethylene and CO<sub>2</sub> evolution rates during ripening and the activities of pectin methyl esterase, polymethyl galacturonase, and polygalacturonase were also higher in fruits of saline-treated plants. The treatment with 6 g/L NaCl shortened the fruit shelf life considerably.

De Pascale *et al.* (2001) revealed that increased EC level may lead to higher contents of vitamin-C and total soluble solid in tomato fruits. Lycopene content may increase with the increasing salinity up to 6-7 dS/m but at excessive salinity inhibition effects may take over, resulting in reduced lycopene. Vitamin-C (L-ascorbic acid) content of tomato fruits was increased with salinity and it was 60% higher in tomatoes grown at EC of 15.7 dS/m, compared with non-salinized control condition.

An experiment was conducted by Petersen *et al.* (1998) with tomato plants irrigated by saline water. Study results revealed that, salinity enhanced the lycopene concentrations up to 4-6 dS/m but restricted at high salinity. This was probably due to a high temperature-induced inhibition of lycopene biosynthesis in tomatoes exposed to high solar radiation arising from smaller leaf area and consequently more fruits directly exposed to sunlight in salinity stressed plants. Vitamin-C content and %Brix of fruits were increased with the increasing salinity levels.

## **2.5 Effect of salinity on agriculture in Bangladesh**

A major challenge towards world agriculture involves production of 70% more food crops for an additional 2.3 billion people by 2050 worldwide (FAO, 2009). Salinity is a major stress limiting the increased demand for growing food crops. More than 20% of cultivated land worldwide is affected by salt stress and the amount is increasing day by day. Climate change due to global warming and its negative consequence on environment and agro ecosystem is a serious concern of global community of recent age. Bangladesh, a deltaic plain of 1, 47, 570 km<sup>2</sup>, has a very flat and low topography. Agriculture is a major sector of Bangladesh's economy and more than 30% of the cultivable land of Bangladesh is in the coastal areas (Karim *et al.*, 1982). Out of 2.86 million ha of coastal and off-shore

lands about 1.056 million ha of arable lands are affected by varying degrees of salinity. Most of the land remains fallow in the dry season (January to May) due to soil salinity, lack of good quality irrigation water and late draining condition (Karim *et al.*, 1990; SRDI, 2001). According to SRMAF Project Report (2010), some of the new land of Satkhira, Patuakhali, Borguna, Barisal, Jhalakathi, Pirojpur, Jessore, Narail, Gopalganj and Madaripur districts are affected by different degrees of salinity, which has reduced agricultural productivity remarkably. Crop production of the salt affected areas in the coastal regions differs considerably from non-saline areas. Because of salinity, special environmental and hydrological situation exists, that restricts the normal crop production throughout the year. In the recent past, with the changing degree of soil and water salinity, normal crop production has become very risky. Crop yields, cropping intensity, production levels and people's quality of livelihood of our coastal regions are much lower than that in other parts of the country. At the same time food demand is increasing with the steady increase in human population. Thus, it has become imperative to explore the possibilities of increasing potential of these saline lands for increased production of food crops combating land salinization problem for food security in the country through adoption of long-term land management strategy. Increased cropping intensity in very and slightly saline areas by analyzing the soil and water salinity intensity, extent, constraints and adopting proper soil and water management practices along with the introduction of different new salt tolerant varieties of crops has to be followed in our coastal areas for the betterment of the country.

## CHAPTER III

### MATERIALS AND METHODS

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This chapter illustrates the informations concerning the research methodologies those were used in the execution of the experiment. The study was conducted as a pot experiment in the area just beside the net house of Genetics and Plant Breeding Department of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period of November, 2017 to March, 2018 to evaluate the performance of four tomatillo genotypes based on their different agromorphogenic, physiological and nutritional traits under three different salinity treatments. A brief description of the experiment comprising of the location of experimental site, planting materials, climate and soil, seed bed preparation, layout and design of the experiment, preparation of main pots, fertilization, transplantation of seedlings, intercultural operations, harvesting of fruits, various data recording processes, different physiological, nutritional and statistical analysis procedures etc. has been stated in this chapter sequentially.

#### **3.1 Experimental site**

The experiment was accomplished in the area just beside the net house of Genetics and Plant Breeding Department of Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during the period from November 2017 to March 2018. Location of the site is 23°74' North latitude and 90°35' East longitude with an elevation of 8 meter from sea level (Anonymous, 2014) in Agro-ecological zone of "Madhupur Tract" (AEZ-28) (Anonymous, 1988). The area was open, thus it was prepared to be suitable for conducting the experiment. A structure was developed using bamboo poles and bamboo sticks. Later, transparent polythene sheets were used to cover the top of the structure to prevent trespassing of rain water but to ensure enough sunlight. And then plastic nets were used in the surrounding of the structure to prevent unwanted entry to the experimental site. The location of the site is shown in the map of AEZ of Bangladesh in (Appendix I).



### **3.2 Planting materials**

A total number of four genotypes of tomatillo were used in the study (Table 1) and they were collected from Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh on October, 2017.

### **3.3 Treatments in the experiment**

A two factorial experiment was conducted to evaluate the performance of four tomatillo genotypes under three different sodium chloride (NaCl) salinity treatments. Salinity treatments were chosen by the classification of saline area given by Soil Research Development Institute, Bangladesh (Report, 2010) (Table 2). According to this classification: Non saline with some very slightly saline (2.0 to 4.0 dS/m), very slightly saline with some slightly saline (4.1 to 8.0 dS/m), slightly saline with some moderately saline (8.1 to 12.0 dS/m), strongly saline with some moderately saline (12.1 to 16.0 dS/m), very strongly saline with some strongly saline (>16.0 dS/m). In this experiment, Factor A was four different tomatillo genotypes (Table 1) and Factor B was three different salinity treatments (Table 2), T<sub>1</sub> (control condition) , T<sub>2</sub> (8.0 dS/m) and T<sub>3</sub> (12.0 dS/m).

### **3.4 Design and layout of the experiment**

The experiment was laid out and evaluated during the Rabi season 2017-'18 in Completely Randomized Design (CRD) using two factors. Factor A included four tomatillo genotypes and Factor B included three salinity treatments. The experiment was conducted in three replications and total 36 plastic pots were used. A pictorial view of the experimental site (shade house) has been presented in Plate 1.

### **3.5 Climate and soil**

Experimental site was located in the subtropical climatic zone, set aparted by plenty of sunshine and moderately low temperature prevails during October to March (Rabi season) which is suitable for growing crops in Bangladesh. The soil was sandy loam in texture having pH of 5.46 to 5.62 and EC of 0.60 dS/m. Weather informations during the time of the experiment of Rabi season (October, 2017 to March, 2018) and physicochemical properties of the soil of experimental

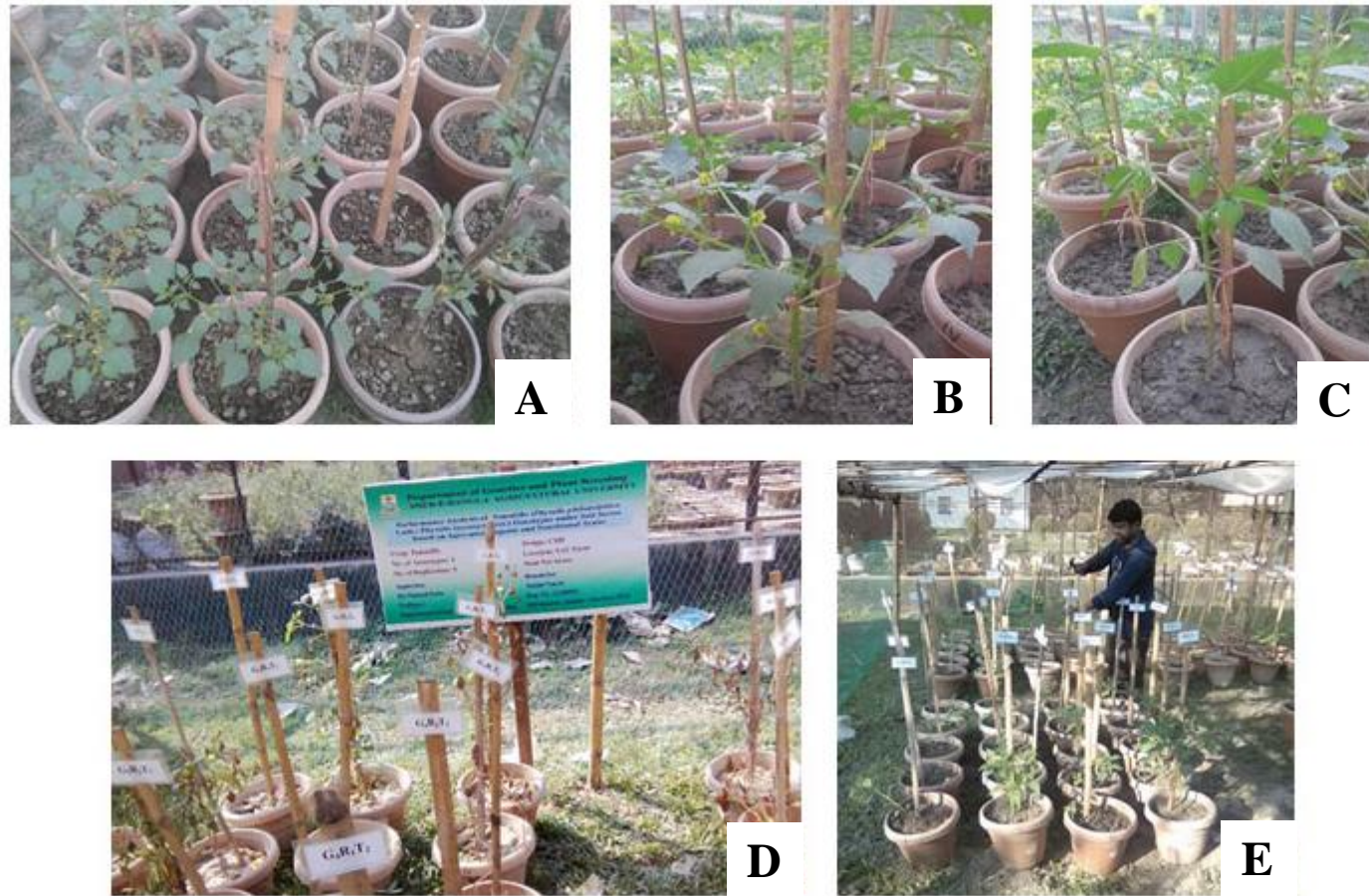
**Table 1. Name and source of tomatillo genotypes (Factor A) used in the present study**

<b>Sl. No.</b>	<b>Genotypes</b>	<b>Name/Accession No.</b>	<b>Source of Collection</b>
1.	G <sub>1</sub>	SAU tomatillo 1	GEPB, SAU
2.	G <sub>2</sub>	SAU tomatillo 2	GEPB, SAU
3.	G <sub>3</sub>	PI003	GEPB, SAU
4.	G <sub>4</sub>	PI004	GEPB, SAU

GEPB=Department of Genetics and Plant Breeding, SAU = Sher-e-Bangla Agricultural University

**Table 2. Different salinity treatments (Factor B) of NaCl used in the present study**

<b>Sl. No.</b>	<b>Salinity Treatments</b>	<b>Electrical Conductivity (dS/m)</b>	<b>Types of Salinity</b>
1.	T <sub>1</sub>	Control	Non-saline
2.	T <sub>2</sub>	8.0	Slightly saline
3.	T <sub>3</sub>	12.0	Moderately saline



**Plate 1. Plants in shade house after transplanting. A) Transplanted plants in main plastic pots, B) Plant with flower, C) Plant bearing fruits, D) Shade house marked with signboard and E) A pictorial view of plants under different salinity treatments**

site are presented in (Appendix II and Appendix III) respectively.

### **3.6 Seed bed preparation and raising of seedling**

The sowing was carried out on November 9, 2017 in the well prepared seedbed. Before sowing, seeds were treated with Bavistin for 5 minutes. Seedlings of all four tomatillo genotypes were raised in the seedbed beside the farm house of Sher-e-Bangla Agricultural University, Dhaka-1207. Before seed sowing, the upper crust of soil (approximately 10 cm of depth) of seedbed was treated with Furadan 5G and Sevin dust. Seeds were sown in rows spaced at 10 cm apart, beds were watered regularly. Seedlings were raised using regular nursery practices. Recommended cultural practices were taken up before and after sowing the seeds. When the seedlings became 21 days old on December 1, 2017, the seedlings were transplanted in the main plastic pots. A pictorial view of seedbed has been presented in Plate 2A.

### **3.7 Manure and fertilizers application**

Soil was well pulverized and dried in the sun and only well decomposed cow dung was mixed with the soil according to the Fertilizer Recommendation Guide released by BARC in 2012. Well decomposed cow dung was calculated for each pot considering the dose of 1 hectare of soil at the depth of 20 cm, one million kg. On an average, each plastic pot was filled with soil containing 100 g of well decomposed cow dung (as 10 tons/ha). Total decomposed cow dung was applied before transplanting the seedlings to plastic pots.

### **3.8 Preparation of pots and transplantation of seedlings**

Weeds and stubbles were completely removed from the soil and soil was treated with Formaldehyde (45%) for 48 hours before transplanting to plastic pots in order to keep soil free from pathogens. Pots were filled up on November 28, 2017, two days before of transplantation operation. Each pot was filled with 10 kg of soil. Height of the pot was 20 cm with top diameter of 30 cm and bottom diameter of 20 cm. Few pores were made in each plastic pot and then the pores were covered by gravels so that excess water could easily drain out. Seedlings of



**A**



**B**



**C**



**D**



**E**



**F**

**Plate 2. Seedbed preparation and intercultural operations. A) Seedlings in the seedbed, B) Transplantation of seedlings, C) Tying seedlings with bamboo stick, D) Measuring EC of saline water, E) application of saline water and F) Measuring pH of soil**

21 days old were transplanted in the main plastic pot on December 1, 2017 (1 plant/pot). A pictorial view of transplanting and sticking of seedlings have been presented in Plate 2B and 2C.

### **3.9 Application of sodium chloride (NaCl)**

Four tomatillo genotypes were executed under three different salinity treatments (T<sub>1</sub>: Control condition; T<sub>2</sub>: 8.0 dS/m and T<sub>3</sub>: 12.0 dS/m). Plants in control treatment (T<sub>1</sub>) were not exposed to salinity; whereas plants of T<sub>2</sub> and T<sub>3</sub> treatments were treated with 8 dS/m (4.4 g of ACI salt/L of water) and 12 dS/m (6.6 g of ACI salt/L of water) level of salinity treatment respectively. Plants in control treatment (T<sub>1</sub>) were always irrigated with fresh (non-saline) water. Saline solution was applied to selected T<sub>2</sub> and T<sub>3</sub> pots at 7 DAT to help the well establishment of young seedlings and later on each pot was watered as per requirement and according to treatments. Electrical conductivity (EC) of different salinity levels in soil was adjusted by a direct reading conductivity meter (EC-meter). Salt solution (calculated) was applied 1 litre/pot in 3 to 4 days interval to maintain the exact salinity level in the soil. When soil in the pots was seemed to reach in water logging condition, then saline water was given after the soil was reached near in dried condition (visual observation). A pictorial view of measuring EC of saline water and its application have been presented in Plate 2D and 2E as well as the determination of soil pH has been presented in Plate 2F.

### **3.10 Intercultural operations**

Necessary intercultural operations were provided as per requirement. Hand weeding and mechanical weeding were performed in all pots as and when required in order to keep plants free from weed infestation. Diseases and pests are limiting factors to crop production. Experimental tomatillo plants were treated with Bavistin DF @ 1 g/L of water and Cupravit 50 WP @ 2 g/L of water to prevent unwanted disease infections. To prevent pest infestation, plants were treated by Malathion 250 EC @ 0.5 mL/L of water. Those fungicides and pesticides were sprayed two times; firstly, at vegetative growth stage and

secondly, at early flowering stage. When plants were well established, staking was performed to each plant of plastic pots by bamboo stick between 25 to 30 DAT to keep the plants erect.

### **3.11 Harvesting and processing**

Harvesting of fruits was done after reaching to its maturity stage. Immature tomatillo fruits are dark green in color and it turns into greenish to light greenish or yellowish when become mature and most often the rupture of the husk occurs as a result of increase in size of fruits with its maturity. Mature fruits were identified and harvested from plants. A number of fruits per plant were allowed to ripe and then seeds were collected from them and stored at 4°C for future use. Harvesting was started from February 17, 2018 and completed by March 10, 2018.

### **3.12 Data recording**

Data were recorded from tomatillo plants of each plastic pot based on their different agromorphogenic, physiological and nutritional traits. Data were recorded in respect of following parameters:

#### **3.12.1 Agromorphogenic traits**

Different agromorphogenic traits related to yield and yield contributing characters were recorded *viz.*, Days to first flowering, Plant height (cm), Days required to maturity, Number of fruits per plant, Average fruit length (mm) per plant, Average fruit diameter (mm) per plant, Average fruit weight (g) per plant and Yield per plant (kg).

##### **3.12.1.1 Days to first flowering**

The required number of days to first flowering of plants was counted from the date of transplantation of seedlings to the date of first flowering of each tomatillo plant in the main plastic pot.

##### **3.12.1.2 Plant height (cm)**

Plant height of each plant in the pot was measured at their maturity stage (65 DAT) in centimeter (cm) unit using a meter scale and the mean values were calculated for further analysis.

#### **3.12.1.3 Days to maturity**

The required number of days to maturity of plants was counted from the date of transplantation of seedlings to the date of first harvesting of mature tomatillo fruits of each plant in the pot.

#### **3.12.1.4 Number of fruits per plant**

The fruits were harvested from each plant and the number of total marketable mature tomatillo fruits was recorded.

#### **3.12.1.5 Average fruit length (mm) per plant**

Average fruit length was measured using Digital Caliper-515 (DC-515) in millimeter (mm) unit and the mean values were calculated (Plate 3A).

#### **3.12.1.6 Average fruit diameter (mm) per plant**

Average fruit diameter was measured using Digital Caliper-515 (DC-515) in millimeter (mm) unit and the mean values were calculated.

#### **3.12.1.7 Average fruit weight (g) per plant**

Average fruit weight was measured by electric precision balance in gram (g) unit. Average fruit weight per plant was recorded by randomly selecting 5 fruits per plant and the mean values were calculated (Plate 3B).

#### **3.12.1.8 Yield per plant (kg)**

Yield per plant was recorded from all harvests (mature tomatillo fruits) of each plant and result was expressed in kilogram (kg) unit per plant.

### **3.12.2 Physiological traits**

Data related to different physiological trait such as Na<sup>+</sup> content (%), K<sup>+</sup> content (%), proline content (µg/g), chlorophyll content (%) and leaf area index (cm<sup>2</sup>) were recorded.

#### **3.12.2.1 Determination of Na<sup>+</sup> and K<sup>+</sup> content (%)**

Oven-dried (at 70°C) tomatillo plant shoot samples were ground in a Wiley Hammer Mill, passed through 40 mesh screens, mixed well and stored in plastic





**Plate 3. Collection of data. A) Measuring fruit length and diameter using Digital Caliper-515, B) Measuring fruit weight using electric precision balance**

vials. The ground plant samples were digested by Micro-Kjeldahl method (Thomas and Nambisan, 1999). 1 g of oven-dried tomatillo plant shoot samples were taken in kjeldahl flasks. About 15 mL of di-acidic mixture ( $\text{HNO}_3 : 60\% \text{HClO}_4 = 2 : 1$ ) were taken in a digestion tube and left to stand for 20 minutes and then transferred to digestion block and continued heating at  $100^\circ\text{C}$ . The temperature was increased up to  $365^\circ\text{C}$  gradually to prevent frothing ( $50^\circ\text{C}$  increase in each step) of sample and left to digest until the solution color turned into yellowish to whitish in color. Then the digestion tubes were removed from the heating source and were allowed to cool down at room temperature ( $25^\circ\text{C}$ ). About 40 mL of de-ionized water was carefully added to the digestion tubes and the content was filtered through Whatman no.40 filter paper. The filtrate was then taken into a 100 mL volumetric flask and the volume was made up to the mark with de-ionized water. The prepared samples were then stored at room temperature in clearly marked containers. Indigenous  $\text{Na}^+$  and  $\text{K}^+$  ion content were determined in percentage (%) using a Flame Photometer (Plate 4A-C).

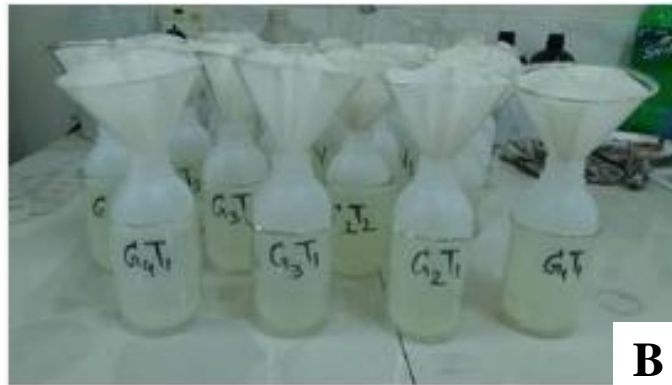
### **3.12.2.2 Determination of proline content ( $\mu\text{g/g}$ )**

#### **3.12.2.2.1 Extraction of proline**

Proline accumulation was determined by the method as described by Sadasivam and Manickam (1996). According to the method, fully expanded fresh tomatillo leaves (0.5 g) of each sample were taken and grinded using mortar and pestle with 10 mL of 3% of sulphosalicylic acid and then the homogenates were centrifuged at  $18000 \times g$ . The homogenates were filtered and 2 mL filtrate of each sample was taken into a test tube to add 2 mL of glacial acetic acid and 2 mL of acid ninhydrin and then, test tubes were kept for 1 hour at  $100^\circ\text{C}$  in water bath, and then followed by ice bath. The mixture was then mixed thoroughly with 4 mL of toluene using a vortex machine. Toluene layer was separated and absorbance was read at 520 nm (Plate 4D-E) using a spectrophotometer. A standard curve of proline was prepared for the calibration of proline content in tomatillo plant leaf samples.



**A**



**B**



**C**



**D**



**E**



**F**



**G**

**Plate 4. Data collection of different physiological traits. A) Digestion of plant sample, B) Dilution of plant sample, C) Estimation of Na<sup>+</sup> and K<sup>+</sup> by Flame photometer, D) Estimation of proline, E) Spectrophotometer reading for proline estimation, F) Leaf area index estimation and G) Chlorophyll estimation by SPAD meter reading**

#### **3.12.2.2.2 Preparation of proline standard curve**

80 mg of pure proline was dissolved into 100 mL of distilled water to get 800 ppm proline stock solution for preparing proline standard curve. By diluting this solution, 50 ppm, 100 ppm, 200 ppm, 400 ppm and 800 ppm solution were prepared in 20 mL of each. The absorbance was measured with the help of a Spectrophotometer at 520 nm. By plotting the concentration of proline (ppm) in 'X' axis and obtained absorbance reading in 'Y' axis a standard curve was prepared (Appendix IV). From the absorbance reading obtained from samples, their respective proline content was estimated in ppm by using proline standard curve and converted into micro gram per gram ( $\mu\text{g/g}$ ) unit using the following formula:

$$\text{Amount of proline } (\mu\text{g/g}) = \frac{x}{2} \times \frac{10}{500} \times 1000$$

#### **3.12.2.3 Determination of leaf area index ( $\text{cm}^2$ )**

The leaf area index is defined as the one sided green leaf area per unit ground area in broadleaf canopies (Hunt, 1990). Leaf area index ( $\text{cm}^2$ ) of tomatillo plants was measured by using Leaf Area Meter (CI-202) (Plate 4F). For this estimation, fully expanded three leaves from each plant (60 DAT) were randomly selected, their leaf area were measured and then the values were averaged for analysis.

#### **3.12.2.4 Measurement of chlorophyll content (%)**

Leaf chlorophyll content (%) of tomatillo plants was measured by SPAD analysis (Soil-Plant Analyses Development) using SPAD-502 plus portable chlorophyll meter (Plate 4G). The chlorophyll content was measured two times in order to observe the difference in its amount, at 30 and 60 days after starting salinity stress application. Reading was taken from two different portions of each three mature leaves and then values were averaged for analysis.

#### **3.12.3 Nutritional traits**

Data were recorded based on different nutritional traits of mature tomatillo fruits *viz.*, fruit pH, %Brix, titratable acid (%) and Vitamin-C content ( $\text{mg}/100 \text{ g}$ ). A

pictorial view of different nutritional data collection methods has been presented in Plate 5.

### **3.12.3.1 Determination of fruit pH**

The pH of mature tomatillo fruits was measured at room temperature (25°C) by using a pH meter (Plate 5B). For the fruit pH estimation, a single mature tomatillo fruit from each replication of the treatments was blended. Then 5 g from each sample was taken and mixed thoroughly with 5 mL of recently done double distilled and de-ionized water (pH: 7.0). The pH of each homogenate was measured and mean was calculated.

### **3.12.3.2 Determination of brix percentage**

Brix content (%) of mature tomatillo fruits were measured by using Portable Hand Refractometer (ERMA, Tokyo, Japan) at room temperature (Plate 5C). For the estimation, a single mature fruit from each replication of the treatments was blended and juice was collected to measure the brix content in percentage (%).

### **3.12.3.3 Determination of titratable acid content (%)**

Amount of titratable acid content was measured in percentage (%) using titration method (Ranganna, 1986) (Plate 5E). For the estimation, a single mature fruit from each replication of the treatments was blended. 5 g of blended pulp from each sample was collected and mixed thoroughly with recently distilled water. The extract was filtrated by Whatman no.1 filter paper. The filtrate was then taken into a 100 mL volumetric flask and volume was made up to the mark using recently distilled water. A definite amount of sample was taken from this preparation and was titrated with 0.1 N NaOH using a few drops of 1% of phenolphthalein solution as indicator. The required amounts of NaOH solution (titre) were noted down for each sample and estimation of titratable acid content (%) was done using following formula:

$$\% \text{ Total acid} = \frac{\text{Titre} \times \text{Eq. wt. of acid} \times \text{Vol. made up} \times 0.1 \times 100}{\text{Extract taken for titration} \times \text{Wt. of sample taken} \times 1000}$$



**Plate 5. Data collection of different nutritional traits. A) Weighting fruit sample, B) Estimation of fruit pH, C) Estimation of brix percentage, D) Estimation of vitamin-C content and E) Estimation of titratable acid content**

#### 3.12.3.4 Determination of vitamin-C content (mg/100 g)

Vitamin-C was measured by using Oxidation Reduction Titration Method (Tee *et al.*, 1988) (Plate 5D). For the estimation, a single mature fruit from each replication of the treatment was blended and then the fruit extract was filtrated by Whatman no.1 filter paper. The fruit juice was then mixed with 3% of metaphosphoric acid solution. The titration was conducted with 2,6-dichlorophenol indophenol, a dye solution and in presence of glacial acetic acid and metaphosphoric acid to inhibit the aerobic oxidation. The mean of observations provided the amount of dye required to oxidize a definite amount of L-ascorbic acid solution of unknown concentration, using an L-ascorbic acid as known sample. Estimation of L-ascorbic acid content of fruit sample was done using the following formula:

$$\text{Amount of vitamin-C (mg/100 g)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Vol. made up} \times 100}{\text{Extract taken for titration} \times \text{Wt. of sample taken}}$$

Here, Dye factor =  $0.5 \div \text{Titre}$  (amount of required dye solution)

#### 3.13 Statistical analysis

Collected data of all agromorphogenic, physiological and nutritional traits of tomatillo were statistically analyzed using MSTAT-C computer package program. Means for every treatment were calculated and analysis of variance (ANOVA) were performed for each character which was analyzed by F-test (Variance Ratio). Comparison between treatment means (all pair comparison) was assessed by Least Significant Difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

## CHAPTER IV

### RESULTS AND DISCUSSION

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The experimental work was accomplished for the performance evaluation of four tomatillo genotypes exposed to three different salinity (NaCl) treatments based on their agromorphogenic, physiological and nutritional traits. In case of stressed condition, the plants were found to be stunted, leaves showed chlorosis, fruits became smaller and plants eventually died. In this chapter the findings of executed experimental work have been put forwarded and discussed. Data have been presented in table(s) and figure(s) for easy discussion, comprehension and understanding. A summary of all the parameters (ANOVAs and increase or decrease percentage of changes) used in this experiment are presented in appendices (Appendix V to Appendix XI). Results have been presented, discussed and possible interpretations are given on the following heads.

#### **4.1 Evaluation of agromorphogenic traits of tomatillo**

Different agromorphogenic traits *viz.*, days to first flowering, plant height, days required to maturity, number of fruits per plant, average fruit length and diameter, average fruit weight and yield per plant of tomatillo were presented and discussed based on their ANOVA, genotype, salinity treatment, genotype-treatment interaction effect and percentage of changes (increase or decrease) in these traits.

##### **4.1.1 Days to first flowering**

The mean values of days to first flowering (from days after transplanting) for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the ANOVA table (Appendix V), it was observed that statistically significant variation was existed among the tomatillo genotypes in respect of days to first flowering after transplantation. The longest period was required for first flowering in genotype G<sub>4</sub> (36.11 days) which was statistically identical with G<sub>2</sub>



(35.00 days) while the shortest required period was in G<sub>3</sub> (32.78 days) which was statistically identical with G<sub>1</sub> (33.22 days) (Table 3).

From the ANOVA table (Appendix V), statistically highly significant variation was found among the three different salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of days to first flowering. The longest period required for first flowering was in T<sub>1</sub> (37.33 days) and the shortest required period was in T<sub>3</sub> (30.84 days) (Table 4). This result showed that days required for first flowering was earlier in treatment T<sub>3</sub> (12 dS/m) than T<sub>1</sub> (control).

Interaction effect between four tomatillo genotypes and three salinity treatments was found statistically non-significant in respect of days to first flowering (Appendix V). Interaction G<sub>4</sub>T<sub>1</sub> (40.00 days) required the maximum period for first flowering after transplantation whereas interaction G<sub>1</sub>T<sub>3</sub> (29.67 days) required the minimum period (Table 5).

The time required for first flowering of four tomatillo genotypes varied significantly under three different salinity treatments and the required first flowering time was decreased gradually with the increase of salinity treatment levels (Appendix IX). The maximum reduction in days to first flowering was observed in genotype G<sub>4</sub> in both cases, at slightly (8 dS/m) and moderately salinity (12 dS/m) stresses (8.33% and 20.83% respectively). The minimum reduction was observed in genotype G<sub>2</sub> (5.31%) at slightly salinity (8 dS/m) stress whereas in G<sub>3</sub> (14.15%) at moderate salinity (12 dS/m) stress (Figure 1). Therefore, genotype G<sub>4</sub> might be considered as a good source for transferring early flowering (earliness) trait in a high yielding genotype of tomatillo under salinity stress condition.

#### **4.1.2 Plant height (cm)**

The mean values of plant height (cm) for four tomatillo genotypes under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. A pictorial view of morphological comparisons of the heights of tomatillo plants between control and stressed conditions has been

**Table 3. Performance of tomatillo genotypes on days to first flowering, plant height, days to maturity and number of fruits per plant<sup>Y</sup>**

<b>Genotype<sup>X</sup></b>	<b>Days to first flowering</b>	<b>Plant height (cm)</b>	<b>Days to maturity</b>	<b>No. of fruits per plant</b>
<b>G<sub>1</sub></b>	33.22 b	65.00 b	87.44 ab	13.22 c
<b>G<sub>2</sub></b>	35.00 a	67.55 a	86.67 b	11.33 d
<b>G<sub>3</sub></b>	32.78 b	65.67 b	90.56 a	18.22 a
<b>G<sub>4</sub></b>	36.11 a	68.50 a	83.33 c	15.11 b
<b>CV %</b>	6.95	4.73	3.69	4.87
<b>LSD 0.05</b>	1.33	1.09	2.14	0.69

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub>

<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

**Table 4. Performance of salinity treatments on days to first flowering, plant height, days to maturity and number of fruits per plant<sup>Y</sup>**

<b>Treatment<sup>X</sup></b>	<b>Days to first flowering</b>	<b>Plant height (cm)</b>	<b>Days to maturity</b>	<b>No. of fruits per plant</b>
<b>T<sub>1</sub> (control)</b>	37.33 a	68.63 a	93.58 a	16.67 a
<b>T<sub>2</sub> (8dS/m)</b>	34.67 b	65.08 b	87.08 b	15.00 b
<b>T<sub>3</sub> (12dS/m)</b>	30.83 c	66.33 b	80.33 c	11.75 c
<b>CV %</b>	6.95	4.73	3.69	4.87
<b>LSD 0.05</b>	2.02	1.67	2.72	0.60

<sup>X</sup>Three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub>

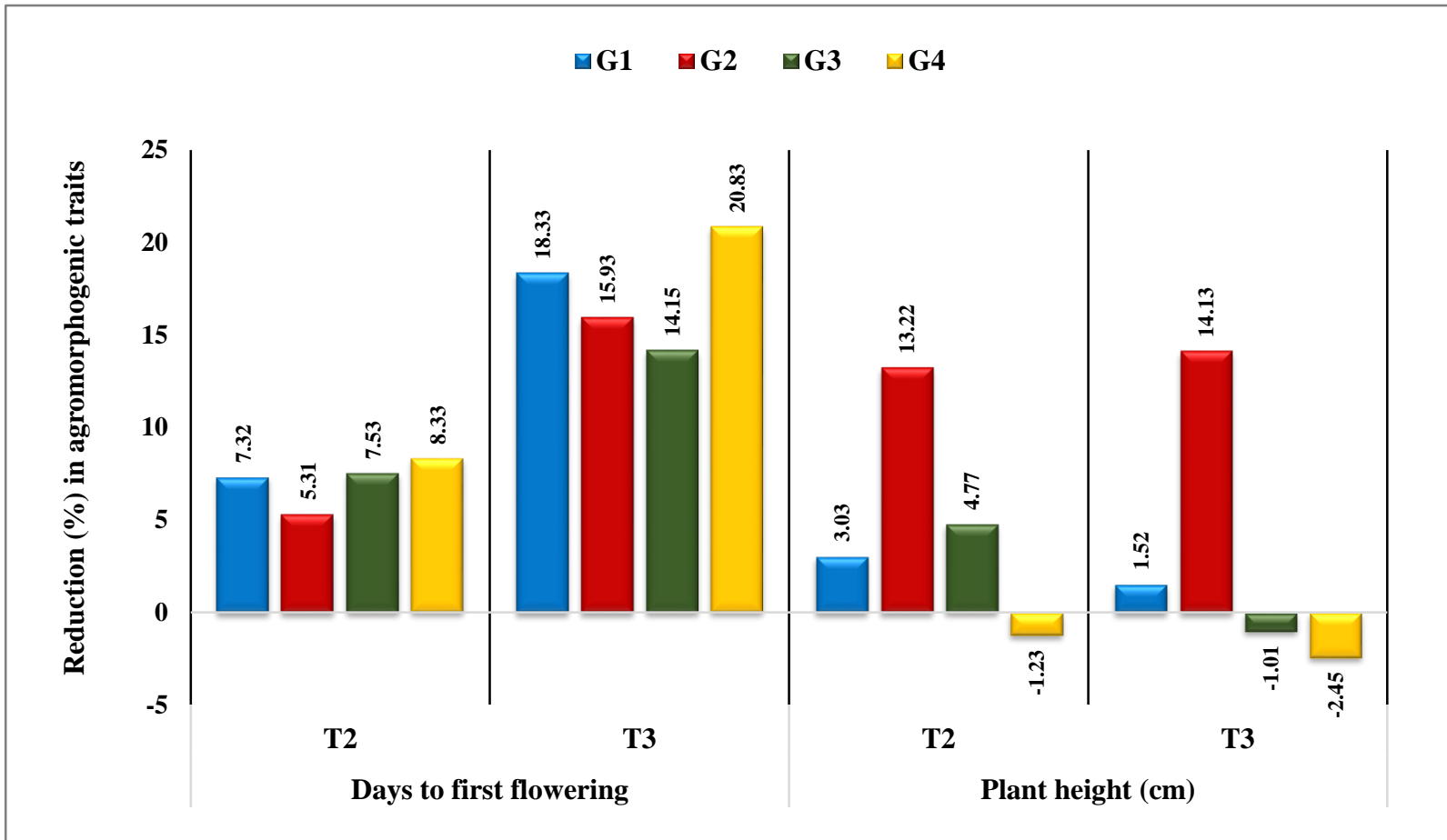
<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

**Table 5. Interaction effect between tomatillo genotypes and salinity treatments on days to first flowering, plant height, days to maturity and number of fruits per plant<sup>Y</sup>**

<b>Interaction<sup>X</sup></b>	<b>Days to first flowering</b>	<b>Plant height (cm)</b>	<b>Days to maturity</b>	<b>No. of fruits per plant</b>
<b>G<sub>1</sub>T<sub>1</sub></b>	36.33	66.00 bc	95.33 ab	16.33 c
<b>G<sub>1</sub>T<sub>2</sub></b>	33.67	64.00 bc	87.67 cde	13.67 ef
<b>G<sub>1</sub>T<sub>3</sub></b>	29.67	65.00 bc	79.33 gh	9.67 h
<b>G<sub>2</sub>T<sub>1</sub></b>	37.67	74.33 a	92.67 bc	13.33 f
<b>G<sub>2</sub>T<sub>2</sub></b>	35.67	64.50 bc	87.00 de	11.67 g
<b>G<sub>2</sub>T<sub>3</sub></b>	31.67	63.83 c	80.33 fgh	9.00 h
<b>G<sub>3</sub>T<sub>1</sub></b>	35.33	66.50 bc	96.00 a	21.33 a
<b>G<sub>3</sub>T<sub>2</sub></b>	32.67	63.33 c	90.00 bcd	18.67 b
<b>G<sub>3</sub>T<sub>3</sub></b>	30.33	67.17 bc	85.67 def	14.67 de
<b>G<sub>4</sub>T<sub>1</sub></b>	40.00	67.67 bc	90.33 bcd	15.67 cd
<b>G<sub>4</sub>T<sub>2</sub></b>	36.67	68.50 bc	83.67 efg	16.00 c
<b>G<sub>4</sub>T<sub>3</sub></b>	31.67	69.33 ab	76.00 h	13.67 ef
<b>CV %</b>	6.95	4.73	3.69	4.87
<b>LSD 0.05</b>	---	5.35	5.44	1.19

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub> and three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub> (T<sub>1</sub>: control, T<sub>2</sub>: 8 dS/m, T<sub>3</sub>: 12 dS/m)

<sup>Y</sup>In a column means containing similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability



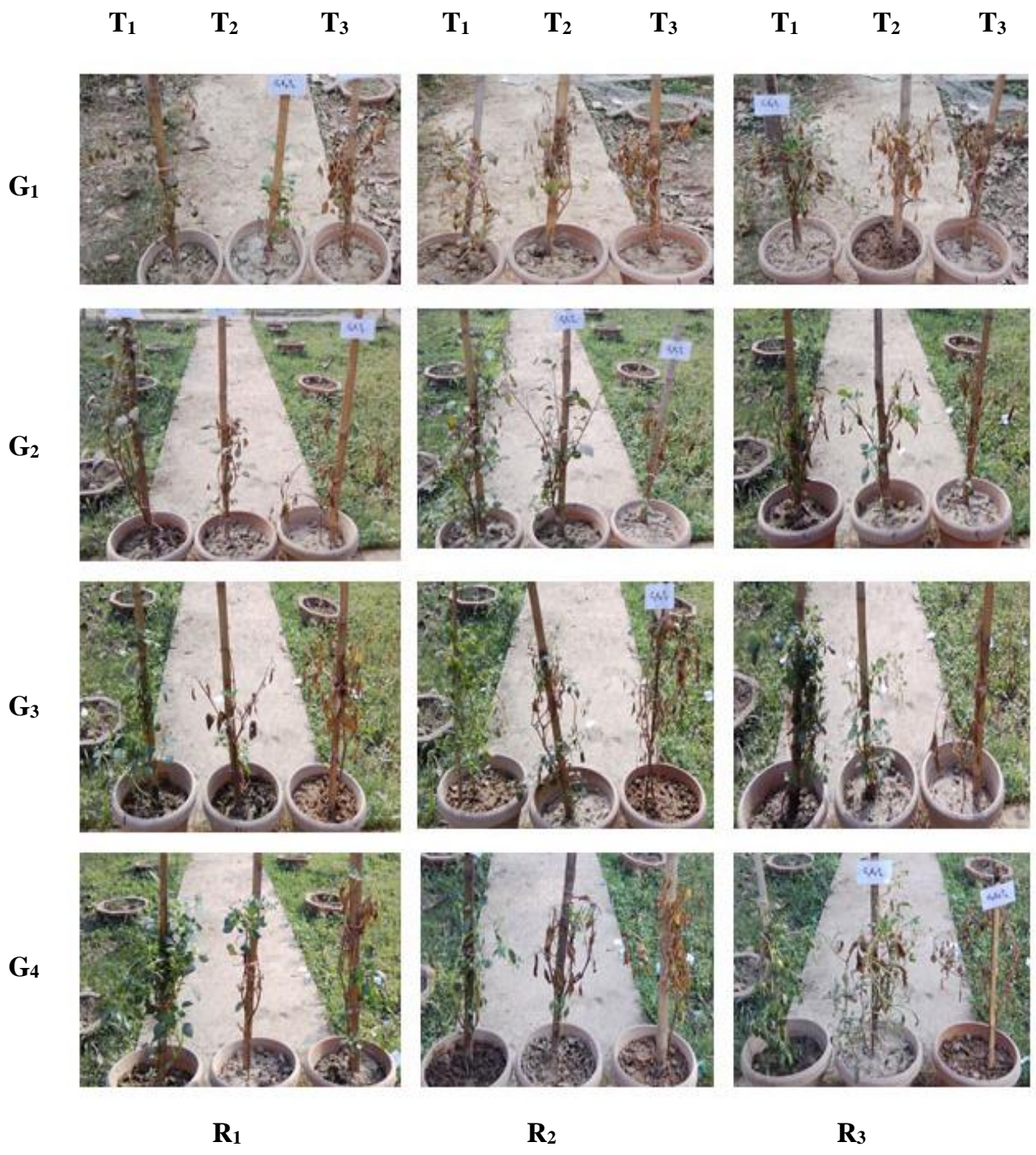
**Figure 1. Reduction percentage in days to first flowering and plant height under increasing salinity stress**

presented in Plate 6. From the ANOVA table (Appendix V), it was observed in this experiment that statistically significant variation was existed among the tomatillo genotypes in respect of plant height (cm). The tallest plant in this experiment was obtained from G<sub>4</sub> (68.50 cm) which was statistically identical with genotype G<sub>2</sub> (67.55 cm) whereas the shortest one was found from G<sub>1</sub> (65.00 cm) which was statistically identical with G<sub>3</sub> (65.67 cm) (Table 3).

From the result of ANOVA table (Appendix V), the tomatillo genotypes showed statistically significant variation to salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of plant height (cm). The tallest plant was found in T<sub>1</sub> (68.63 cm) whereas the shortest plant was from treatment T<sub>2</sub> (65.08 cm) which was statistically identical with T<sub>3</sub> (66.33 cm) (Table 4). This result showed that plant height was decreased under the increasing levels of salinity treatment. Study referred that plant height was found to decrease gradually with an increase of salinity levels and the reduction in plant growth exposed to salinity may be attributed to the reduction in water content and water potential of plant tissues, which resulted in internal water deficit to plants (Hishida *et al.*, 2013). The suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of NaCl (Munns *et al.*, 2002). Accumulation of Na<sup>+</sup>, Cl<sup>-</sup> and retardation in the uptake of macronutrients especially Na<sup>+</sup> and Ca<sup>2+</sup> causing a reduction in plant growth (Juan *et al.*, 2005; Dasgan *et al.*, 2002).

The plant height (cm) performed significant variation among the effect of interaction between four tomatillo genotypes and three salinity treatments (Appendix V). The tallest plant was found in interaction G<sub>2</sub>T<sub>1</sub> (74.33 cm) which was statistically identical with G<sub>4</sub>T<sub>3</sub> (69.33 cm) while the shortest plant was found in interaction G<sub>3</sub>T<sub>2</sub> (63.33 cm) which was statistically identical with G<sub>2</sub>T<sub>3</sub> (63.83 cm) (Table 5).

The plant height of four tomatillo genotypes varied significantly under three different salinity treatments and the height was mainly decreased gradually with the increase of salinity treatment levels (Appendix IX). The maximum reduction



**Plate 6. Morphological comparisons of height of tomatillo plants between control and salinity stress condition**

in plant height was observed in genotype G<sub>2</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (13.22% and 14.13% respectively) and the minimum reduction was observed in genotype G<sub>1</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (3.03% and 1.52% respectively). Genotype G<sub>4</sub> showed increase in plant height at both slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (-1.23% and -2.45% respectively) (Figure 1). According to Naidoo *et al.* (1995), the stimulatory effect of moderate salinity on growth of some plants can improve their growth and it may be due to the improved shoot osmotic status as a result of increasing ions uptake. The obtained results were matched with those obtained by Achilea, 2002; Agong *et al.*, 2004; Zaki *et al.*, 1987. Similar results were also reported by Ashraf and Sharif (1998).

#### **4.1.3 Days to maturity**

The mean values of days to maturity (from days after transplanting to days of first harvesting) for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix V), it was observed in this experiment that days required to maturity showed statistically highly significant variation among different tomatillo genotypes. The longest maturity (first harvesting) period was required in genotype G<sub>3</sub> (90.56 days) which was statistically identical with G<sub>1</sub> (87.44 days) whereas the shortest maturity period was required for genotype G<sub>4</sub> (83.33 days) (Table 3).

From the ANOVA table (Appendix V), the tomatillo genotypes showed statistically highly significant variation to salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of days to maturity. The earliest fruit harvesting was performed in treatment T<sub>3</sub> (80.33 days) and the most delayed harvesting was performed in treatment T<sub>1</sub> (93.58 days) (Table 4). This result showed that maturity time was decreased under the increased level of salinity treatments and other ions in the root zone of tomatillo plant. Similar study results were also found by Agarwal *et al.*, 2005 and Ghadiri *et al.*, 2005.

Interaction effect between four tomatillo genotypes and three salinity treatments was found statistically significant in respect of days to maturity (Appendix V). The earliest fruit harvesting period was observed in interaction G<sub>4</sub>T<sub>3</sub> (76.00 days) which was statistically identical with G<sub>1</sub>T<sub>3</sub> (79.33 days) and G<sub>2</sub>T<sub>3</sub> (80.33 days) whereas interaction G<sub>3</sub>T<sub>1</sub> (96.00 days) was the most delayed one which was statistically identical with G<sub>1</sub>T<sub>1</sub> (95.33 days) (Table 5).

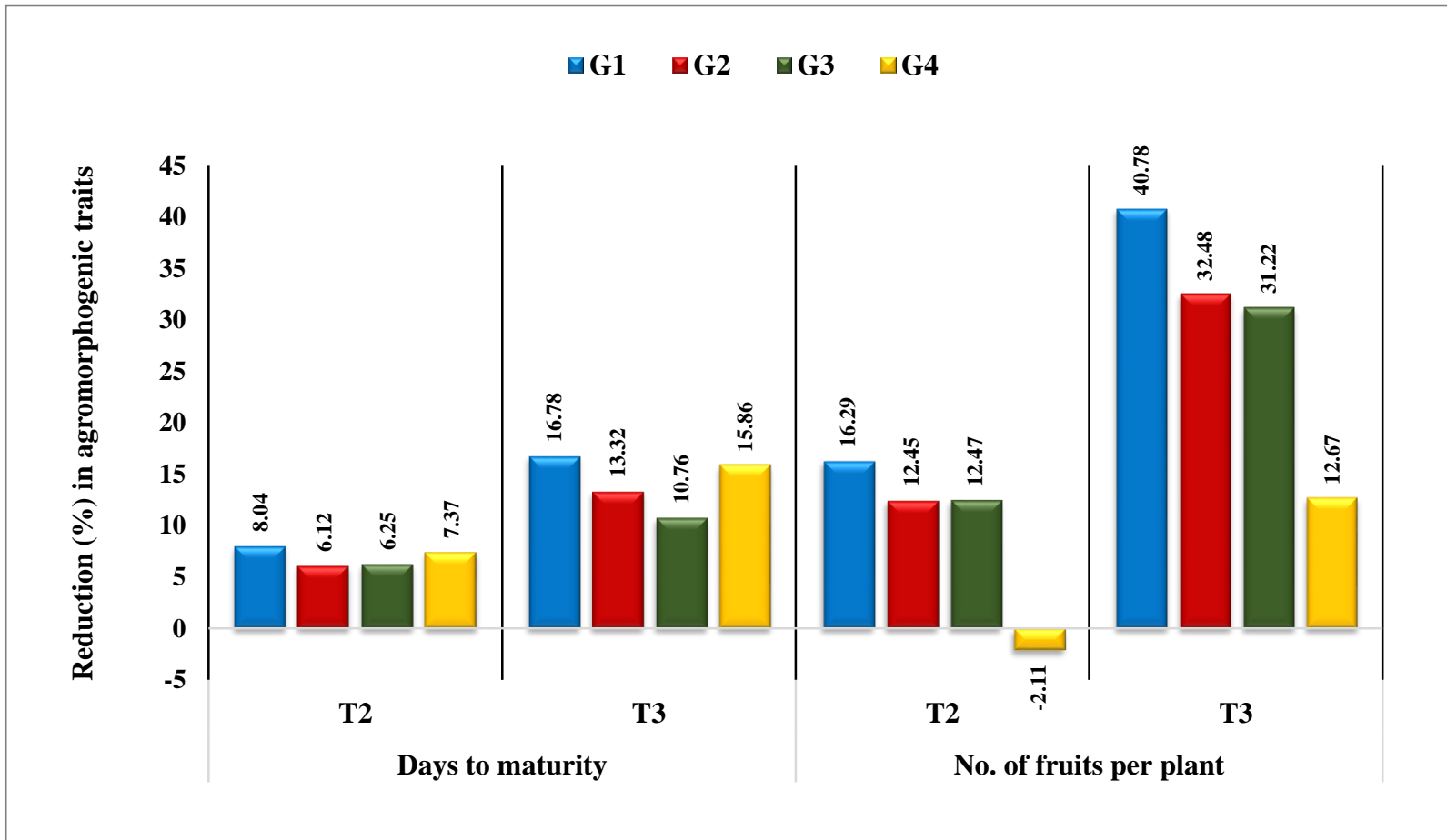
The time required for days to maturity or first harvesting of four tomatillo genotypes varied significantly under three different salinity treatment levels and the required maturity period was decreased gradually with the increase of salinity treatment levels (Appendix IX). The maximum reduction in case of days to maturity was observed in the genotype G<sub>1</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (8.04% and 16.78% respectively) and the minimum reduction was observed in genotype G<sub>2</sub> (6.12%) at slightly (8 dS/m) salinity stress whereas in G<sub>3</sub> (10.76%) at moderately (12 dS/m) salinity stress (Figure 2). Therefore, genotype G<sub>1</sub> might be considered as a good source for transferring the early maturity trait in a high yielding genotype of tomatillo under salinity stress condition.

#### **4.1.4 Number of fruits per plant**

The mean values of number of fruits per plant for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the ANOVA table (Appendix V), it was observed in this experiment that the number of fruits per plant showed statistically highly significant variation among different tomatillo genotypes. The maximum number of fruits was obtained from genotype G<sub>3</sub> (18.22 fruits/plant) whereas the minimum number of fruits was found in G<sub>2</sub> (11.33 fruits/plant) (Table 3).

From the result of ANOVA table (Appendix V), the tomatillo genotypes showed statistically highly significant variation to salinity treatment levels; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of number of fruits per plant. The highest number of fruits per plant was found in treatment T<sub>1</sub> (16.67 fruits/plant) and the





**Figure 2. Reduction percentage in days to maturity and number of fruits per plant under increasing salinity stress**

lowest number of fruits was found in T<sub>3</sub> (11.75 fruits/plant) (Table 4). This result showed that number of fruits per plant was decreased under the increase of salinity treatment levels and other ions in the root zone of tomatillo plant. According to Islam *et al.* (2011), the maximum number of fruits per plant were found in control and was decreased gradually with the increase of salinity stress levels. Similar results were also found by Siddiky *et al.* (2012) and Al-Yahyai *et al.* (2010).

Interaction effect between four tomatillo genotypes and three salinity treatments was found statistically highly significant in respect of number of fruits per plant (Appendix V). The highest number of fruits was obtained from interaction G<sub>3</sub>T<sub>1</sub> (21.33 fruits/plant) whereas the lowest number of fruits was obtained from G<sub>2</sub>T<sub>3</sub> (9.00 fruits/plant) which was statistically identical with interaction G<sub>1</sub>T<sub>3</sub> (9.67 fruits/plant) (Table 5).

The number of fruits obtained from per plant of four tomatillo genotypes varied significantly under three different salinity treatments and the number of fruits was decreased gradually with the increase of salinity treatment levels (Appendix IX). The maximum reduction in number of fruits per plant was found in genotype G<sub>1</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (16.29% and 40.78% respectively) whereas the minimum reduction was found in G<sub>2</sub> (12.45%) at slightly (8 dS/m) salinity stress and in G<sub>4</sub> (12.76%) at moderately (12 dS/m) salinity stress. Genotype G<sub>4</sub> (-2.11%) showed increased number of fruits per plant at slightly (8 dS/m) salinity level (Figure 2). Such stimulatory effect of low salinity levels on yield and its components were mentioned by Babu and Thirumurugan (2001) who noted that yield components were increased under low salinity level; further increase in salinity, decreased the yield parameters. The obtained results were also matched with those reported by Maggio *et al.*, 2007; Al-Harbi *et al.*, 2009; Al-Omran *et al.*, 2010 and Al-Harbi *et al.*, 2015. Similar findings were recorded on wheat and canola respectively by Ozoris and Robishy (1984) as well as Francois (1994). Therefore, genotype G<sub>4</sub> might be considered as a good source of parent material as it showed

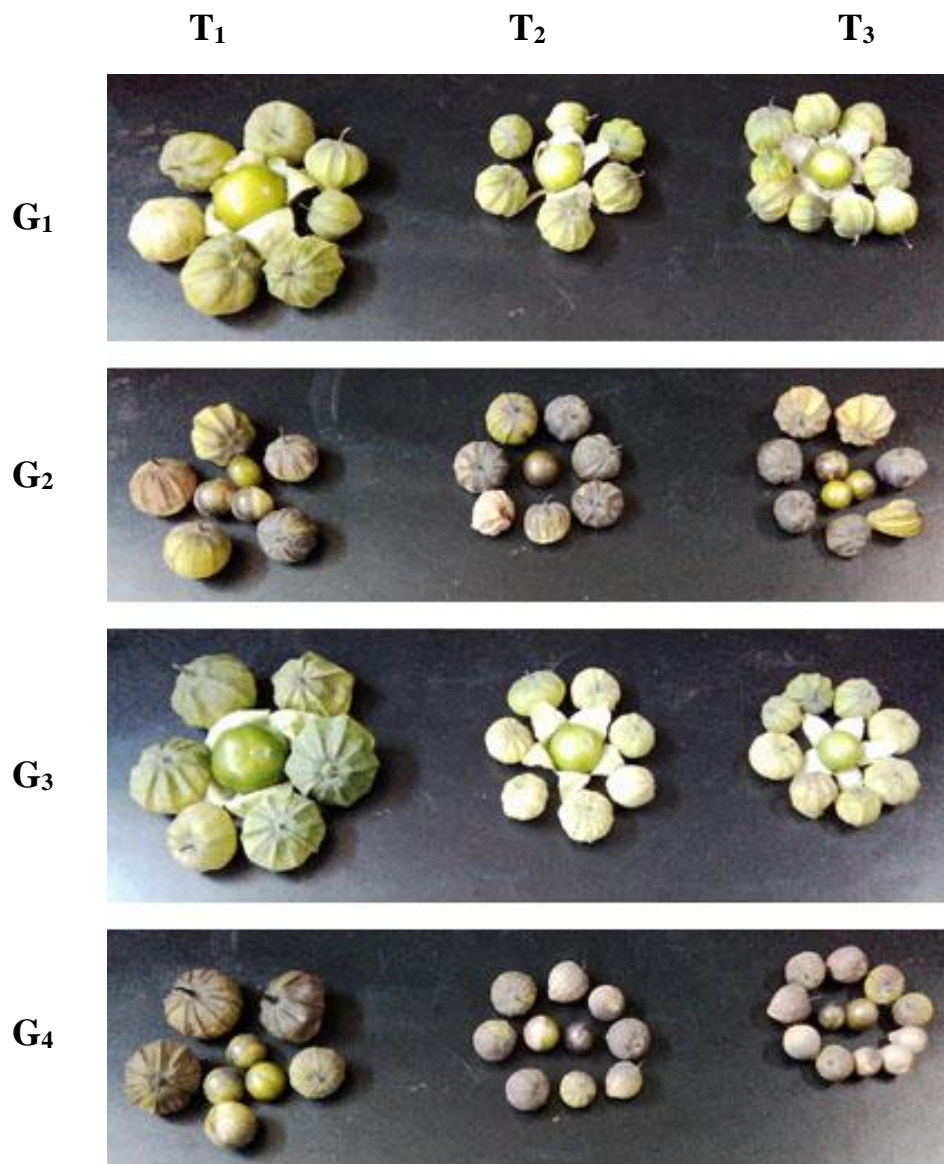
the minimum reduction (at 12 dS/m) and even increase (at 8 dS/m) in case of number of fruits per plant under the salinity stress condition.

#### **4.1.5 Average fruit length (mm)**

The mean values of average fruit length (mm) per plant for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. A pictorial view of morphological comparisons of tomatillo fruits between control and stressed conditions has been presented in Plate 7. From the result of ANOVA table (Appendix V), it was observed in this experiment that statistically highly significant variation was found for average fruit length per plant among tomatillo genotypes. The longest fruit was found from genotype G<sub>1</sub> (25.69 mm) which was statistically identical with G<sub>3</sub> (25.42 mm) while the shortest one was found from genotype G<sub>4</sub> (15.52 mm) (Table 6).

From the ANOVA table (Appendix V), the tomatillo genotypes showed statistically highly significant variation to different salinity treatment levels; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of average fruit length (mm) per plant. The longest fruit was found in treatment T<sub>1</sub> (27.05 mm) while the shortest fruit was found in T<sub>3</sub> (16.52 mm) (Table 7). This result showed that average fruit length of tomatillo was decreased under the increase of salinity treatment levels and other ions in the root zone of tomatillo plant. According to Cuartero and Fernandez-Munoz (1999), the fruit growth can greatly reduce by the increase of salinity level. The cell division phase of salt treated fruit is normal but salt have a deleterious effect on cell expansion phase due to low water content in the fruit. Supply of water into the fruit under saline conditions is restricted by lower water potential in the plant (Johnson *et al.*, 1992). Thus, higher levels of salinity can reduce the fruit size and marketable yield (Hao *et al.*, 2000). The reduction in fruit length due to the increase of salinity levels was also found by Edris *et al.* (2012) and Magan *et al.* (2008).

Interaction effect between four tomatillo genotypes and three salinity treatments was found statistically highly significant in respect of average fruit length (mm)



**Plate 7. Morphological comparisons of tomatillo fruits between control and salinity stress condition**

**Table 6. Performance of tomatillo genotypes on average fruit length, average fruit diameter, average fruit weight and yield per plant<sup>Y</sup>**

<b>Genotype<sup>X</sup></b>	<b>Average fruit length (mm)</b>	<b>Average fruit diameter (mm)</b>	<b>Average fruit weight (g)</b>	<b>Yield/Plant (kg)</b>
<b>G<sub>1</sub></b>	25.69 a	29.52 a	30.57 a	0.404 b
<b>G<sub>2</sub></b>	20.20 b	24.28 b	18.67 c	0.212 c
<b>G<sub>3</sub></b>	25.42 a	30.48 a	28.71 b	0.523 a
<b>G<sub>4</sub></b>	15.52 c	18.04 c	12.10 d	0.183 d
<b>CV %</b>	5.85	4.67	3.08	6.59
<b>LSD 0.05</b>	1.24	1.17	0.68	0.02

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub>

<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

**Table 7. Performance of salinity treatments on average fruit length, average fruit diameter, average fruit weight and yield per plant<sup>Y</sup>**

<b>Treatment<sup>X</sup></b>	<b>Average fruit length (mm)</b>	<b>Average fruit diameter (mm)</b>	<b>Average fruit weight (g)</b>	<b>Yield/Plant (kg)</b>
<b>T<sub>1</sub> (control)</b>	27.05 a	31.44 a	26.34 a	0.439 a
<b>T<sub>2</sub> (8dS/m)</b>	21.57 b	25.21 b	22.49 b	0.337 b
<b>T<sub>3</sub> (12dS/m)</b>	16.52 c	20.08 c	18.71 c	0.220 c
<b>CV %</b>	5.85	4.67	3.08	6.59
<b>LSD 0.05</b>	1.08	1.01	0.59	0.02

<sup>X</sup>Three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub>

<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

per plant (Appendix V). The longest fruit was found from interaction G<sub>1</sub>T<sub>1</sub> (34.01 mm) whereas the shortest fruit was found from interaction G<sub>4</sub>T<sub>3</sub> (10.89 mm) (Table 8).

The average fruit length (mm) of four tomatillo genotypes varied significantly under three different salinity treatment levels and the fruit length was mainly decreased gradually with the increase of salinity treatment levels (Appendix IX). The maximum reduction in average fruit length per plant was observed in genotype G<sub>1</sub> (28.46%) at slightly (8 dS/m) salinity stress and in G<sub>4</sub> (45.25%) at moderately (12 dS/m) salinity stress whereas the minimum reduction was observed in genotype G<sub>3</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (12.06% and 28.78% respectively) (Figure 3). Therefore, genotype G<sub>3</sub> might be considered as a good source of parent material as it showed the minimum reduction in case of average fruit length (mm) per plant under both slightly (8 dS/m) and moderately (12 dS/m) salinity stress condition.

#### **4.1.6 Average fruit diameter (mm)**

The mean values of average fruit diameter (mm) per plant for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix V), it was observed in this experiment that statistically highly significant variation was found for average fruit diameter per plant among tomatillo genotypes. The maximum diameter of fruit was found in genotype G<sub>3</sub> (30.48 mm) which was statistically identical with G<sub>1</sub> (29.52 mm) while the minimum fruit diameter was found in genotype G<sub>4</sub> (18.04 mm) (Table 6).

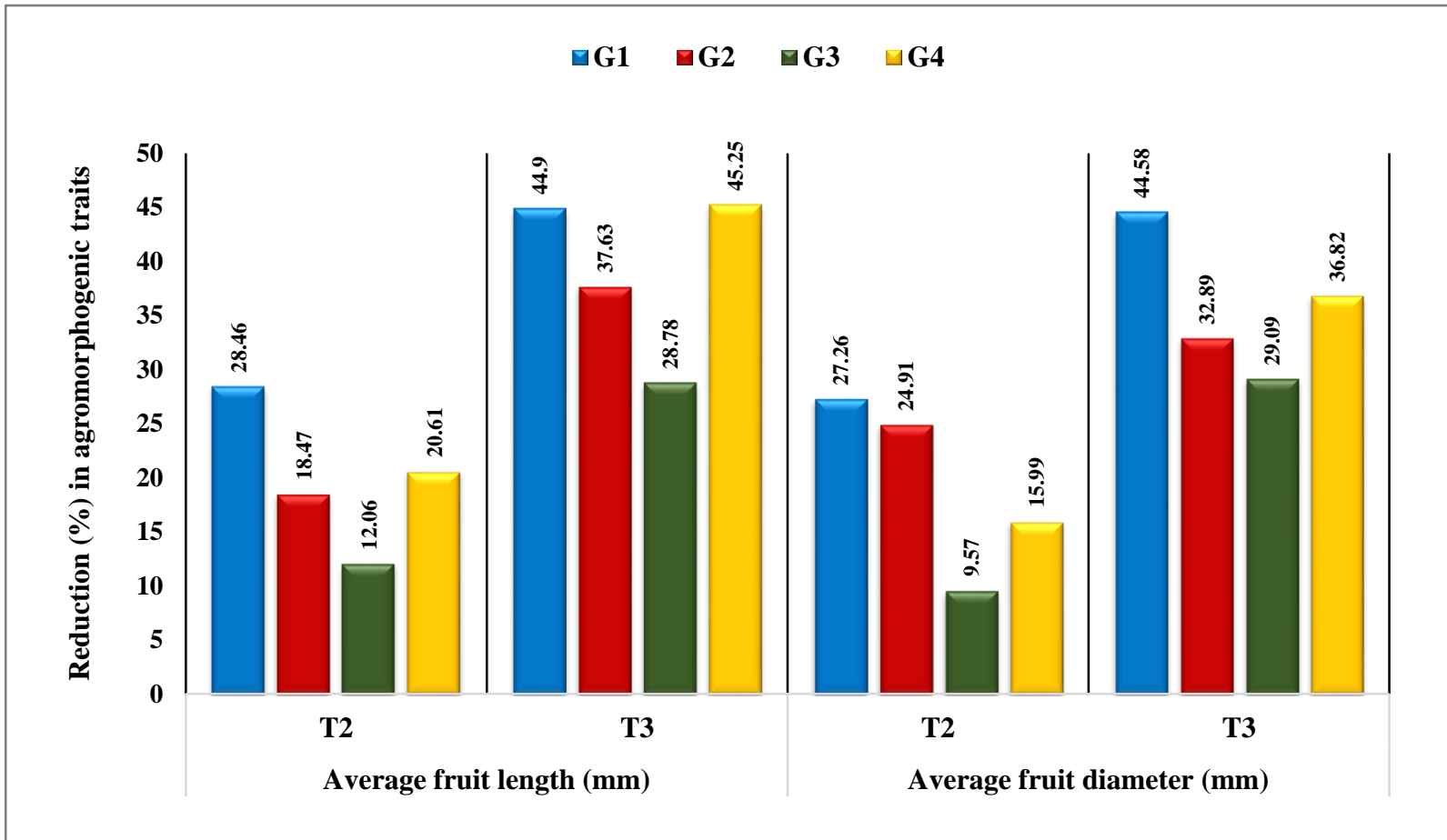
From the result of ANOVA (Appendix V), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of average fruit diameter (mm) per plant. The widest fruit was found in treatment T<sub>1</sub> (31.44 mm) while the narrowest fruit was found in T<sub>3</sub> (20.08 mm) (Table 7). This result showed that average fruit diameter per plant of tomatillo fruit was decreased under the increasing salinity treatment levels and other ions in the root zone of

**Table 8. Interaction effect between tomatillo genotypes and salinity treatments on average fruit length, average fruit diameter, average fruit weight and yield per plant<sup>Y</sup>**

<b>Interaction<sup>X</sup></b>	<b>Average fruit length (mm)</b>	<b>Average fruit diameter (mm)</b>	<b>Average fruit weight (g)</b>	<b>Yield/Plant (kg)</b>
<b>G<sub>1</sub>T<sub>1</sub></b>	34.01 a	38.81 a	35.29 a	0.576 b
<b>G<sub>1</sub>T<sub>2</sub></b>	24.33 c	28.23 d	30.53 c	0.417 d
<b>G<sub>1</sub>T<sub>3</sub></b>	18.74 e	21.51 fg	25.88 e	0.250 g
<b>G<sub>2</sub>T<sub>1</sub></b>	24.85 c	30.07 cd	22.25 g	0.297 f
<b>G<sub>2</sub>T<sub>2</sub></b>	20.26 de	22.58 f	18.92 h	0.221 gh
<b>G<sub>2</sub>T<sub>3</sub></b>	15.50 f	20.18 gh	14.85 i	0.134 i
<b>G<sub>3</sub>T<sub>1</sub></b>	29.43 b	34.99 b	33.17 b	0.708 a
<b>G<sub>3</sub>T<sub>2</sub></b>	25.88 c	31.64 c	28.41 d	0.530 c
<b>G<sub>3</sub>T<sub>3</sub></b>	20.96 d	24.81 e	24.55 f	0.360 e
<b>G<sub>4</sub>T<sub>1</sub></b>	19.89 de	21.89 fg	14.64 i	0.229 gh
<b>G<sub>4</sub>T<sub>2</sub></b>	15.79 f	18.39 h	12.11 j	0.194 h
<b>G<sub>4</sub>T<sub>3</sub></b>	10.89 g	13.83 i	9.54 k	0.130 i
<b>CV %</b>	5.85	4.67	3.08	6.59
<b>LSD 0.05</b>	2.15	2.02	1.18	0.04

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub> and three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub> (T<sub>1</sub>: control, T<sub>2</sub>: 8 dS/m, T<sub>3</sub>: 12 dS/m)

<sup>Y</sup>In a column means containing similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability



**Figure 3. Reduction percentage in average fruit length and average fruit diameter under increasing salinity stress**



the plant. Higher levels of salinity can reduce fruit size and marketable yield (Hao *et al.*, 2000). High salinity can reduce the fruit growth rate and final fruit size by an osmotic effect. High salinity induces lower water potential in the plant which reduces the water flow in the fruit and therefore, the rate of fruit expansion becomes restricted (Ephevelink, 2005). Reduction in fruit diameter due to the increase of salinity levels was also found by Edris *et al.* (2012).

The average fruit diameter (mm) per plant performed highly significant variation in respect of the effect of interaction between four tomatillo genotypes and three salinity treatment levels (Appendix V). The maximum diameter of fruit was obtained from interaction G<sub>1</sub>T<sub>1</sub> (38.81 mm) whereas the minimum fruit diameter was from interaction G<sub>4</sub>T<sub>3</sub> (13.83 mm) (Table 8).

The average fruit diameter (mm) per plant of four tomatillo genotypes varied significantly under three different salinity treatments and the diameter of fruit was mainly decreased gradually with the increase of salinity treatment levels (Appendix IX). The maximum reduction in average fruit diameter (mm) per plant was observed in genotype G<sub>1</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (27.26% and 44.58% respectively) whereas the minimum reduction was observed in genotype G<sub>3</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (9.57% and 29.09% respectively) (Figure 3). Therefore, genotype G<sub>3</sub> might be considered as a good source of parent material as it showed the minimum reduction in case of average fruit diameter (mm) per plant under both at slightly (8 dS/m) and moderately (12 dS/m) salinity stress condition.

#### **4.1.7 Average fruit weight (g)**

The mean values of average fruit weight (g) per plant for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix V), it was observed in this experiment that statistically highly significant variation was found for average fruit weight per plant among the tomatillo genotypes. The maximum weight of tomatillo fruit was found in

genotype G<sub>1</sub> (30.57 g) and the minimum fruit weight was found in genotype G<sub>4</sub> (12.10 g) (Table 6).

From the result of ANOVA (Appendix V), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatment levels; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of average fruit weight (g) per plant. The maximum weight of fruit was found in treatment T<sub>1</sub> (26.34 g) while the minimum fruit weight was found in treatment T<sub>3</sub> (18.71 g) (Table 7). This result showed that average fruit weight of tomatillo fruit was decreased under the increase of salinity treatment levels and other ions in the root zone of the plant. Reduction in single fruit weight per plant due to the increase of salinity levels was found by Al-Yahyai *et al.* (2010) and Islam *et al.* (2011). In saline area the plants are affected by excessive amount of salt (mainly NaCl). Excessive amounts of soluble salts in the root environment cause osmotic stress, which may result in disturbance of the plant water relations, in the uptake and utilization of essential nutrients, and also in toxic ion accumulation. Supply of water into the fruit under saline conditions is restricted by a lower water potential in the plant. Less water flow in the fruit cause reduction in fruit size, thus reduces the fruit weight (Munns, 2002).

Interaction effect between four tomatillo genotypes and three salinity treatments was found statistically highly significant in respect of average fruit weight (g) per plant (Appendix V). The maximum weight of fruit was found from interaction G<sub>1</sub>T<sub>1</sub> (35.29 g) whereas the minimum fruit weight was found from interaction G<sub>4</sub>T<sub>3</sub> (9.54 g) (Table 8).

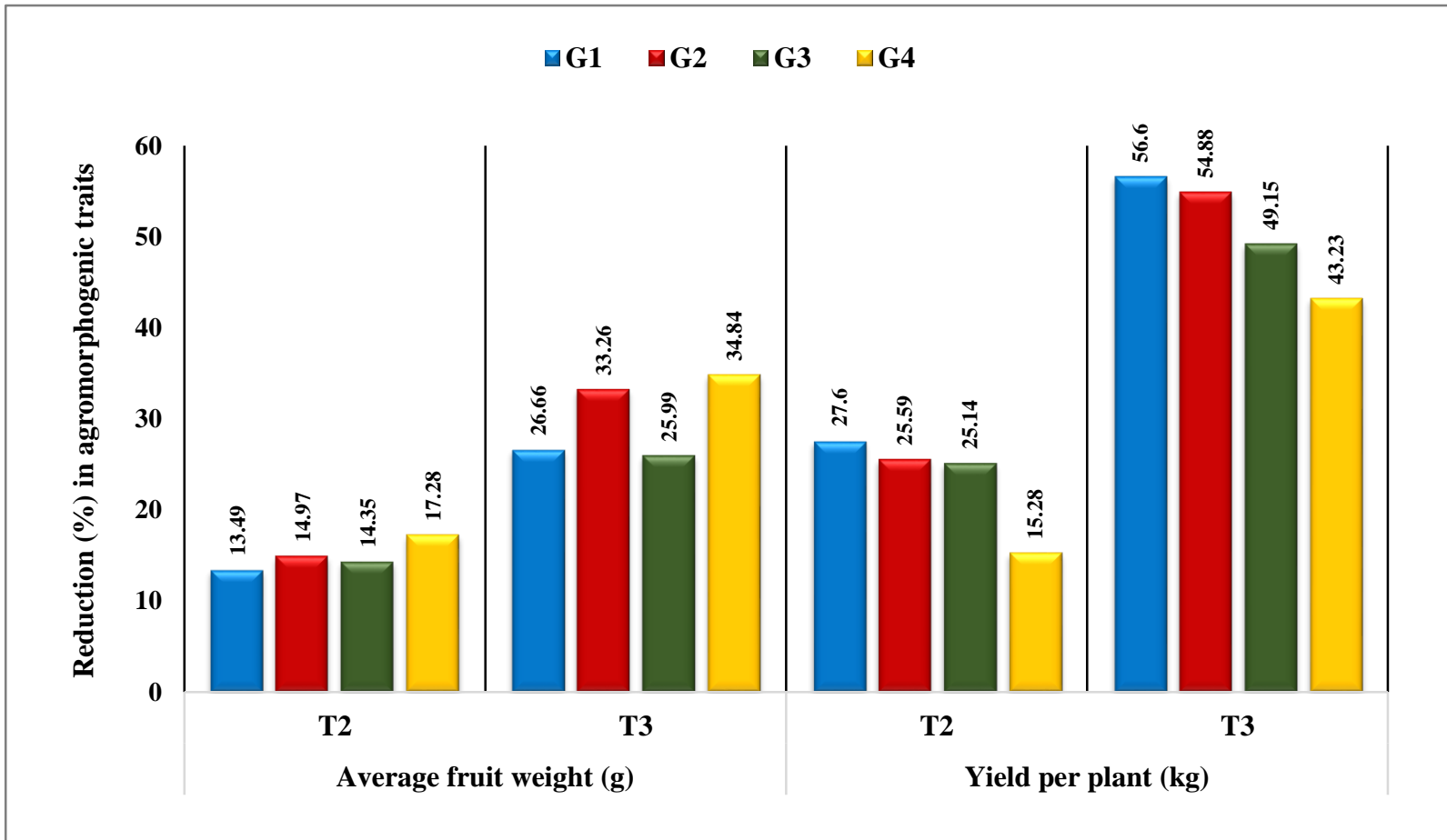
The average fruit weight (g) per plant of four tomatillo genotypes varied significantly under three different salinity treatments and the weight of fruit was mainly decreased gradually with the increase of salinity treatment levels (Appendix IX). The maximum reduction in average fruit weight per plant was observed in genotype G<sub>4</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (17.28% and 34.84% respectively) whereas the minimum reduction was observed in genotype G<sub>1</sub> (13.49%) at slightly (8 dS/m) salinity

stress and in G<sub>3</sub> (25.99%) at moderately (12 dS/m) salinity stress (Figure 4). Therefore, genotype G<sub>1</sub> and G<sub>3</sub> might be considered as good source of parent materials as these showed the minimum reduction in case of average fruit weight (g) per plant under slightly (8 dS/m) and moderately (12 dS/m) salinity stress condition respectively.

#### **4.1.8 Yield per plant (kg)**

The mean values of yield of fruit per plant (kg) for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix V), it was observed in this experiment that statistically highly significant variation was found for yield of fruit per plant among the tomatillo genotypes. The highest yield per plant of tomatillo was obtained from genotype G<sub>3</sub> (0.523 kg/plant) and the lowest yield per plant was from genotype G<sub>4</sub> (0.183 kg/plant) (Table 6).

From the result of ANOVA table (Appendix V), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of yield of fruit per plant (kg). The highest yield of fruit was found in treatment T<sub>1</sub> (0.439 kg/plant) while the lowest fruit yield was found in treatment T<sub>3</sub> (0.220 kg/plant) (Table 7). This result showed that yield of fruit per plant of tomatillo was decreased under the increasing salinity treatment levels and other ions in the root zone of the plant. Salinity stress can reduce the fruit number and average fruit weight per plant and thus, in case of high salinity levels the total fruit weight per plant can be reduced (Siddiky *et al.*, 2012; Islam *et al.*, 2011). The negative effect of high salinity levels on yield losses may be attributed to fact that plants grown under saline environments were directly exposed to osmotic stress resulting from low external water potential induced by high salt concentration in the soil (Khalil, 2006). It was also revealed that the suppressive effect of high salinity was a consequence of several physiological responses including variation in photosynthetic products translocation toward roots, modification of ion balance,



**Figure 4. Reduction percentage in average fruit weight and yield per plant under increasing salinity stress**

water status, total or partial stomatal enclosure, disrupted photosynthetic efficiency, allocation and utilization of carbon and ultimately yield.

Interaction effect between four tomatillo genotypes and three salinity treatments was found statistically highly significant in respect of yield of fruit per plant (kg) (Appendix V). The highest yield of fruit was found in interaction G<sub>3</sub>T<sub>1</sub> (0.708 kg/plant) whereas the lowest fruit yield was found in interaction G<sub>4</sub>T<sub>3</sub> (0.130 kg/plant) which was statistically identical with G<sub>2</sub>T<sub>3</sub> (0.134 kg/plant) (Table 8).

The yield of fruit per plant (kg) of four tomatillo genotypes varied significantly under three different salinity treatments and the yield per plant was mainly decreased gradually with the increase of salinity treatment levels (Appendix IX). The maximum reduction in yield of fruit per plant was observed in genotype G<sub>1</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stress (27.60% and 56.60% respectively) whereas the minimum reduction was observed in genotype G<sub>4</sub> at both slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (15.28% and 43.23% respectively) (Figure 4). Therefore, genotype G<sub>4</sub> might be considered as a good source of parent material as it showed the minimum reduction in case of yield (kg) per plant under both at slightly (8 dS/m) and moderately (12 dS/m) salinity stress condition.

## **4.2 Evaluation of physiological traits of tomatillo**

Different physiological traits of tomatillo plant *viz.*, indigenous sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) ion content, proline content, leaf area index and chlorophyll content (30 and 60 days after providing salinity stress) were presented and discussed based on their ANOVA, genotype, salinity treatment, genotype-treatment interaction effect and percentage of changes (increase or decrease) in these traits.

### **4.2.1 Na<sup>+</sup> content (%)**

The mean values of Na<sup>+</sup> content (%) for four genotypes of tomatillo under three different salinity treatment levels, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix

VI), it was observed in this experiment that statistically highly significant variation was found for indigenous  $\text{Na}^+$  content in plant shoots among the tomatillo genotypes. The highest amount of  $\text{Na}^+$  content was found in genotype  $G_4$  (1.53%) whereas the lowest amount of  $\text{Na}^+$  content was found in genotype  $G_1$  (1.35%) which was statistically identical with  $G_3$  (1.38%) (Table 9).

From the result of ANOVA table (Appendix VI), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatment levels;  $T_1$  (Control),  $T_2$  (8 dS/m) and  $T_3$  (12 dS/m) in respect of the amount of indigenous  $\text{Na}^+$  content (%) in plant shoots. The highest amount of  $\text{Na}^+$  content was found in treatment  $T_3$  (1.86%) while the lowest amount of  $\text{Na}^+$  content was found in treatment  $T_1$  (0.10%) (Table 10). This result showed that the amount of indigenous  $\text{Na}^+$  content in the plant of tomatillo was increased gradually under the increase of salinity treatment levels. When excessive amounts of salt enter into the plant through water, salt concentration will eventually rise to toxic levels in the older transpiring leaves, causing premature senescence, and increasing of the indigenous  $\text{Na}^+$  concentration in both shoot and root zone of plants exposed to salinity stress (Siddiky *et al.*, 2012; Hajiboland *et al.*, 2010; Dasgan *et al.*, 2002).

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically significant in respect of the amount of indigenous  $\text{Na}^+$  content (%) (Appendix VI). The highest amount of  $\text{Na}^+$  content was found in interaction  $G_4T_3$  (1.97%) which was statistically identical with  $G_2T_3$  (1.91%) whereas the lowest amount of  $\text{Na}^+$  content was found in the interaction  $G_1T_1$  (0.91%) which was statistically identical with  $G_3T_1$  (0.97%) and  $G_2T_1$  (1.02%) (Table 11).

The amount of  $\text{Na}^+$  content (%) of four tomatillo genotypes varied significantly under three different salinity treatments and the amount of  $\text{Na}^+$  was mainly increased gradually with the increase of salinity treatment levels (Appendix X). The maximum increase in the amount of indigenous  $\text{Na}^+$  content was observed in the genotype  $G_1$  in both cases, at slightly (8 dS/m) and moderately (12 dS/m)

**Table 9. Performance of tomatillo genotypes on Na<sup>+</sup> content, K<sup>+</sup> content and proline content<sup>Y</sup>**

<b>Genotype<sup>X</sup></b>	<b>Na<sup>+</sup> content (%)</b>	<b>K<sup>+</sup> content (%)</b>	<b>Proline content (µg/g)</b>
<b>G<sub>1</sub></b>	1.35 c	3.41 a	1771.80 d
<b>G<sub>2</sub></b>	1.46 b	2.55 b	2380.70 c
<b>G<sub>3</sub></b>	1.38 c	3.24 a	2803.10 b
<b>G<sub>4</sub></b>	1.53 a	2.45 b	3088.80 a
<b>CV %</b>	4.63	6.68	2.35
<b>LSD 0.05</b>	0.06	0.19	57.70

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub>

<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

**Table 10. Performance of salinity treatments on Na<sup>+</sup> content, K<sup>+</sup> content and proline content<sup>Y</sup>**

<b>Treatment<sup>X</sup></b>	<b>Na<sup>+</sup> content (%)</b>	<b>K<sup>+</sup> content (%)</b>	<b>Proline content (µg/g)</b>
<b>T<sub>1</sub> (control)</b>	0.10 c	3.70 a	1268.30 c
<b>T<sub>2</sub> (8dS/m)</b>	1.44 b	2.91 b	2402.50 b
<b>T<sub>3</sub> (12dS/m)</b>	1.86 a	2.13 c	3862.50 a
<b>CV %</b>	4.63	6.68	2.35
<b>LSD 0.05</b>	0.06	0.16	49.97

<sup>X</sup>Three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub>

<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

**Table 11. Interaction effect between tomatillo genotypes and salinity treatments on Na<sup>+</sup> content, K<sup>+</sup> content and proline content<sup>Y</sup>**

<b>Interaction<sup>X</sup></b>	<b>Na<sup>+</sup> content (%)</b>	<b>K<sup>+</sup> content (%)</b>	<b>Proline content (µg/g)</b>
<b>G<sub>1</sub>T<sub>1</sub></b>	0.91 g	4.12 a	652.20 j
<b>G<sub>1</sub>T<sub>2</sub></b>	1.39 e	3.42 bc	1569.90 h
<b>G<sub>1</sub>T<sub>3</sub></b>	1.74 c	2.68 d	3093.30 d
<b>G<sub>2</sub>T<sub>1</sub></b>	1.02 fg	3.58 b	1342.30 i
<b>G<sub>2</sub>T<sub>2</sub></b>	1.46 de	2.32 e	2230.50 f
<b>G<sub>2</sub>T<sub>3</sub></b>	1.91 ab	1.75 f	3569.30 c
<b>G<sub>3</sub>T<sub>1</sub></b>	0.97 fg	3.97 a	1361.50 i
<b>G<sub>3</sub>T<sub>2</sub></b>	1.35 e	3.23 c	2814.00 e
<b>G<sub>3</sub>T<sub>3</sub></b>	1.82 bc	2.51 de	4233.80 b
<b>G<sub>4</sub>T<sub>1</sub></b>	1.08 f	3.13 c	1717.20 g
<b>G<sub>4</sub>T<sub>2</sub></b>	1.54 d	2.67 d	2995.70 d
<b>G<sub>4</sub>T<sub>3</sub></b>	1.97 a	1.56 f	4553.70 a
<b>CV %</b>	4.63	6.68	2.35
<b>LSD 0.05</b>	0.11	0.33	99.93

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub> and three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub> (T<sub>1</sub>: control, T<sub>2</sub>: 8 dS/m, T<sub>3</sub>: 12 dS/m)

<sup>Y</sup>In a column means containing similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

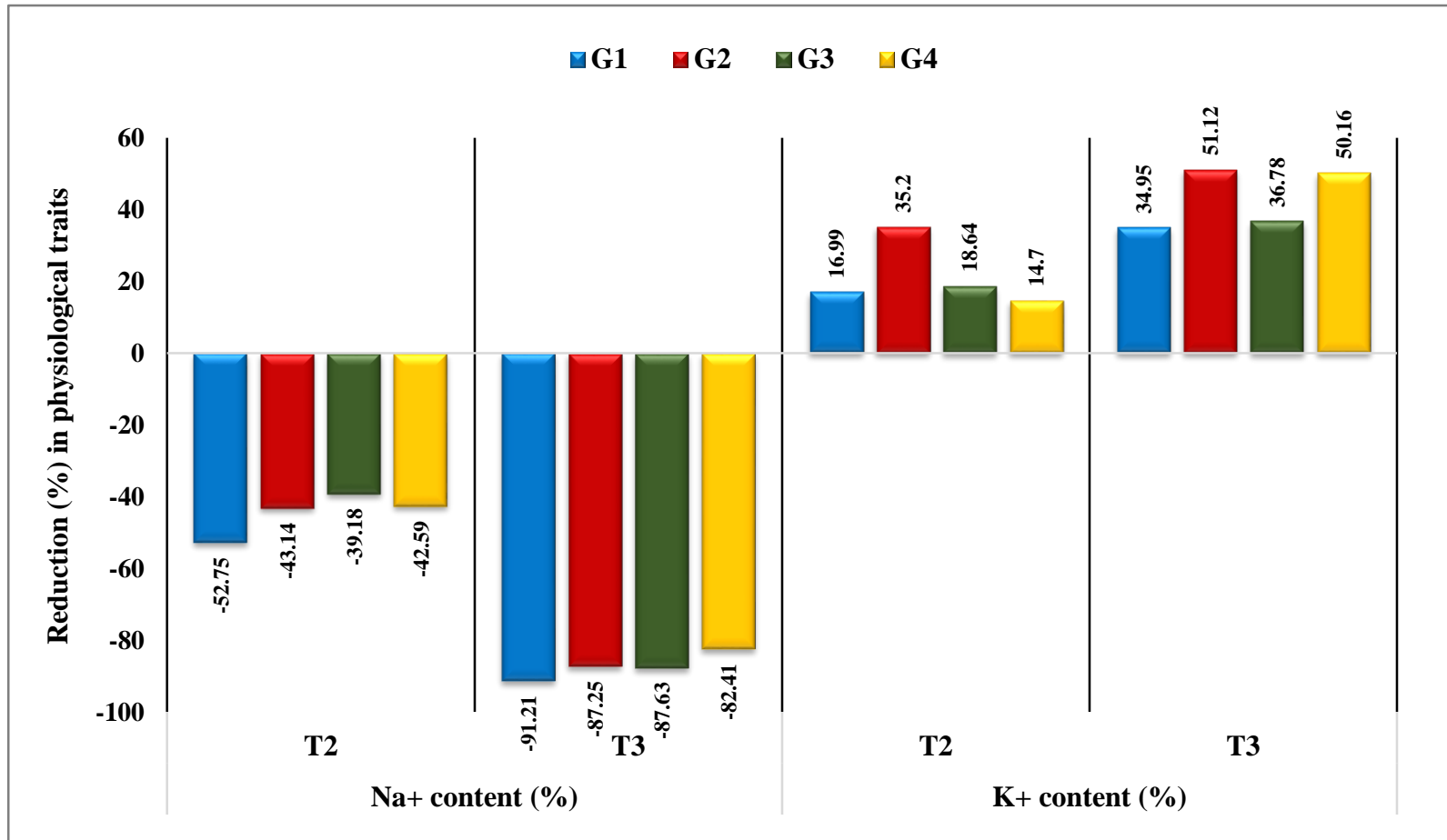


salinity stresses (-52.75% and -91.21% respectively) whereas the minimum increase was observed in genotype G<sub>3</sub> (-39.18%) at slightly (8 dS/m) salinity stress and in G<sub>4</sub> (-82.41%) at moderately (12 dS/m) salinity stress (Figure 5). Therefore, genotype G<sub>3</sub> and G<sub>4</sub> might be considered as desirable genotypes as they showed the minimum increase in indigenous Na<sup>+</sup> content (%) in plant shoots under slightly (8 dS/m) and moderately (12 dS/m) salinity stress conditions respectively.

#### **4.2.2 K<sup>+</sup> content (%)**

The mean values of K<sup>+</sup> content (%) for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix VI), it was observed in this experiment that statistically highly significant variation was found for indigenous K<sup>+</sup> content in plant shoots among the tomatillo genotypes. The highest amount of K<sup>+</sup> content was found in genotype G<sub>1</sub> (3.41%) which was statistically identical with G<sub>3</sub> (3.24%) whereas the lowest amount of K<sup>+</sup> content was found in genotype G<sub>4</sub> (2.45%) which was statistically identical with G<sub>2</sub> (2.55%) (Table 9).

From the result of ANOVA table (Appendix VI), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of the amount of indigenous K<sup>+</sup> content (%) in plant shoots. The highest amount of indigenous K<sup>+</sup> content was found in treatment T<sub>1</sub> (3.70%) and the lowest amount of indigenous K<sup>+</sup> content was found in treatment T<sub>3</sub> (2.13%) (Table 10). This result showed that the amount of indigenous K<sup>+</sup> content in the shoot of tomatillo plants was decreased gradually under the increasing levels of salinity treatment. The increase of Na<sup>+</sup> concentration in the root zone of plant gradually decreases the uptake of K<sup>+</sup> in plants and thus, reduce the indigenous K<sup>+</sup> concentration in stressed plant shoots (Edris *et al.*, 2012; Hajiboland *et al.*, 2010; Dasgan *et al.* 2002; Akinci *et al.* 2004).



**Figure 5. Reduction percentage in indigenous Na<sup>+</sup> and K<sup>+</sup> ion content under increasing salinity stress**

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically significant in respect of the amount of indigenous K<sup>+</sup> content (%) in plant shoots (Appendix VI). The highest amount of indigenous K<sup>+</sup> content was found in interaction G<sub>1</sub>T<sub>1</sub> (4.12%) which was statistically identical with G<sub>3</sub>T<sub>1</sub> (3.97%) whereas the lowest amount of indigenous K<sup>+</sup> content was found in the interaction G<sub>4</sub>T<sub>3</sub> (1.56%) which was statistically identical with G<sub>2</sub>T<sub>3</sub> (1.75%) (Table 11).

The amount of indigenous K<sup>+</sup> content (%) in plant shoots of four tomatillo genotypes varied significantly under three different salinity treatments and the amount of indigenous K<sup>+</sup> was mainly decreased gradually with the increase of salinity treatment levels (Appendix X). The maximum reduction in the amount of indigenous K<sup>+</sup> content was observed in genotype G<sub>2</sub> in both cases, at slightly (8dS/m) and moderately (12 dS/m) salinity stresses (35.20% and 51.12% respectively) while the minimum reduction was observed in genotype G<sub>4</sub> (14.70%) at slightly (8 dS/m) salinity stress and in G<sub>1</sub> (34.95%) at moderately (12 dS/m) salinity stress (Figure 5). Therefore, genotype G<sub>4</sub> and G<sub>1</sub> might be considered as desirable genotypes as they showed the minimum reduction in indigenous K<sup>+</sup> content (%) in plant shoots under slightly (8 dS/m) and moderately (12 dS/m) salinity stress conditions respectively.

#### **4.2.3 Proline content (µg/g)**

The mean values of proline content (µg/g) for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. A pictorial view of proline content of four tomatillo genotypes under three different salinity treatments has been presented in Plate 8. From the result of ANOVA table (Appendix VI), it was observed in this experiment that statistically highly significant variation was found for proline content among the tomatillo genotypes. The highest amount of proline content was found in genotype G<sub>4</sub> (3088.80 µg/g) whereas the lowest amount of proline was found in the genotype G<sub>1</sub> (1771.80 µg/g) (Table 9). Thus, from the



**Plate 8. A pictorial view of proline content of four tomatillo genotypes under three different salinity treatments**

observed result, G<sub>4</sub> was found to be the highest proline containing genotype of tomatillo.

From the result of ANOVA table (Appendix VI), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of the amount of proline content ( $\mu\text{g/g}$ ). The highest amount of proline content was found in treatment T<sub>3</sub> (3862.50  $\mu\text{g/g}$ ) while the lowest amount of proline content was found in treatment T<sub>1</sub> (1268.30  $\mu\text{g/g}$ ) (Table 10). This result showed that the amount of stress protein proline content in the plant of tomatillo was increased gradually under the increase of salinity treatment levels. Plants accumulate proline in their leaves as a nontoxic and protective osmolyte under salinity stress conditions (Mahdi and El-Katony, 2001). Proline accumulation under stress might occurs due to an increase in pyrroline-5-carboxylate synthetase (P5CS), the rate-limiting enzyme in proline biosynthesis (Singh *et al.*, 2000) and a decrease of proline dehydrogenase (PDH) activity (Spoljarevic *et al.*, 2011).

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically highly significant in respect of the amount of proline content ( $\mu\text{g/g}$ ) (Appendix VI). The highest amount of proline content was found in interaction G<sub>4</sub>T<sub>3</sub> (4553.70  $\mu\text{g/g}$ ) whereas the lowest amount of proline content was found in the interaction G<sub>1</sub>T<sub>1</sub> (652.20  $\mu\text{g/g}$ ) (Table 11).

The amount of proline content ( $\mu\text{g/g}$ ) of four tomatillo genotypes varied significantly under three different salinity treatments and the amount of proline content was mainly increased gradually with the increase of salinity treatment levels (Appendix X). The maximum increase in the amount of proline content was observed in genotype G<sub>1</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (-140.71% and -374.29% respectively) whereas the minimum increase was observed in genotype G<sub>2</sub> (-66.17%) at slightly (8 dS/m) salinity stress and in G<sub>4</sub> (-165.18%) at moderately (12 dS/m) salinity stress (Figure 6). Therefore, the genotype G<sub>1</sub> might be considered as a good source of

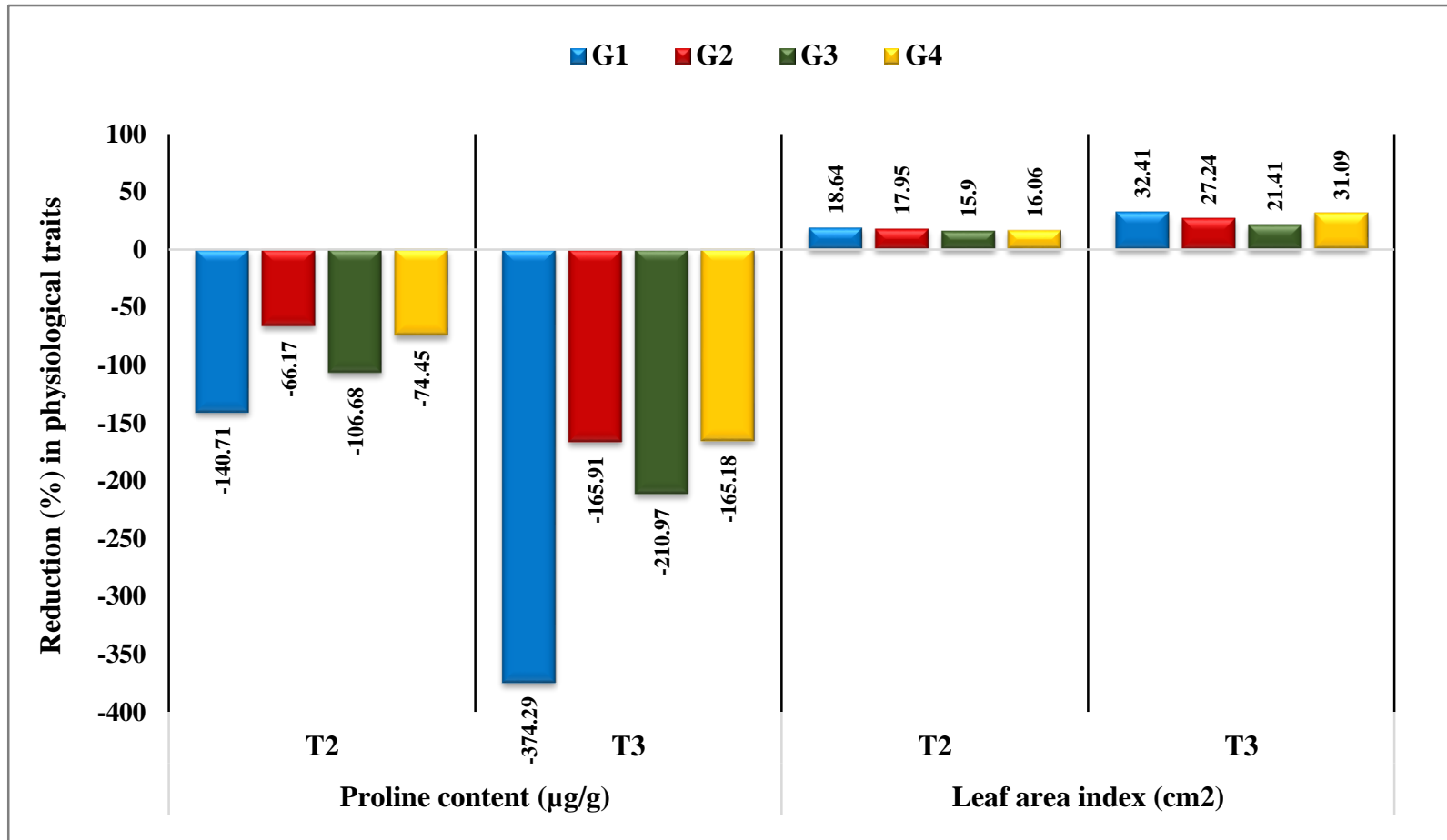


Figure 6. Reduction percentage in proline content and leaf area index under increasing salinity stress

parent material as it showed the maximum increase in proline content under both at slightly (8 dS/m) and moderately (12 dS/m) salinity stress conditions.

#### **4.2.4 Leaf area index (cm<sup>2</sup>)**

The mean values of leaf area index (cm<sup>2</sup>) for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the ANOVA table (Appendix VI), it was observed in this experiment that statistically highly significant variation was found for leaf area index in plant among the tomatillo genotypes. The highest amount of leaf area was found in genotype G<sub>1</sub> (19.28 cm<sup>2</sup>) whereas the lowest amount of leaf area was found in genotype G<sub>4</sub> (15.53 cm<sup>2</sup>) which was statistically identical with G<sub>2</sub> (15.99 cm<sup>2</sup>) (Table 12).

From the result of ANOVA table (Appendix VI), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of the amount of leaf area index (cm<sup>2</sup>). The highest amount of leaf area was found in treatment T<sub>1</sub> (19.84 cm<sup>2</sup>) and the lowest amount of leaf area was found in treatment T<sub>3</sub> (14.23 cm<sup>2</sup>) (Table 13). This result showed that the amount of leaf area in the tomatillo plant was decreased gradually under the increasing levels of salinity treatment. Soil salinity causes growth inhibition through low external water potentials, ion toxicity and ion imbalance (Munns, 1993). The lower leaf area index causes with the increase of higher salinity stress due to salt effect which is responsible for a decrease in leaf growth (Walker and Bernal, 2008). Reduction in leaf growth rate has been related to a reduction in cell turgor, cell wall rheological properties and a reduction in photosynthetic rate (Munns, 1993). At relatively low salinities, this can result in a transient reduction in turgor and leaf growth rate (Yeo *et al.*, 1991).

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically significant in respect of the amount of leaf area index (cm<sup>2</sup>) of tomatillo plant (Appendix VI). The highest amount of leaf area was found in interaction G<sub>1</sub>T<sub>1</sub> (23.23 cm<sup>2</sup>) while the lowest amount of leaf area

**Table 12. Performance of tomatillo genotypes on leaf area index and chlorophyll content after 30 days and 60 days of providing salinity stress<sup>Y</sup>**

Genotype <sup>X</sup>	Leaf area index (cm <sup>2</sup> )	Chlorophyll content (%)	
		30 days after stress	60 days after stress
G <sub>1</sub>	19.28 a	61.84 b	55.88 b
G <sub>2</sub>	15.99 c	80.23 a	73.01 a
G <sub>3</sub>	16.52 b	62.67 b	57.06 b
G <sub>4</sub>	15.53 c	62.60 b	56.78 b
CV %	4.13	5.69	6.69
LSD 0.05	0.68	3.72	3.97

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub>

<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

**Table 13. Performance of salinity treatments on leaf area index and chlorophyll content after 30 days and 60 days of providing salinity stress<sup>Y</sup>**

Treatment <sup>X</sup>	Leaf area index (cm <sup>2</sup> )	Chlorophyll content (%)	
		30 days after stress	60 days after stress
T <sub>1</sub> (control)	19.84 a	90.80 a	84.63 a
T <sub>2</sub> (8dS/m)	16.42 b	64.78 b	58.64 b
T <sub>3</sub> (12dS/m)	14.23 c	44.93 c	38.78 c
CV %	4.13	5.69	6.69
LSD 0.05	0.59	3.22	3.44

<sup>X</sup>Three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub>

<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability



was found in the interaction G<sub>4</sub>T<sub>3</sub> (12.70 cm<sup>2</sup>) which was statistically identical with G<sub>2</sub>T<sub>3</sub> (13.70 cm<sup>2</sup>) (Table 14).

The amount of leaf area index (cm<sup>2</sup>) of four tomatillo genotypes varied significantly under three different salinity treatments and the amount of leaf area was mainly decreased gradually with the increase of salinity treatment levels (Appendix X). The maximum reduction in the amount of leaf area was observed in genotype G<sub>1</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (18.64% and 32.41% respectively) whereas the minimum reduction was observed in genotype G<sub>3</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (15.90% and 21.41% respectively) (Figure 6). Therefore, genotype G<sub>3</sub> might be considered as a good source of parent material as it showed the minimum reduction in leaf area index (cm<sup>2</sup>) under both at slightly (8 dS/m) and moderately (12 dS/m) salinity stress conditions.

#### **4.2.5 Chlorophyll content (%)**

##### **4.2.5.1 Chlorophyll content after 30 days of applying salinity stress**

The mean values of chlorophyll content (%) of leaves (SPAD reading) after 30 days of starting the application of stress for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the ANOVA table (Appendix VI), it was observed in this experiment that statistically highly significant variation was found for chlorophyll content in the leaves of tomatillo plant among the tomatillo genotypes. The highest amount of chlorophyll content was found in genotype G<sub>2</sub> (80.23%) whereas the lowest amount of chlorophyll content was found in genotype G<sub>1</sub> (61.84%) which was statistically identical with G<sub>4</sub> (62.60%) and G<sub>3</sub> (62.67%) (Table 12). Thus, from the observed result of the experiment, G<sub>2</sub> was the maximum chlorophyll (%) containing genotype.

From the result of ANOVA table (Appendix VI), statistically highly significant variation was found in the tomatillo genotypes exposed to three different salinity

**Table 14. Interaction effect between tomatillo genotypes and salinity treatments on leaf area index and chlorophyll content after 30 days and 60 days of providing salinity stress<sup>Y</sup>**

Interaction <sup>X</sup>	Leaf area index (cm <sup>2</sup> )	Chlorophyll content (%)	
		30 days after stress	60 days after stress
<b>G<sub>1</sub>T<sub>1</sub></b>	23.23 a	85.47 bc	79.13 b
<b>G<sub>1</sub>T<sub>2</sub></b>	18.90 b	63.90 d	57.33 d
<b>G<sub>1</sub>T<sub>3</sub></b>	15.70 c	36.17 g	31.17 g
<b>G<sub>2</sub>T<sub>1</sub></b>	18.83 b	102.43 a	95.67 a
<b>G<sub>2</sub>T<sub>2</sub></b>	15.45 c	79.20 c	72.13 c
<b>G<sub>2</sub>T<sub>3</sub></b>	13.70 de	59.07 de	51.23 de
<b>G<sub>3</sub>T<sub>1</sub></b>	18.87 b	85.93 b	80.50 b
<b>G<sub>3</sub>T<sub>2</sub></b>	15.87 c	62.23 d	57.20 d
<b>G<sub>3</sub>T<sub>3</sub></b>	14.83 cd	39.83 fg	33.47 fg
<b>G<sub>4</sub>T<sub>1</sub></b>	18.43 b	89.37 b	83.20 b
<b>G<sub>4</sub>T<sub>2</sub></b>	15.47 c	53.77 e	47.90 e
<b>G<sub>4</sub>T<sub>3</sub></b>	12.70 e	44.67 f	39.23 f
<b>CV %</b>	4.13	5.69	6.69
<b>LSD 0.05</b>	1.18	6.44	6.88

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub> and three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub> (T<sub>1</sub>: control, T<sub>2</sub>: 8 dS/m, T<sub>3</sub>: 12 dS/m)

<sup>Y</sup>In a column means containing similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

treatment levels; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of chlorophyll content (%) of leaves (SPAD reading) after 30 days of starting stress application. The highest amount of chlorophyll content was found in treatment T<sub>1</sub> (90.80%) and the lowest amount of chlorophyll content was found in treatment T<sub>3</sub> (44.93%) (Table 13). This result showed that the amount of chlorophyll content in the leaves of tomatillo plant was decreased gradually under the increasing levels of salinity treatment. Gradual decrease in chlorophyll content causes due to the increase of salinity treatment levels (Hajer *et al.*, 2006; Al-Sobhi, 2005). Reduction in chlorophyll content is probably due to the inhibitory effect of the accumulated ions of salts on the biosynthesis of the different chlorophyll fractions. Salinity affects the strength of the forces bringing the complex pigment protein-liquid, in the chloroplast structure. As the chloroplast in membrane bound its stability is dependent on the membrane stability which under high salinity condition seldom remains intact and reduces the chlorophyll content (Edris *et al.*, 2012; Hajiboland *et al.*, 2010; Amini and Ehsnapour, 2006).

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically highly significant in respect of chlorophyll content (%) of plant leaves (SPAD reading) after 30 days of starting stress application (Appendix VI). The highest amount of chlorophyll content was found in interaction G<sub>2</sub>T<sub>1</sub> (102.43%) while the lowest amount of chlorophyll content was found in the interaction G<sub>1</sub>T<sub>3</sub> (36.17%) which was statistically identical with G<sub>3</sub>T<sub>3</sub> (39.83%) (Table 14).

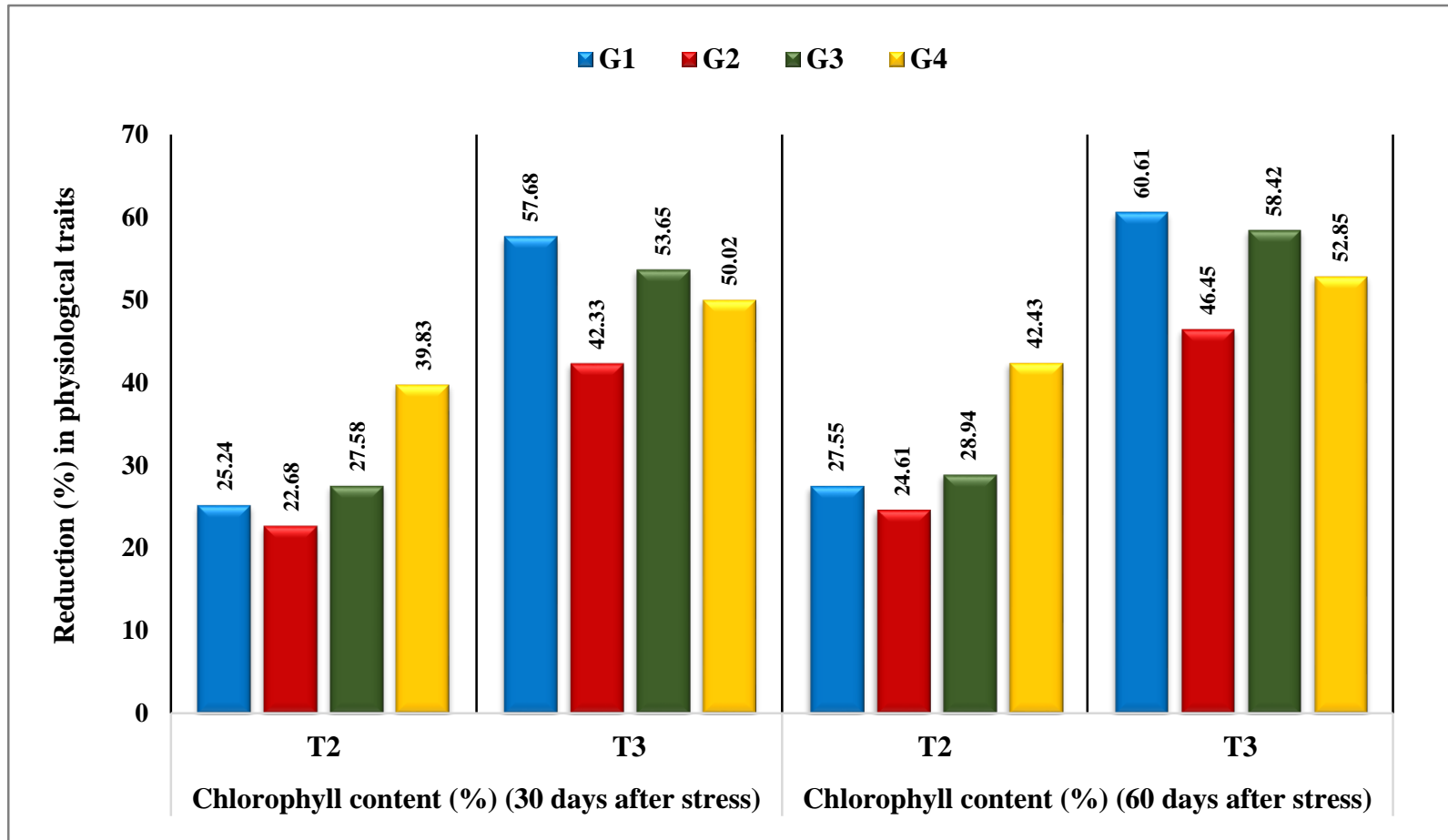
The amount of chlorophyll content (%) of plant leaves (SPAD reading) after 30 days of starting stress application of four tomatillo genotypes varied significantly under three different salinity treatments and the amount of chlorophyll content was mainly decreased gradually with the increase of salinity treatment levels (Appendix X). The maximum reduction in the amount of chlorophyll content was observed in genotype G<sub>4</sub> (39.83%) at slightly (8 dS/m) salinity stress and in G<sub>1</sub> (57.68%) at moderately (12 dS/m) salinity stress whereas the minimum

reduction was observed in genotype G<sub>2</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (22.68% and 42.33% respectively) (Figure 7). Therefore, genotype G<sub>2</sub> might be considered as a good source of parent material as it showed the minimum reduction in chlorophyll (%) content (SPAD reading) under both at slightly (8 dS/m) and moderately (12 dS/m) salinity stress conditions.

#### **4.2.5.2 Chlorophyll content after 60 days of applying salinity stress**

The mean values of chlorophyll content (%) of leaves (SPAD reading) after 60 days of starting the application of stress for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the ANOVA table (Appendix VI), it was observed in this experiment that statistically highly significant variation was found for chlorophyll content in the leaves of tomatillo plant among the tomatillo genotypes. The highest amount of chlorophyll content was found in genotype G<sub>2</sub> (73.01%) whereas the lowest amount of chlorophyll content was found in genotype G<sub>1</sub> (55.88%) which was statistically identical with G<sub>4</sub> (56.78%) and G<sub>3</sub> (57.06%) (Table 12). Thus, from the observed result, G<sub>2</sub> was the maximum chlorophyll (%) containing genotype.

From the result of ANOVA table (Appendix VI), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatment levels; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of chlorophyll content (%) of leaves (SPAD reading) after 60 days of starting stress application. The highest amount of chlorophyll content was found in treatment T<sub>1</sub> (84.63%) and the lowest amount of chlorophyll content was found in treatment T<sub>3</sub> (38.78%) (Table 13). This result showed that the amount of chlorophyll content in the leaves of tomatillo plants was decreased gradually under the increasing levels of salinity treatment. Gradual decrease in chlorophyll content causes due to the increase of salinity treatment levels (Hajer *et al.*, 2006; Al-Sobhi, 2005). Reduction in the chlorophyll content may probably due to the inhibitory effects of the accumulation of ions of salts on the biosynthesis of the



**Figure 7. Reduction percentage in chlorophyll content (after 30 days and 60 days of applying treatment) under increasing salinity stress**

different chlorophyll fractions. Salinity affects the strength of the forces bringing the complex pigment protein-liquid, in the chloroplast structure. As the chloroplast in membrane bound its stability is dependent on the membrane stability which under high salinity condition seldom remains intact and reduces the chlorophyll content (Edris *et al.*, 2012; Hajiboland *et al.*, 2010; Amini and Ehsnapour, 2006).

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically significant in respect of chlorophyll content (%) of plant leaves (SPAD reading) after 60 days of starting stress application (Appendix VI). The highest amount of chlorophyll content was found in interaction G<sub>2</sub>T<sub>1</sub> (95.67%) while the lowest amount of chlorophyll content was found in the interaction G<sub>1</sub>T<sub>3</sub> (31.17%) which was statistically identical with G<sub>3</sub>T<sub>3</sub> (33.47%) (Table 14).

The amount of chlorophyll content (%) of plant leaves (SPAD reading) after 60 days of starting stress application of four tomatillo genotypes varied significantly under three different salinity treatments and the amount of chlorophyll content (SPAD reading) was mainly decreased gradually with the increase of salinity treatment levels (Appendix X). The maximum reduction in the amount of chlorophyll content was observed in genotype G<sub>4</sub> (42.43%) at slightly (8 dS/m) salinity stress and in G<sub>1</sub> (60.61%) at moderately (12 dS/m) salinity stress whereas the minimum reduction was observed in genotype G<sub>2</sub> in both cases, at slightly (8 dS/m) and moderately (8 dS/m) salinity stresses (24.61% and 46.45% respectively) (Figure 7). Therefore, genotype G<sub>2</sub> might be considered as a good source of parent material as it showed the minimum reduction in chlorophyll (%) content (SPAD reading) under both at slightly (8 dS/m) and moderately (12 dS/m) salinity stress conditions.

#### **4.3 Evaluation of nutritional traits of tomatillo**

Different nutritional traits of tomatillo fruits *viz.*, fruit pH, brix percentage, titratable acid and vitamin C content were presented and discussed based on their

ANOVA, genotype, salinity treatment, genotype-treatment interaction effect and percentage of changes (increase or decrease) in these traits.

#### **4.3.1 Fruit pH**

The mean values of fruit pH for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix VII), it was observed in this experiment that statistically non-significant variation was found for fruit pH among the tomatillo genotypes. The highest value of fruit pH was found in genotype G<sub>3</sub> (4.18) whereas the lowest value of fruit pH was found in genotype G<sub>1</sub> (3.89) (Table 15).

From the result of ANOVA table (Appendix VII), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of the pH value of tomatillo fruits. The highest value of fruit pH was found in treatment T<sub>1</sub> (4.29) while the lowest value of fruit pH was found in treatment T<sub>3</sub> (3.74) (Table 16). This result showed that the value of pH in the fruit of tomatillo was decreased gradually under the increase of salinity treatment levels. The decrease in water content and turgidity of the plant under saline irrigation can increase the acidity of the fruit (Saied *et al.*, 2005). Husk tomato fruits grown under saline water irrigation showed high titratable acidity which may be attributed to the accumulation of organic acids, thus maintaining lower fruit pH (Helaly *et al.*, 2017). Similar results were also reported by Janse, 1989; Chartzoulakis, 1992; Adams, 1991; Yungfu and Dashu, 2002; Krauss *et al.*, 2006; Al-Harbi *et al.*, 2015.

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically non-significant in respect of the value of fruit pH (Appendix VII). The highest value of fruit pH was found in interaction G<sub>4</sub>T<sub>1</sub> (4.38) whereas the lowest value of fruit pH was found in the interaction G<sub>1</sub>T<sub>3</sub> (3.52) (Table 17).

**Table 15. Performance of tomatillo genotypes on fruit pH, brix percentage, titratable acid content and vitamin-C content<sup>Y</sup>**

<b>Genotype<sup>X</sup></b>	<b>Fruit pH</b>	<b>Brix percentage</b>	<b>Titratable acid content (%)</b>	<b>Vitamin-C content (mg/100 g)</b>
<b>G<sub>1</sub></b>	3.89	6.14 b	0.87 b	17.98 b
<b>G<sub>2</sub></b>	4.03	6.53 b	0.91 b	20.07 a
<b>G<sub>3</sub></b>	4.18	7.20 a	0.99 a	21.70 a
<b>G<sub>4</sub></b>	4.06	5.68 c	0.79 c	16.27 b
<b>CV %</b>	6.85	7.58	4.75	10.04
<b>LSD 0.05</b>	---	0.47	0.04	1.86

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub>

<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

**Table 16. Performance of salinity treatments on fruit pH, brix percentage, titratable acid content and vitamin-C content<sup>Y</sup>**

<b>Treatment<sup>X</sup></b>	<b>Fruit pH</b>	<b>Brix percentage</b>	<b>Titratable acid content (%)</b>	<b>Vitamin-C content (mg/100 g)</b>
<b>T<sub>1</sub> (control)</b>	4.29 a	3.81 c	0.54 c	14.36 c
<b>T<sub>2</sub> (8dS/m)</b>	4.10 b	6.53 b	0.87 b	19.41 b
<b>T<sub>3</sub> (12dS/m)</b>	3.74 c	8.83 a	1.26 a	23.24 a
<b>CV %</b>	6.85	7.58	4.75	10.04
<b>LSD 0.05</b>	0.23	0.41	0.04	1.62

<sup>X</sup>Three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub>

<sup>Y</sup>In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability



**Table 17. Interaction effect between tomatillo genotypes and salinity treatments on fruit pH, brix percentage, titratable acid content and vitamin-C content<sup>Y</sup>**

<b>Interaction<sup>X</sup></b>	<b>Fruit pH</b>	<b>Brix percentage</b>	<b>Titratable acid content (%)</b>	<b>Vitamin-C content (mg/100 g)</b>
<b>G<sub>1</sub>T<sub>1</sub></b>	4.27	3.70 gh	0.51 hi	13.40 hi
<b>G<sub>1</sub>T<sub>2</sub></b>	3.89	6.60 ef	0.85 e	18.50 def
<b>G<sub>1</sub>T<sub>3</sub></b>	3.52	8.12 c	1.25 b	22.05 bc
<b>G<sub>2</sub>T<sub>1</sub></b>	4.17	3.90 gh	0.56 h	14.75 ghi
<b>G<sub>2</sub>T<sub>2</sub></b>	4.06	6.50 ef	0.89 e	20.85 cd
<b>G<sub>2</sub>T<sub>3</sub></b>	3.87	9.20 b	1.28 ab	24.60 ab
<b>G<sub>3</sub>T<sub>1</sub></b>	4.34	4.30 g	0.64 g	17.50 efg
<b>G<sub>3</sub>T<sub>2</sub></b>	4.21	7.10 de	0.97 d	21.80 bc
<b>G<sub>3</sub>T<sub>3</sub></b>	3.99	10.21 a	1.34 a	25.80 a
<b>G<sub>4</sub>T<sub>1</sub></b>	4.38	3.33 h	0.46 i	11.80 i
<b>G<sub>4</sub>T<sub>2</sub></b>	4.23	5.90 f	0.77 f	16.50 fgh
<b>G<sub>4</sub>T<sub>3</sub></b>	3.58	7.80 cd	1.15 c	20.50 cde
<b>CV %</b>	6.85	7.58	4.75	10.04
<b>LSD 0.05</b>	---	0.82	0.07	3.23

<sup>X</sup>Four tomatillo genotypes coded from G<sub>1</sub> to G<sub>4</sub> and three salinity treatments coded from T<sub>1</sub> to T<sub>3</sub> (T<sub>1</sub>: control, T<sub>2</sub>: 8 dS/m, T<sub>3</sub>: 12 dS/m)

<sup>Y</sup>In a column means containing similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

The value of fruit pH of four tomatillo genotypes varied significantly under three different salinity treatments and the fruit pH value was mainly decreased gradually with the increase of salinity treatment levels (Appendix XI). The maximum reduction in the fruit pH value was observed in genotype G<sub>1</sub> (-8.90%) at slightly (8 dS/m) salinity stress and in G<sub>4</sub> (-18.26%) at moderately (12 dS/m) salinity stress while the minimum reduction was observed in genotype G<sub>2</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (-2.64% and -7.19% respectively) (Figure 8). The lowering of fruit pH value indicates the increase of sourness and the quality of fruits. The preference of the fruit pH value should be as per requirement.

#### **4.3.2 Brix percentage**

The mean values of brix content (%) for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix VII), it was observed in this experiment that statistically highly significant variation was found for brix content (%) among the tomatillo genotypes. The highest amount of brix content was found in genotype G<sub>3</sub> (7.20%) whereas the lowest amount of brix content was found in genotype G<sub>4</sub> (5.68%) (Table 15).

From the result of ANOVA table (Appendix VII), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatment levels; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of the brix content (%) of tomatillo fruit. The highest amount of brix content was found in treatment T<sub>3</sub> (8.83%) while the lowest amount of brix content was found in treatment T<sub>1</sub> (3.81%) (Table 16). This result showed that the amount of brix content (%) in tomatillo fruit was increased gradually under the increase of salinity treatment levels. Salinity stress can up-regulate sucrose transporter expression in source leaves and increase the activity of ADP-glucose pyrophosphorylase (AGPase) in fruits during early development stages that enhance carbohydrate accumulation (Yong-Gen *et al.*, 2009). The increase in total soluble solid of husk tomato under salinity were recorded by Shakhov, 1956

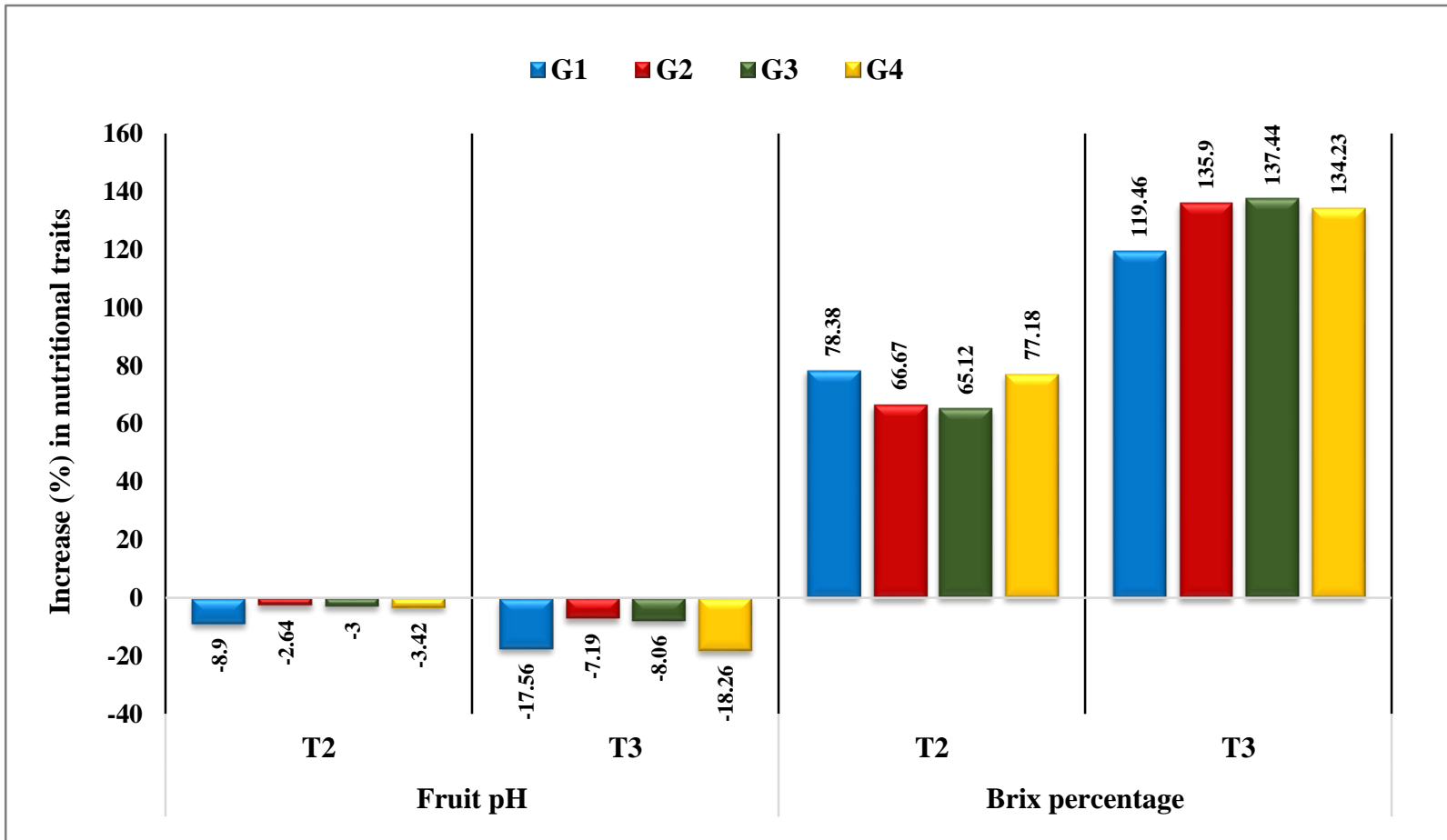


Figure 8. Increase percentage in fruit pH and brix percentage under increasing salinity stress

and he mentioned that salt ions especially the  $\text{Na}^+$  might increase the hydrophilous properties of plasma colloids that played a very important role to protect the bio-colloids and plasma from the effect of higher salinity. These observations were also in agreement with those obtained by Medhat, 2002; Fathy *et al.*, 2005; Khalil, 2006; Saito *et al.*, 2008; Flores *et al.*, 2003 and Taha *et al.*, 2011.

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically highly significant in respect of the amount of brix content (%) (Appendix VII). The highest amount of brix content was found in interaction  $G_3T_3$  (10.21%) whereas the lowest amount of brix content was found in the interaction  $G_4T_1$  (3.33%) which was statistically identical with  $G_1T_1$  (3.70%) and  $G_2T_1$  (3.90%) (Table 17).

The amount of brix content (%) of four tomatillo genotypes varied significantly under three different salinity treatments and the amount of brix content was mainly increased gradually with the increase of salinity treatment levels (Appendix XI). The maximum increase in the brix content amount was observed in genotype  $G_1$  (78.38%) at slightly (8 dS/m) salinity stress and in  $G_3$  (137.44%) at moderately (12 dS/m) salinity stress while the minimum increase was observed in genotype  $G_3$  (65.12%) at slightly (8 dS/m) salinity stress and in  $G_1$  (119.46%) at moderately (12 dS/m) salinity stress (Figure 8). Therefore, genotype  $G_1$  and  $G_3$  might be considered as good source of parent materials as these showed the maximum increase in brix percentage of tomatillo fruits under slightly (8 dS/m) and moderately (12 dS/m) salinity stress conditions respectively.

#### **4.3.3 Titratable acid content (%)**

The mean values of titratable acid content (%) for four genotypes of tomatillo under three different salinity treatments,  $T_1$  (Control),  $T_2$  (8 dS/m) and  $T_3$  (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix VII), it was observed in this experiment that statistically highly significant variation was found for titratable acid (%) content among the

tomatillo genotypes. The highest amount of titratable acid content was found in genotype G<sub>3</sub> (0.99%) whereas the lowest amount of titratable acid content was found in genotype G<sub>4</sub> (0.79%) (Table 15).

From the result of ANOVA table (Appendix VII), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatment levels; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of the titratable acid content (%) of tomatillo fruit. The highest amount of titratable acid content was found in treatment T<sub>3</sub> (1.26%) while the lowest amount of titratable acid content was found in treatment T<sub>1</sub> (0.54%) (Table 16). This result showed that the amount of titratable acid content (%) in tomatillo fruits was increased gradually under the increase of salinity treatment levels. Higher values of acidity in fruit juice from salinized plants means that the quality of the products is better than control. The decrease in water content and turgidity of the plant under saline irrigation can increase the acidity of fruits (Saied *et al.*, 2005). The increase in acidity of fruit juice as a result of salinity treatments were also reported by Janse, 1989; Chartzoulakis, 1992; Adams, 1991; Yungfu and Dashu, 2002; Krauss *et al.*, 2006; Al-Harbi *et al.*, 2015 and Helaly *et al.*, 2017.

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically highly significant in respect of the amount of titratable acid content (%) (Appendix VII). The highest amount of titratable acid content was found in interaction G<sub>3</sub>T<sub>3</sub> (1.34%) which was statistically identical with G<sub>2</sub>T<sub>3</sub> (1.28%) whereas the lowest amount of titratable acid content was found in the interaction G<sub>4</sub>T<sub>1</sub> (0.46 %) which was statistically identical with G<sub>1</sub>T<sub>1</sub> (0.51%) (Table 17).

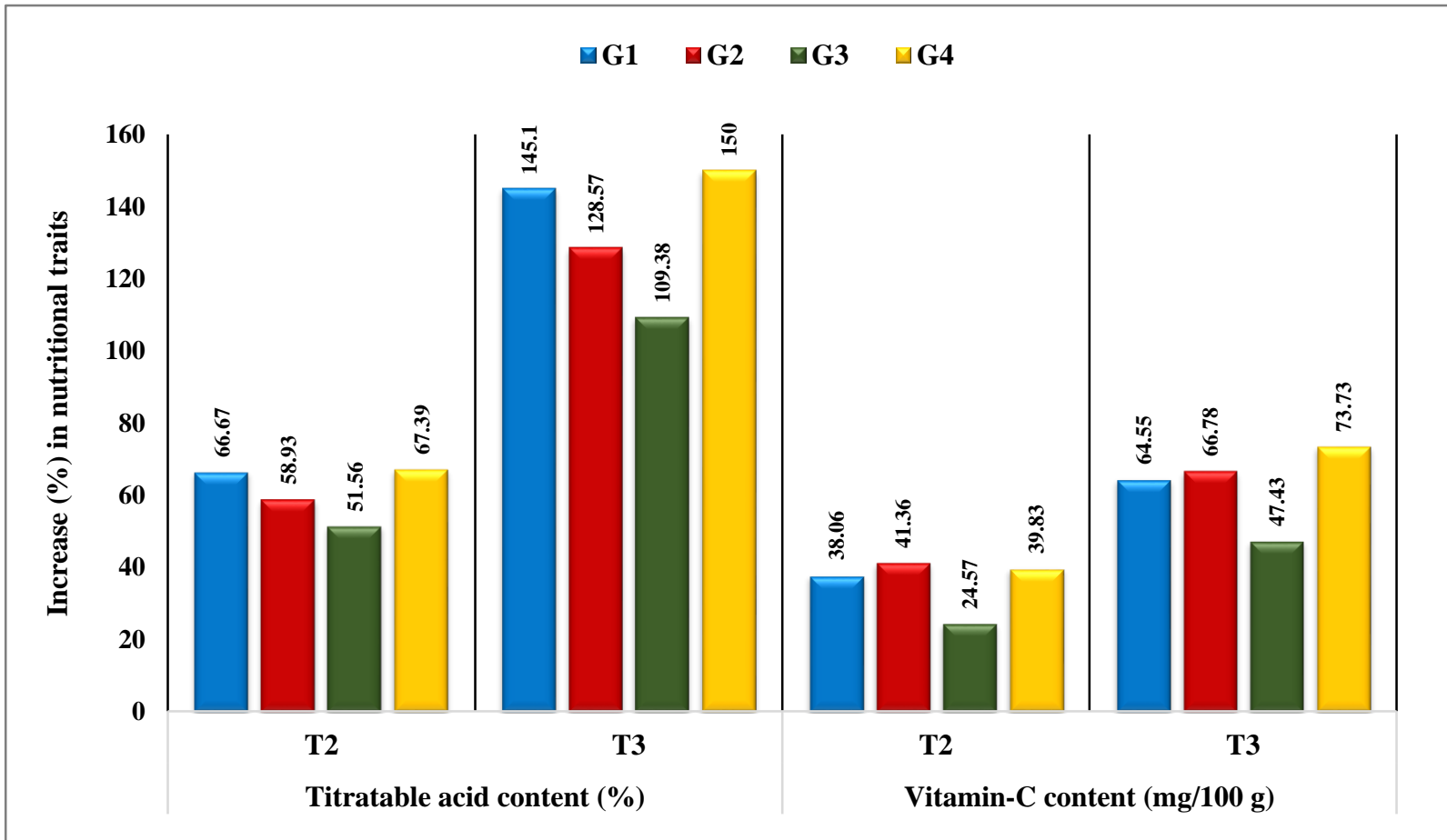
The amount of titratable acid content (%) of four tomatillo genotypes varied significantly under three different salinity treatments and the amount of titratable acid content was mainly increased gradually with the increase of salinity treatment levels (Appendix XI). The maximum increase in the titratable acid content amount was observed in genotype G<sub>4</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (67.39% and 150.00% respectively)

while the minimum increase was observed in genotype G<sub>3</sub> in both cases, at slightly (8 dS/m) and moderately (8 dS/m) salinity stresses (51.56% and 109.38% respectively) (Figure 9). Therefore, genotype G<sub>4</sub> might be considered as a good source of parent material as it showed the maximum increase in titratable acid (%) content of tomatillo fruits under both slightly (8 dS/m) and moderately (12 dS/m) salinity stress conditions.

#### **4.3.4 Vitamin-C content (mg/100 g)**

The mean values of vitamin-C content (mg/100 g) for four genotypes of tomatillo under three different salinity treatments, T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) are presented in Appendix VIII. From the result of ANOVA table (Appendix VII), it was observed in this experiment that statistically highly significant variation was found for vitamin-C content (mg/100 g) among the fruits of tomatillo genotypes. The highest amount of vitamin-C content was found in genotype G<sub>3</sub> (21.70 mg/100 g) which was statistically identical with G<sub>2</sub> (20.07 mg/100 g) whereas the lowest amount of vitamin-C content was found in genotype G<sub>4</sub> (16.27 mg/100 g) which was statistically identical with G<sub>1</sub> (17.98 mg/100 g) (Table 15).

From the result of ANOVA table (Appendix VII), statistically highly significant variation was found in tomatillo genotypes exposed to three different salinity treatments; T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m) and T<sub>3</sub> (12 dS/m) in respect of the vitamin-C content (mg/100 g) of tomatillo fruit. The highest amount of vitamin-C content was found in treatment T<sub>3</sub> (23.24 mg/100 g) while the lowest amount of vitamin-C content was found in treatment T<sub>1</sub> (14.36 mg/100 g) (Table 16). This result showed that the amount of vitamin-C content (mg/100 g) in tomatillo fruits was increased gradually under the increase of salinity treatment levels. The increase in salinity concentration in the nutrient solutions improves the fruit quality by increasing the sugar, organic acid and antioxidants content like vitamin-C (L-ascorbic acid) (Flores *et al.*, 2003; Cuartero *et al.*, 2003; De Pascale *et al.*, 2001; Petersen *et al.*, 1998).



**Figure 9. Increase percentage in titratable acid and vitamin-C content under increasing salinity stress**

The interaction effect between four tomatillo genotypes and three salinity treatments was found statistically significant in respect of the amount of vitamin-C content (mg/100 g) in tomatillo fruits (Appendix VII). The highest amount of vitamin-C content was found in interaction G<sub>3</sub>T<sub>3</sub> (25.80 mg/100 g) which was statistically identical with G<sub>2</sub>T<sub>3</sub> (24.60 mg/100 g) whereas the lowest amount of vitamin-C content was found in the interaction G<sub>4</sub>T<sub>1</sub> (11.80 mg/100 g) which was statistically identical with G<sub>1</sub>T<sub>1</sub> (13.40 mg/100 g) and G<sub>2</sub>T<sub>1</sub> (14.75 mg/100 g) (Table 17).

The amount of vitamin-C content (mg/100 g) of four tomatillo genotypes varied significantly under three different salinity treatments and the amount of vitamin-C content was mainly increased gradually with the increase of salinity treatment levels (Appendix XI). The maximum increase in the vitamin-C content amount was observed in genotype G<sub>2</sub> (41.37%) at slightly (8 dS/m) salinity stress and in G<sub>4</sub> (73.73%) at moderately (12 dS/m) salinity stress whereas the minimum increase was observed in genotype G<sub>3</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (24.57% and 47.43% respectively) (Figure 9). Therefore, genotype G<sub>2</sub> and G<sub>4</sub> might be considered as good source of parent materials as these showed the maximum increase in vitamin-C (mg/100 g) content of tomatillo fruits under slightly (8 dS/m) and moderately (12 dS/m) salinity stress conditions respectively.



## CHAPTER V

### SUMMARY AND CONCLUSION

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Tomatillo is an important crop of Mexico, and nowadays have been introduced and appreciated across the world. It belongs to the Solanaceae family and recently has been introduced in our country. After being introduced, this new crop species has shown remarkable increase in yield than in its origin and thus drawn huge interest. A large amount of area of the southern region of Bangladesh has still remained uncultivated due to high level of soil salinity and it is increasing rapidly due to global climate change. On the other hand, the rapid growth of population needs an increase in food production. To overcome this problem, cultivation of modern high yielding salt tolerant variety and to bring the uncultivable saline lands under cultivation is apparent. Thus, the screening and selection as well as the introduction and development of new salt tolerant genotypes and crops are major goal of global agriculture now-a-days. As a newly introduced crop of our country, tomatillo was taken to consideration for this experiment to observe its tolerance capacity to salinity stress levels and whether it is possible to recommend this crop for cultivation in our salinity affected southern regions.

A pot experiment was conducted to observe the performance of four tomatillo genotypes under three different salinity treatment levels. The experiment was conducted in the area just beside the net house of Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh, during the months of November, 2017 to March, 2018. A two factorial experiment was conducted which included four tomatillo genotypes (Factor A) *viz.* G<sub>1</sub> (SAU tomatillo 1), G<sub>2</sub> (SAU tomatillo 2), G<sub>3</sub> (PI003), G<sub>4</sub> (PI004) and three different salinity treatments (Factor B) *viz.* T<sub>1</sub> (Control), T<sub>2</sub> (8 dS/m), T<sub>3</sub> (12 dS/m) and that was outlined in Completely Randomized Design (CRD) with three replications. The collected data were statistically analyzed for the evaluation of salinity treatment levels as well as for detecting the suitable

tomatillo genotypes to grow under higher salinity levels (sodium chloride, NaCl).

The observed results of the current study showed that, salinity treatment levels significantly influenced different agromorphological, physiological and nutritional traits of tomatillo genotypes at all stages of growth and development. Observation of these traits plays important role in selecting suitable genotypes of crops for future breeding purpose. Among the interaction effect of different agromorphological traits of tomatillo, earliest flowering was observed in the interaction G<sub>1</sub>T<sub>3</sub> (29.67 days). The tallest plant was found in the interaction G<sub>2</sub>T<sub>1</sub> (74.33 cm) and G<sub>4</sub>T<sub>3</sub> (69.33 cm) whereas the shortest plant was found from G<sub>3</sub>T<sub>2</sub> (63.33 cm). Earliest harvesting period was observed in interaction G<sub>4</sub>T<sub>3</sub> (76.00 days), G<sub>1</sub>T<sub>3</sub> (79.33 days) and G<sub>2</sub>T<sub>3</sub> (80.33 days). The maximum number of fruits was obtained from the interaction G<sub>3</sub>T<sub>1</sub> (21.33 fruits/plant). The longest as well as the widest fruit was recorded from the interaction G<sub>1</sub>T<sub>1</sub> (34.01 mm length and 38.81 mm diameter respectively). The maximum average fruit weight was obtained from the interaction G<sub>1</sub>T<sub>1</sub> (35.29 g) and the maximum fruit yield was obtained from the interaction G<sub>3</sub>T<sub>1</sub> (0.708 kg/plant). In case of studying different physiological traits of tomatillo, the minimum amount of indigenous Na<sup>+</sup> content was found in interaction G<sub>1</sub>T<sub>1</sub> (0.91%), G<sub>3</sub>T<sub>1</sub> (0.97%) and G<sub>2</sub>T<sub>1</sub> (1.02%) and the maximum indigenous K<sup>+</sup> ion content was found in interaction G<sub>1</sub>T<sub>1</sub> (4.12%) and in G<sub>3</sub>T<sub>1</sub> (3.97%). Considering the amount of stress protein proline content, the maximum amount was observed in interaction G<sub>4</sub>T<sub>3</sub> (4553.70 µg/g). The maximum area of leaf was observed in the interaction G<sub>1</sub>T<sub>1</sub> (23.23 cm<sup>2</sup>). The maximum amount of chlorophyll content in tomatillo leaves was found in the interaction G<sub>2</sub>T<sub>1</sub> in both cases, 30 days and 60 days after starting the application of salinity treatments (102.43% and 95.67% respectively). In studying the interaction effect among different fruit parameters of tomatillo, the maximum value of fruit pH was obtained from the interaction G<sub>4</sub>T<sub>1</sub> (4.38) while the minimum value of fruit pH was found in interaction G<sub>1</sub>T<sub>3</sub> (3.52). Brix (%), titratable acid and vitamin-C content in tomatillo fruits were maximum in interaction G<sub>3</sub>T<sub>3</sub> (10.21%, 1.34% and 25.80 mg/100 g respectively).

In the observed results of the present study, the maximum reduction in days to first flowering after transplanting was found in genotype G<sub>4</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stress levels (8.33% and 20.83% respectively). Plant height (cm) was found to be increased in genotype G<sub>4</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stress levels (-1.23% and -2.45% respectively). The maximum reduction in days to maturity was found in genotype G<sub>1</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity treatment levels (8.04% and 16.78% respectively). Considering the yield parameters, the number of fruits per plant showed the minimum reduction in genotype G<sub>4</sub> (12.76%) at moderately (12 dS/m) salinity level and the number was increased (-2.11%) at slightly (8 dS/m) salinity. Average fruit length (mm) and diameter (mm) showed the minimum reduction in genotype G<sub>3</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity treatment levels (for length, 12.06% and 28.78% whereas for diameter, 9.57% and 29.09% respectively). Average fruit weight (g) per plant showed the minimum reduction in genotype G<sub>1</sub> (13.49%) at slightly (8 dS/m) salinity and in G<sub>3</sub> (25.99%) at moderately (12 dS/m) salinity treatment. Yield per plant (kg) showed the minimum reduction in genotype G<sub>4</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (15.28% and 43.23% respectively).

Indigenous ion content of plant is an important indicator of salinity tolerance. The minimum increase in indigenous Na<sup>+</sup> (%) content was found in genotype G<sub>3</sub> (-39.18%) and in G<sub>4</sub> (-82.41%) at slightly (8 dS/m) and moderately (12 dS/m) salinity treatments respectively. The minimum reduction in indigenous K<sup>+</sup> (%) content was observed in genotype G<sub>4</sub> (14.70%) at slightly (8 dS/m) salinity and in G<sub>1</sub> (34.95%) at moderately (12 dS/m) salinity treatment. The proline content was found to be increased under high salinity conditions and the maximum increase was found in genotype G<sub>1</sub> under both slightly (8 dS/m) and moderately (12 dS/m) salinity treatments (-140.71% and -374.29% respectively). The minimum reduction in leaf area index was observed in genotype G<sub>3</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity treatment levels (15.90% and 21.41% respectively). The minimum reduction in chlorophyll content (%)

was observed in genotype G<sub>2</sub> at both slightly (8 dS/m) and moderately (12 dS/m) salinity stress levels for both cases, after 30 days (22.68% and 42.33% respectively) and 60 days (24.61% and 46.45% respectively) of starting the application of salinity treatment. The nutritional traits of tomatillo fruits were increased under higher salinity treatment levels and the increase indicated the improved quality of tomatillo fruits. The maximum increase in %Brix was observed in genotype G<sub>1</sub> (78.38%) at slightly (8 dS/m) salinity and in G<sub>3</sub> (137.44%) at moderately (12 dS/m) salinity treatment. The maximum increase in titratable acid content (%) was observed in genotype G<sub>4</sub> in both cases, at slightly (8 dS/m) and moderately (12 dS/m) salinity stresses (67.39% and 150.00% respectively). The maximum increase vitamin-C content (mg/100 g) was observed in genotype G<sub>2</sub> (41.36%) at slightly (8 dS/m) salinity and in G<sub>4</sub> (73.73%) at moderately (12 dS/m) salinity treatment level.

Based on the above research findings, the following conclusions and recommendations can be drawn for this executed study:

- Genotype G<sub>1</sub> and G<sub>3</sub> showed minimum reduction in the parameters *viz.*, fruit numbers, fruits length, fruit diameter, fruit weight and yield under slightly and moderately salinity stresses. These two genotypes also showed minimum reduction in leaf area index under slight to moderate salinity. They also showed minimum increase in indigenous Na<sup>+</sup> and maximum increase in K<sup>+</sup> content. As these two ion content are the salt tolerance indicator and with the decrease of indigenous Na<sup>+</sup> content, other agromorphogenic parameters showed upsurge, thus these two genotypes could be recommended for cultivation (G<sub>1</sub>) and further trial (G<sub>3</sub>) in the Southern region of Bangladesh.
- Genotype G<sub>1</sub> showed maximum increase in proline content under slightly and moderately salinity stresses. As proline is a stress tolerance indicator under salinity and with the increase of proline, yield and its contributing traits were also increased, thus G<sub>1</sub> could also be recommended to the farmers of coastal belt of Bangladesh.

- Genotype G<sub>3</sub> along with G<sub>4</sub> and G<sub>1</sub> showed maximum increase in %Brix, titratable acid and vitamin-C content under slightly and moderately salinity treatments, thus indicating G<sub>3</sub> is better for fruit quality as well as for yield and yield components and could be recommended for further trial.
- Maximum reduction in days to maturity was observed in G<sub>1</sub> followed by G<sub>4</sub> under slightly and moderately salinity stresses, thus indicating it's owing of short duration behavior and could be served as parent materials for further hybridization or genetic transformation program.
- Minimum reduction in chlorophyll content was observed in G<sub>2</sub> under both slightly and moderately salinity stresses, thus indicating it could be served as parent material for further hybridization program.

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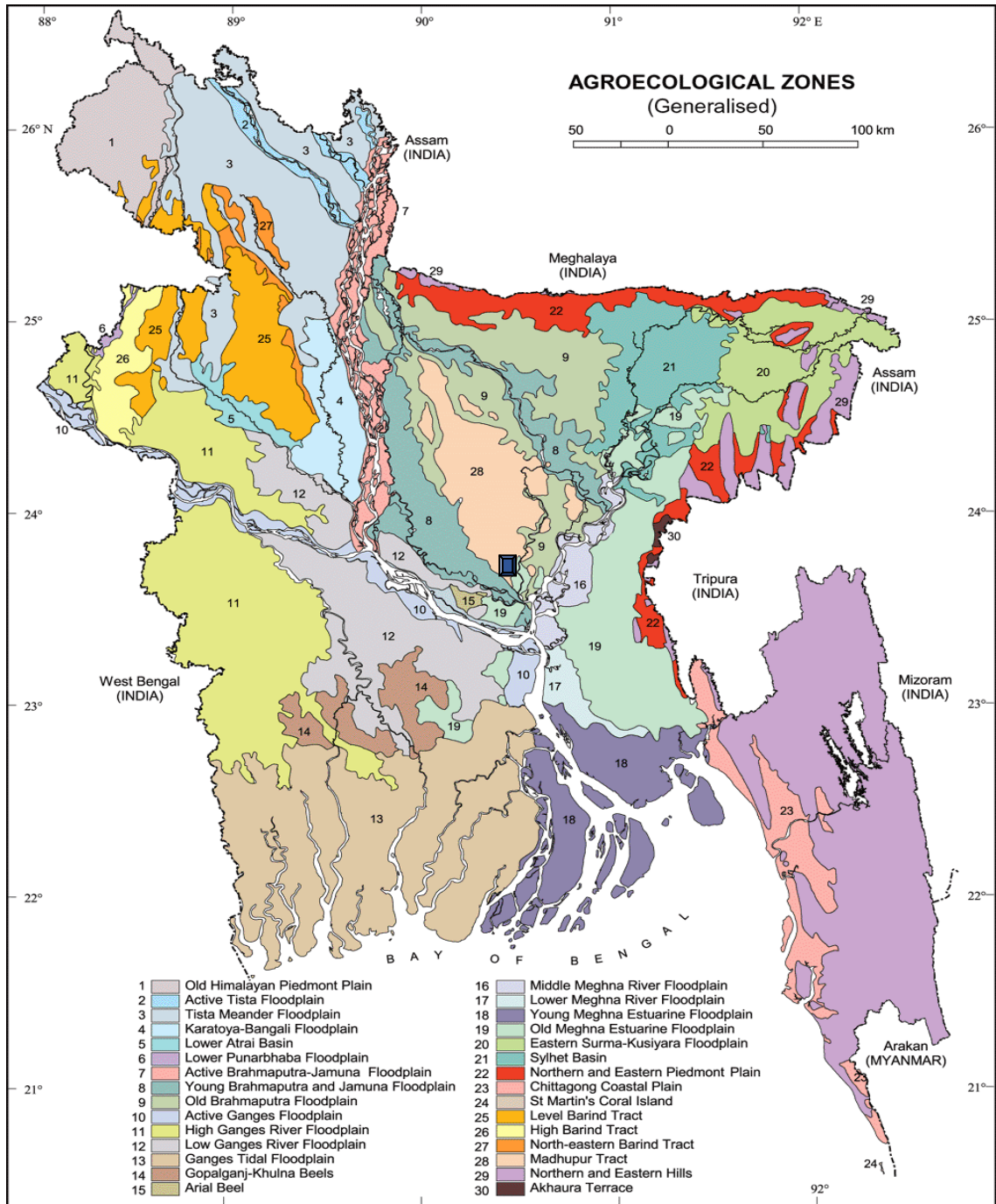
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# APPENDICES

## Appendix I. Map showing the experimental site under the executed study



The experimental site under the executed study

**Appendix II. Monthly records of air temperature, relative humidity, rainfall and sunshine hours during the period from October, 2017 to March, 2018**

Month	Year	Monthly average air temperature (°C)			Average relative humidity (%)	Total rainfall (mm)	Total sunshine (hours)
		Maximum	Minimum	Mean			
Oct	2017	35	18.89	28.89	80	4.0	195.50
Nov	2017	33.89	15	26.11	74	Trace	218.50
Dec.	2017	28.89	12.78	22.22	79	4.0	212.00
Jan.	2018	27.22	7.22	18.89	75	3.0	216.50
Feb.	2018	33.89	12.78	24.44	66	Trace	225.50
Mar.	2018	35	15	28.89	63	Trace	235.50

**Source:** Bangladesh Meteorological Department (Climate and Weather division), Agargaon, Dhaka-1212

**Appendix III. The mechanical and chemical characteristics of soil (0-15 cm depth) of the experimental site as observed prior to experimentation**

**Mechanical composition:**

<b>Particle size</b>	<b>Constitution</b>
Sand	40%
Silt	40%
Clay	20%
Texture	Loamy

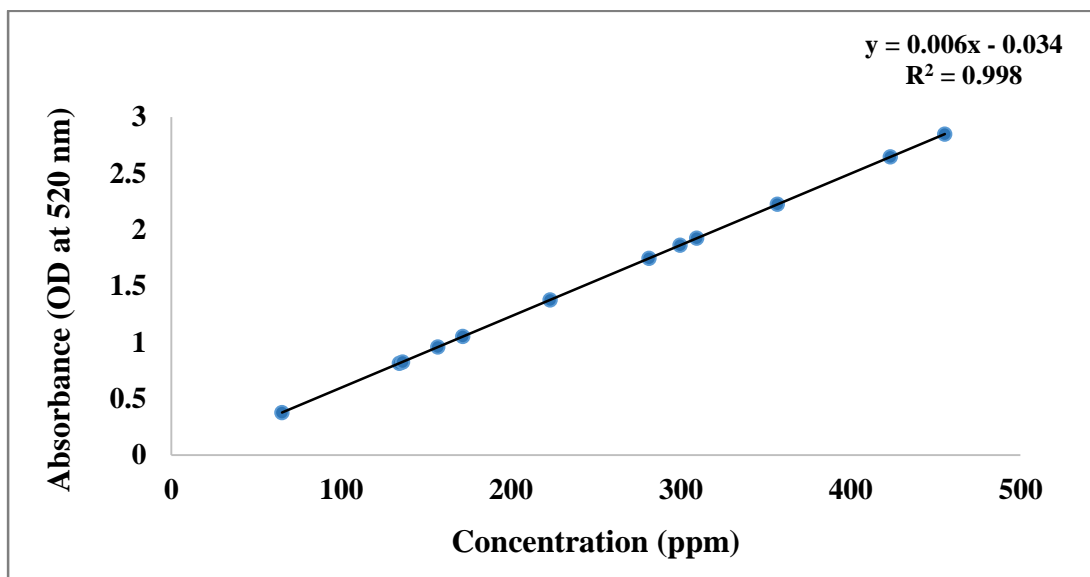
**Chemical composition:**

<b>Soil characters</b>	<b>Value</b>
Organic matter	1.44%
Potassium (K)	0.15 meq/100 g soil
Calcium (Ca)	3.60 meq/100 g soil
Magnesium (Mg)	1.00 meq/100 g soil
Total nitrogen (N)	0.072
Phosphorus (P)	22.08 µg/g soil
Sulphur (S)	25.98 µg/g soil
Boron (B)	0.48 µg/g soil
Copper (Cu)	3.54 µg/g soil
Iron (Fe)	262.6 µg/g soil
Manganese (Mn)	164 µg/g soil
Zinc (Zn)	3.32 µg/g soil

**Source:** Soil Resources Development Institute (SRDI), Khamarbari, Dhaka



#### Appendix IV. Proline standard curve



**Appendix V. Analysis of variance of the data on agromorphogenic traits of tomatillo**

Sources of variation	Degrees of freedom	Mean Sum Square of			
		Days to first flowering	Plant height (cm)	Days to maturity	No. of fruits per plant
<b>Factor A (genotype)</b>	3	21.741*	33.785*	79.185**	77.657**
<b>Factor B (salinity)</b>	2	128.111**	38.715*	526.750**	75.028**
<b>A × B</b>	6	1.741 <sup>NS</sup>	27.549*	40.935*	3.880**
<b>Error</b>	22	5.679	9.967	10.303	0.498

\*\*Significance at 0.01 level of probability

\*Significance at 0.05 level of probability

<sup>NS</sup>Non-significance

**Appendix V (cont'd).**

<b>Sources of variation</b>	<b>Degrees of freedom</b>	<b>Mean Sum Square of</b>			
		<b>Average fruit length (mm)</b>	<b>Average fruit diameter (mm)</b>	<b>Average fruit weight (g)</b>	<b>Yield per plant (kg)</b>
<b>Factor A (genotype)</b>	3	210.711**	294.321**	679.396**	0.242**
<b>Factor B (salinity)</b>	2	332.202**	387.963**	174.807**	0.164**
<b>A × B</b>	6	9.152**	16.553**	2.763**	0.012**
<b>Error</b>	22	1.613	1.426	0.482	0.001

\*\*Significance at 0.01 level of probability

\*Significance at 0.05 level of probability

<sup>NS</sup>Non-significance

**Appendix VI. Analysis of variance of the data on physiological traits of tomatillo**

Sources of variation	Degrees of freedom	Mean Sum Square of		
		Na <sup>+</sup> content (%)	K <sup>+</sup> content (%)	Proline content (µg/g)
<b>Factor A (genotype)</b>	3	0.062**	2.075**	9477.870**
<b>Factor B (salinity)</b>	2	2.245**	7.442**	5786.620**
<b>A × B</b>	6	0.018*	0.119*	1277.210**
<b>Error</b>	22	0.004	0.038	382.880

\*\*Significance at 0.01 level of probability

\*Significance at 0.05 level of probability

<sup>NS</sup>Non-significance

**Appendix VI (cont'd).**

Sources of variation	Degrees of freedom	Mean Sum Square of		
		Leaf area index (cm <sup>2</sup> )	Chlorophyll content (%)	
			30 days after salinity stress	60 days after salinity stress
<b>Factor A (genotype)</b>	3	25.398**	719.190**	210.440**
<b>Factor B (salinity)</b>	2	95.881**	6349.490**	344.080**
<b>A × B</b>	6	1.755*	54.750**	51.680*
<b>Error</b>	22	0.483	14.470	16.500

\*\*Significance at 0.01 level of probability

\*Significance at 0.05 level of probability

<sup>NS</sup>Non-significance

**Appendix VII. Analysis of variance of the data on nutritional traits of tomatillo**

Sources of variation	Degrees of freedom	Mean Sum Square of			
		Fruit pH	Brix percentage	Titrateable acid content (%)	Vitamin-C content (mg/100 g)
<b>Factor A (genotype)</b>	3	0.125 <sup>NS</sup>	3.751**	0.058**	50.797**
<b>Factor B (salinity)</b>	2	0.935**	75.871**	1.526**	237.798**
<b>A × B</b>	6	0.064 <sup>NS</sup>	6.531**	0.225**	13.549*
<b>Error</b>	22	0.077	0.234	0.002	3.639

\*\*Significance at 0.01 level of probability

\*Significance at 0.05 level of probability

<sup>NS</sup>Non-significance

**Appendix VIII. Mean values of different agromorphogenic, physiological and nutritional traits of tomatillo under control and salinity stress condition**

	<b>Days to first flowering</b>	<b>Plant height (cm)</b>	<b>Days to maturity</b>	<b>No. of fruits per plant</b>	<b>Average fruit length (mm)</b>	<b>Average fruit diameter (mm)</b>
<b>G<sub>1</sub>T<sub>1</sub></b>	36.33	66.00	95.33	16.33	34.01	38.81
<b>G<sub>1</sub>T<sub>2</sub></b>	33.67	64.00	87.67	13.67	24.33	28.23
<b>G<sub>1</sub>T<sub>3</sub></b>	29.67	65.00	79.33	9.67	18.74	21.51
<b>G<sub>2</sub>T<sub>1</sub></b>	37.67	74.33	92.67	13.33	24.85	30.07
<b>G<sub>2</sub>T<sub>2</sub></b>	35.67	64.50	87.00	11.67	20.26	22.58
<b>G<sub>2</sub>T<sub>3</sub></b>	31.67	63.83	80.33	9.00	15.50	20.18
<b>G<sub>3</sub>T<sub>1</sub></b>	35.33	66.50	96.00	21.33	29.43	34.99
<b>G<sub>3</sub>T<sub>2</sub></b>	32.67	63.33	90.00	18.67	25.88	31.64
<b>G<sub>3</sub>T<sub>3</sub></b>	30.33	67.17	85.67	14.67	20.96	24.81
<b>G<sub>4</sub>T<sub>1</sub></b>	40.00	67.67	90.33	15.67	19.89	21.89
<b>G<sub>4</sub>T<sub>2</sub></b>	36.67	68.50	83.67	16.00	15.79	18.39
<b>G<sub>4</sub>T<sub>3</sub></b>	31.67	69.33	76.00	13.67	10.89	13.83

**Appendix VIII (cont'd).**

	<b>Average fruit weight (g)</b>	<b>Yield per plant (kg)</b>	<b>Na<sup>+</sup> content (%)</b>	<b>K<sup>+</sup> content (%)</b>	<b>Proline content (µg/g)</b>	<b>Leaf area index (cm<sup>2</sup>)</b>
<b>G<sub>1</sub>T<sub>1</sub></b>	35.29	0.576	0.91	4.12	652.20	23.23
<b>G<sub>1</sub>T<sub>2</sub></b>	30.53	0.417	1.39	3.42	1569.90	18.90
<b>G<sub>1</sub>T<sub>3</sub></b>	25.88	0.250	1.74	2.68	3093.30	15.70
<b>G<sub>2</sub>T<sub>1</sub></b>	22.25	0.297	1.02	3.58	1342.30	18.83
<b>G<sub>2</sub>T<sub>2</sub></b>	18.92	0.221	1.46	2.32	2230.50	15.45
<b>G<sub>2</sub>T<sub>3</sub></b>	14.85	0.134	1.91	1.75	3569.30	13.70
<b>G<sub>3</sub>T<sub>1</sub></b>	33.17	0.708	0.97	3.97	1361.50	18.87
<b>G<sub>3</sub>T<sub>2</sub></b>	28.41	0.530	1.35	3.23	2814.00	15.87
<b>G<sub>3</sub>T<sub>3</sub></b>	24.55	0.360	1.82	2.51	4233.80	14.83
<b>G<sub>4</sub>T<sub>1</sub></b>	14.64	0.229	1.08	3.13	1717.20	18.43
<b>G<sub>4</sub>T<sub>2</sub></b>	12.11	0.194	1.54	2.67	2995.70	15.47
<b>G<sub>4</sub>T<sub>3</sub></b>	9.54	0.130	1.97	1.56	4553.70	12.70



**Appendix VIII (cont'd).**

	<b>Chlorophyll content (%)</b>		<b>Fruit pH</b>	<b>Brix percentage</b>	<b>Titrateable acid content (%)</b>	<b>Vitamin-C content (mg/100 g)</b>
	<b>30 days after stress</b>	<b>60 days after stress</b>				
<b>G<sub>1</sub>T<sub>1</sub></b>	85.47	79.13	4.27	3.70	0.51	13.40
<b>G<sub>1</sub>T<sub>2</sub></b>	63.90	57.33	3.89	6.60	0.85	18.50
<b>G<sub>1</sub>T<sub>3</sub></b>	36.17	31.17	3.52	8.12	1.25	22.05
<b>G<sub>2</sub>T<sub>1</sub></b>	102.43	95.67	4.17	3.90	0.56	14.75
<b>G<sub>2</sub>T<sub>2</sub></b>	79.20	72.13	4.06	6.50	0.89	20.85
<b>G<sub>2</sub>T<sub>3</sub></b>	59.07	51.23	3.87	9.20	1.28	24.60
<b>G<sub>3</sub>T<sub>1</sub></b>	85.93	80.50	4.34	4.30	0.64	17.50
<b>G<sub>3</sub>T<sub>2</sub></b>	62.23	57.20	4.21	7.10	0.97	21.80
<b>G<sub>3</sub>T<sub>3</sub></b>	39.83	33.47	3.99	10.21	1.34	25.80
<b>G<sub>4</sub>T<sub>1</sub></b>	89.37	83.20	4.38	3.33	0.46	11.80
<b>G<sub>4</sub>T<sub>2</sub></b>	53.77	47.90	4.23	5.90	0.77	16.50
<b>G<sub>4</sub>T<sub>3</sub></b>	44.67	39.23	3.58	7.80	1.15	20.50

**Appendix IX. Reduction percentage in agromorphogenic traits of tomatillo under increasing salinity stress**

	Days to first flowering		Plant height (cm)		Days to maturity		No. of fruits per plant	
	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>
<b>G<sub>1</sub></b>	7.32	18.33	3.03	1.52	8.04	16.78	16.29	40.78
<b>G<sub>2</sub></b>	5.31	15.93	13.22	14.13	6.12	13.32	12.45	32.48
<b>G<sub>3</sub></b>	7.53	14.15	4.77	-1.01	6.25	10.76	12.47	31.22
<b>G<sub>4</sub></b>	8.33	20.83	-1.23	-2.45	7.37	15.86	-2.11	12.76

**Appendix IX (cont'd).**

	Average fruit length (mm)		Average fruit diameter (mm)		Average fruit weight (g)		Yield per plant (kg)	
	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>
<b>G<sub>1</sub></b>	28.46	44.90	27.26	44.58	13.49	26.66	27.60	56.60
<b>G<sub>2</sub></b>	18.47	37.63	24.91	32.89	14.97	33.26	25.59	54.88
<b>G<sub>3</sub></b>	12.06	28.78	9.57	29.09	14.35	25.99	25.14	49.15
<b>G<sub>4</sub></b>	20.61	45.25	15.99	36.82	17.28	34.84	15.28	43.23

**Appendix X. Reduction percentage in physiological traits of tomatillo under increasing salinity stress**

	<b>Na<sup>+</sup> content (%) (Increasing trait)</b>		<b>K<sup>+</sup> content (%)</b>		<b>Proline content (µg/g) (Increasing trait)</b>	
	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>
<b>G<sub>1</sub></b>	-52.75	-91.21	16.99	34.95	-140.71	-374.29
<b>G<sub>2</sub></b>	-43.14	-87.25	35.20	51.12	-66.17	-165.91
<b>G<sub>3</sub></b>	-39.18	-87.63	18.64	36.78	-106.68	-210.97
<b>G<sub>4</sub></b>	-42.59	-82.41	14.70	50.16	-74.45	-165.18

**Appendix X (cont'd).**

	Leaf area index (cm <sup>2</sup> )		Chlorophyll content (%)			
			30 days after stress		60 days after stress	
	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>
<b>G<sub>1</sub></b>	18.64	32.41	25.24	57.68	27.55	60.61
<b>G<sub>2</sub></b>	17.95	27.24	22.68	42.33	24.61	46.45
<b>G<sub>3</sub></b>	15.90	21.41	27.58	53.65	28.94	58.42
<b>G<sub>4</sub></b>	16.06	31.09	39.83	50.02	42.43	52.85

**Appendix XI. Increase percentage in nutritional traits of tomatillo under increasing salinity stress**

	Fruit pH (Reducing trait)		Brix percentage		Titratable acid content (%)		Vitamin-C content (mg/100 g)	
	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	T <sub>3</sub>
<b>G<sub>1</sub></b>	-8.90	-17.56	78.38	119.46	66.67	145.10	38.06	64.55
<b>G<sub>2</sub></b>	-2.64	-7.19	66.67	135.90	58.93	128.57	41.36	66.78
<b>G<sub>3</sub></b>	-3.00	-8.06	65.12	137.44	51.56	109.38	24.57	47.43
<b>G<sub>4</sub></b>	-3.42	-18.26	78.18	134.23	67.39	150.00	39.83	73.73