# EVALUATION OF TOMATILLO (*Physalis ixocarpa* Brot./*Physalis philadelphica*) GENOTYPES AGAINST DROUGHT

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# *CERTIFICATE*

*This is to certify that thesis entitled, "Evaluation of tomatillo (Physalis ixocarpa Brot./Physalis philadelphica) genotypes based on agromorphogenic, physiological, antioxidant and nutritional traits against drought" 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 Sharmin Sultana, Registration No.: 12-04749 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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**Dated: June, 2018**
**Place: Dhaka, Bangladesh**

**(Prof. Dr. Md. Ashaduzzaman Siddikee)**
**Supervisor**

***Dedicated***
***to***
***My Beloved***
***Parents, Teachers***
***and***
***Brother Taibul Islam***

## Some commonly used abbreviations

| Full word | Abbreviations | Full word | Abbreviations |
|---|---|---|---|
| Agricultural | *Agril.* | Leaf area index | LAI |
| Agriculture | *Agric.* | Milligram per liter | mg/L |
| And others | *et al.* | Milligram (s) | mg |
| Applied | *App.* | Millimeter | mm |
| Bangladesh Agricultural Research Institute | BARI | Milliliter | mL |
| | | Microgram per gram | µg/g |
| | | Natural | *Nat.* |
| Bangladesh Bureau of Statistics | BBS | Negative logarithm of hydrogen ion concentration ($-\log[H^+]$) | pH |
| Biochemical | *Biochem.* | | |
| Biological | *Biol.* | | |
| Biology | *Biol.* | Nature Genetics | *Nat. Genet.* |
| Biotechnology | *Biotechnol.* | Number | No. |
| Centimeter | cm | Nutrition | *Nutr.* |
| Completely randomized design | CRD | Percentage | % |
| | | Pharmaceutical | *Pharm.* |
| Days after sowing | DAS | Particular page | p. |
| Days after transplanting | DAT | Particular pages | pp. |
| | | Physiology | *Physiol.* |
| Degree Celsius | °C | Parts per million | ppm |
| Environment | *Environ.* | Relative water content | RWC |
| Ecology | *Ecol.* | | |
| Etcetera | etc. | Review | *Rev.* |
| Food and Agriculture Organization | FAO | Research and Resource | *Res.* |
| Food Chemistry | *Food Chem.* | Science | *Sci.* |
| Genetics | *Genet.* | Serial | Sl. |
| Gram | g | Soil Resource Development Institute | SRDI |
| Gram per liter | g/L | | |
| Horticulture | *Hort.* | Technology | *Technol.* |
| International | *Intl.* | That is | i.e. |
| Journal | *J.* | Ton per hectare | t/ha |
| Kilogram | Kg | Total soluble solid | TSS |
| Least Significant Difference | LSD | United States of America | U.S.A. |
| Liter | L | Videlicet (namely) | viz. |

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**EVALUATION OF TOMATILLO (*Physalis ixocarpa* Brot./*Physalis philadelphica*) GENOTYPES AGAINST DROUGHT**

**BY**

**SHARMIN SULTANA**

## ABSTRACT

A pot experiment was conducted near the net house of the Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, during the months of October 2017 to March 2018 to observe the performances of four tomatillo genotypes under three different drought treatments. Two factorial experiment including four tomatillo genotypes *viz*., $G_1$ (SAU Tomatillo 1), $G_2$ (PI003), $G_3$ (SAU Tomatillo 2) and $G_4$ (PI004) and three drought treatments *viz*., $T_1$ (Control), $T_2$ (30 days withholding of water/moderate stress) and $T_3$ (45 days withholding of water/severe stress) were outlined in completely randomized design (CRD) with five replications. Days to first flowering, days to maturity, plant height (cm), number of fruits per plant, average fruit weight per plant (g), leaf length (cm), leaf width (cm), leaf length × width ($cm^2$), leaf length/width, number of seeds per fruit, average fruit length (cm), average fruit diameter (cm), yield per plant (kg), relative water content, RWC (%), proline content (µg/g), Brix (%) and Vitamin C content were studied in the experiment. The results showed that both the tomatillo genotypes and drought treatments had significant influence independently and dependently on agromorphogenic, physiological, antioxidant and nutritional traits of tomatillo plant. Most of the traits responded negatively as the drought level increased except days to first flowering, days to maturity, leaf L/W, proline content (µg/g) and Brix (%). Among the genotypes, $G_1$ could be cultivated at moderate drought condition for early harvesting, the highest average fruit weight, maximum yield, maximum proline content and maximum Brix (%) content. Regarding antioxidant and nutritional traits such as for Vitamin C content, $G_3$ could be recommended for moderate as well as severe drought stress regions in Bangladesh. These genotypes ($G_1$ and $G_3$) could also be used in future hybridization programs.

# CHAPTER I

# INTRODUCTION

Drought is a natural frequent and complex phenomenon that is a condition of dry weather, along with inadequate rainfall. Although an increase in temperature is beneficial for crop productivity in some cooler regions of the world, drought still significantly reduces national cereal production by 9-10% on a global scale (Lesk *et al.*, 2016) via negative effects on plant growth, physiology, and grain development (Fahad *et al.*, 2017; Matiu *et al.*, 2017; Farooq *et al.*, 2014). It happens when the rate of evaporation and transpiration go beyond the rate of rainfall for a specific time period over an area. Due to global warming and climate change, the occurrence and severity of drought has increased in many parts of the world (He *et al.*, 2013). It is predicted that the global mean temperature will increase 0.2 ℃ per decade, but such a temperature rise will vary regionally (Anonymous, 2007). The latest prediction indicates that worldwide temperatures will increase between 0.3 ℃ and4.8 ℃ by the end of twenty-first century (Anonymous, 2014a; Hartmann *et al.*, 2013). Bangladesh is one of the disaster prone countries in the world. Like flood, cyclones, coastal erosion, sea level rise, salinity intrusion and storm surge, drought is also a major disaster in Bangladesh. Some studies indicate that the rate of warming in Bangladesh is higher than the present rate of global warming and it is predicted to continue warming over the next few decades (Rahman and Lateh, 2016; Shahid, 2010; Ahmad and Warrick, 1996), which can increase water demand and thus drought severity in the country. Natural disasters, particularly meteorological disasters can cause immense losses of approximately 85% (Sun *et al.*, 2014). Previously, Bangladesh has experienced different degrees of drought that have mainly affected agricultural land, resulting in the loss of huge food grains. Moreover, it affects the socio-environment and development activities of the country (Shahid and Behrawan, 2008). Severe historical droughts have occurred in Bangladesh in the years 1951, 1957, 1961, 1973, 1975, 1979, 1981, 1982, 1984, 1989 and 1995 (Shahid and Behrawan, 2008; Paul, 1998), leading to a net loss of 377,000 tons of Aman rice during the year by 1995 drought (Ahmed, 2006). Ahmed (2006) mentioned that about 47% of the country is drought-prone and 53% of the total population are living in these areas. According to Habiba *et al*. (2013), in the north-western part of Bangladesh, the average crop production reduced 25-30% due to the effect of drought. It is reported that although less attention has been paid for drought preparedness and management in Bangladesh than other disasters, drought is more damaging than floods and losses from drought are higher than floods (Shahid and Behrawan, 2008; Rahman and Saha, 2007; Alexander, 1995). We have to bring uncultivable land under cultivation by selecting some drought tolerant crops. We need to screen crops with high yielding, drought tolerant, nutritious and having medicinal value.

Tomatillo is herbaceous, indeterminate, sprawling, annual plant native to Mexico and Guatemala. It is a relative of tomato, eggplant, pepper, potato and tobacco and belongs to the *Solanaceae* family and *Physalis* species. Mexico, with about 50 species of *Physalis* growing in its territory, is considered the center of origin, diversity (D'Arcy, 1991), and domestication of this genus (Santiaguillo *et al*., 1994). It can be extensively used in salad as well as for culinary purposes and a unique crop which provides a variety of processed products, namely, juice, pickles, paste, puree, sauces, soup, ketchup etc. The fruits of many species of *Physalis* have been important elements in the culinary traditions of the people of Mesoamerica, consumed fresh or cooked, and calyces and leaves have been used in folk medicine since pre-Columbian times. Calyces are also consumed as seasoning and leaves as food (Hernández and Yáñez, 2009). The tomatillo fruit grows faster that the husk that covers it, breaking it open as it reaches full size. Depending on the cultivar, when the fruit ripens it can turn light green, yellow or purple, and it will get sweet losing its tangy flavor. It is a new crop in Bangladesh introducing from Mexico. It takes 12 to 14 weeks in the field from planting to harvesting, if seeding is direct or 8 to 10 weeks if seedling transplanted (Karim, 2016; Reza, 2016; Brito *et al*., 1985). Thus, it may be a good option to our agriculture due to the short growth season present in this area. Generally, tomatillo is grown during Rabi season in Bangladesh and inadequate soil moisture in this season limits the use of fertilizers and consequently results in decreased yield. Deficiency of water considered as one of the major constraints to successful upland crop production in Bangladesh (Islam and Noor, 1982). The growth, yield and fruit quality of tomatillos can be affected by drought stress, a common abiotic stress for crop plants. The cultivation of tomatillo requires proper supply of water and this requirement can meet by applying irrigation. In spite of its broad adaptation, production is concentrated in a few area and rather dry area (Cuortero and Fernandez, 1999). The screening of drought tolerant lines to identify a tolerant genotype is quiet necessary which may hopefully sustain a reasonable yield on drought affected soils. Screening is considered as an easier method to determine drought tolerant genotypes. With conceiving the above scheme in mind, the present research work has been undertaken in order to fulfill the following objectives:

- To compare the yield potentiality of the different genotypes of tomatillo.
- To determine the response of genotype-treatment interaction on different yield and yield contributing characters.
- To compare the tolerance of genotype, treatment and genotype-treatment interaction for proline accumulation as the drought tolerance indicator.
- To identify the best drought tolerent genotypes based on agromorphogenic, physiological and biochemical traits.

## CHAPTER II

## REVIEW OF LITERATURE

Tomatillo (*Physalis ixocarpa* Brot.) is a small edible fruit in the Solanaceae family but little known to our country as it is introduced recently from Mexico. A tan to straw colored calyx covers the fruit like a husk, giving rise to the common name of “husk tomato.” Tomatillo may have potential as a special crop in Bangladesh. The crop has received much attention in worldwide by the researchers on various aspects of its production under different adverse conditions. There is not even a single, combined, constructive review report available about the different Bangladeshi species of genus *Physalis* L. evaluated by using agromorphological, ethnobotanical, phytochemical and biological activities. The work so far done in Bangladesh is not adequate and conclusive. However, some of the important and informative works and research findings on this aspect have been reviewed in this chapter under the followings:

### 2.1.1 Nomenclature, origin and distribution of tomatillo

The tomatillo (*Physalis ixocarpa* Brot.), known as the Husk Tomato or Mexican Husk Tomato, is a plant of the nightshade family, related to the cape gooseberry, bearing small, spherical and green or green-purple fruit. Tomatillos originated in Mexico and are a staple of Mexican cuisine. The tomatillo is also known as the husk tomato, jamberry, husk cherry, or Mexican tomato, but the latter is more appropriately used to describe the relative of which bears smaller fruit. These names can also refer to other species in the *Physalis* genus. In Spanish, it is called tomate de cáscara, tomate de fresadilla, tomate milpero, tomate verde, tomatillo, miltomate (Mexico, Guatemala), or simply tomate. Tomatillos are grown as annuals throughout the Western Hemisphere and are generally eaten fried, boiled or steamed. Short life cycle of tomatillo has allowed it to be introduced in some other countries such as: Austria, France, Hungary, Italy, Poland, Russia, Spain, Turkey and United States (Abak *et al.*, 1994; Cantwell *et al.*, 1992;

Porcelli and Proto, 1991). The tomatillo fruit is surrounded by an inedible, paper-like husk formed from the calyx. As the fruit matures, it fills the husk and can split it open by harvest. The husk turns brown, and the fruit can be several colors when ripe, including yellow, red, green, or even purple. Purple and red-ripening cultivars often have a slight sweetness, unlike the green and yellow ripening cultivars, and are therefore more suitable for fruit-like uses like jams and preserves. Tomatillo plants are highly self-incompatible, and two or more plants are needed for proper pollination. Ripe tomatillos can be kept refrigerated for about two weeks. They will keep even longer if the husks are removed and the fruits are placed in sealed plastic bags and refrigerated.

### 2.1.2 Trade and common name

Winter cherry, Cape goose berry, Hogweed, Balloon cherry, Coqueret, Strawberry tomato, Cut leaf ground cherry, Wild tomato, Winter tomato, Winter cherry, Cow pops, Chinese lantern, Mullaca, Koropo, Camapu etc.

### 2.1.3 Geographical distribution and habitat

There are about 120 species of *Physalis* (L.) distributed worldwide. Among them *P. alkekengi* has an unknown center of origin and it is old world species originated from Asia. Other species viz. *P. angulata, P. peruviana* and *P. minima* are originated from tropical America (Jose *et al*., 2003). *P. peruviana* (L.) found most commonly in Brazil. There are six species of *Physalis* (L.) present in India, viz: *P. alkekengi* (L.); *P. angulata* (L.); *P. ixocarpa* Brot.; *P. longifolia* Nutt.; *P. peruviana* (L.) as cultivated species and *P. minima* (L.) as common weed (Deb, 1979). The various species of genus *Physalis* and their hybrids are now well established weeds that disturbed landscapes and crops throughout the tropics, including Asia. (Vatsavaya *et al*., 2007).

### 2.1.4 Cytological status

Cytological variations among the medicinal plants species caused by environmental stress, genetic recombination and mutation. The *Physalis* (L.)

is extensively studied by various researchers from India and other countries. The cytological analysis of first Indian species viz., *P. alkekengi* (L.) was done by various researchers world-wide and reported 2n=2x=24 (Badr *et al.*, 1997; Pogan *et al.*, 1989; Kliphuis and Wieffering, 1979). The second Indian species *P. angulata* (L.) was cytologically well-studied and reported 2n=4x=48 (Pedrosa, 1999; Ganapathi *et al.*, 1991; Husaini and Iwo, 1990; Lydia and Rao, 1982). The third species *P. longifolia* reported to have chromosome count 2n=4x=48, whereas 2n=2x=24 was also reported for *P. longifolia* var. *longifolia* (Tuteja and Bhatt, 1984). The fourth Indian species *P. minima* (L.) was reported to have 2n=4x=48 and 2n=6x=72 chromosome numbers (Kumar and Sinha, 1989; Gupta and Roy, 1981). The fifth Indian species *P. peruviana* (L.) was reported tetraploid and hexaploid i.e. 2n=4x=48 and 2n=6x=72 (Panda and Rao, 1983). The sixth Indian species i.e. *P. ixocarpa* Brot. was cytologically examined and showed diploid (2n=2x=24) chromosome numbers (Quiros, 1984; Lydia and Rao, 1981; Rao, 1979). From the cytological data it is clear that the Indian species of the genus *Physalis* (L.) exhibit different (2x, 4x and 6x) ploidy levels.

### 2.1.5 Morphological description

*P. ixocarpa* plant is an annual branched herb having weedy appearance. It gets 3-6 feet (0.9-1.8 m) tall and falls over and sprawls on the ground if not given support. The flowers are yellow with purple markings. The fruit develops inside a green and purple bladder like calyx that looks like a small Chinese lantern hanging from the stem. (Kirtikar and Basu, 2008; Khare, 2007; Pandey, 2005; Parmar and Kaushal, 1982). Due to the high morphological variation and the abundance of wild populations, *P. ixocarpa* is considered as a species in a current domestication process (Tavares *et al.*, 2015). Some authors consider that *P. ixocarpa* and *P. hiladelphica* L. are synonymous names (Santiaguillo and Yáñez, 2009), whereas others suggest that they are separate taxonomic entities (Tavares *et al.*, 2015; Lagos *et al.*, 2005). Most varieties of tomatillo have been typified by their morphological and agronomical attributes (Osuna *et al.*, 2015; Valerio *et al.*, 2012). Several authors reported that *P. ixocarpa* is a species with a high genetic variability (Osuna *et al.*, 2015; Santiaguillo *et al.*, 2004). Commercial tomatillo varieties grown in an open field experiment yielded 5.6-6.4 kg/$m^2$ in New Hampshire, U.S.A. (Freyre and Loy, 2000); 1.1-1.9 kg per plant in Georgia, U.S.A. (Perez *et al.*, 2005); 1.1-2.6 kg per plant in Mexico (Godina *et al.*, 2013); and 1.8 kg plant in the European part of Russia (Mamedov, 2017). Naumova *et al.* (2018) also found similar yield in the south of West Siberia, Russia. They studied field-grown tomatillo yield and fruit properties and their relationship with soil chemistry and temperature, at five experimental sites. At each site, a microplot experiment with two cultivars was conducted. Basic soil chemical properties and fruit pH and dry matter, total carbon, nitrogen, and ascorbic acid content were determined. Both cultivars grew and yielded very well, producing on average 70 fruits, 1.46 kg per plant, with 14 mg ascorbic acid per 100 g fresh weight and 9.0% dry matter. Tomatillo production in California was reported as ranging from 1.5 to 3.5 t/acre; i.e., 0.4-0.9 kg/$m^2$, which is extremely low and even seems to be erroneous (Smith et al., 1999). Somewhat higher, but still low, yields of 2.0-2.4 kg/$m^2$ were reported for Florida (Maynard, 1993).

## 2.2 Drought

Drought can be referred as the absence of adequate moisture necessary for a plant to grow normally and complete its life cycle (Zhu *et al.*, 2004). Meteorological drought is a short but recurring natural disaster that originates from a lack of rainfall (Pal *et al.*, 2000). The World Bank defines drought as more than 30 % rainfall deficiency in a year compared to the mean of a prolonged period (Venkateswarlu, 1992). Water deficit leads to the agitation of most of the physiological and biochemical processes and consequently reduces plant growth and yield (Boutraa, 2010). Caused by reduced precipitation and increased temperature (Parry, *et al.*, 2007) drought has been the most important limiting factor for crop productivity and, ultimately, for food security worldwide (Daryanto, *et al*., 2017). It has been widely reported that increasing water use efficiency at field level is one of the alternatives to cope with rainfall uncertainty and scarce water availability (Causape and Aragués, 2014; Gómez *et al*., 2014; Nair *et al*., 2012). The productivity of the crop related to physiological attributes such as transpiration rate, photosynthetic rate, relative water content (RWC) etc. Rapid early growth and maintenance of RWC at reasonably higher level during reproductive phase greatly influences the yield (Haloi and Baldev, 1986). Cornic also reported that water deficit reduces the rate of photosynthesis in plants (Cornic, 2000). Reduction of transpiration rate under drought causes increment of leaf temperature is deleterious effect for plants. They also influence on the distribution and density of populations as people won't settle in the areas experiencing shortage of life supports like rainfall and water (Rakib *et al*., 2014). In accordance to the IPCC special report on climate change and its probable effects on rainfall pattern and warmer climate in Bangladesh (Annonymous, 2007), the country may experience a 56% increase of rainfall by 2050 owing to the increase of snow melting attributed to more intense monsoon which is accompanied with prolonged periods of heavy flooding and followed by increased monsoon drought. Of the most recent 19 drought events experienced in Bangladesh, the 1973, 1978, 1979, 1981, 1982, 1992, 1994, 1995, 2000 and 2006 were the most severe (Habiba *et al*., 2012). In general, Bangladesh is one of the most vulnerable countries to climate change that is the long term variation of climatic parameters- temperature, precipitation, humidity, wind direction and pressure, and it's enhancing of natural flooding, drought and cyclone disasters. It is the country which experiences the highest frequency of droughts in a year. The mean temperatures recorded in Bangladesh ranges from 26.9 to 31.1°C during summer and 17 to 20.6°C during winter. Mean precipitation in the country varies from 1,329 mm in the northwest to 4,338 mm in the northeast (Shahid *et al.*, 2005). The average rainfall in the western part is approximately 2,044 mm; much lower than in others parts of the country (Shahid and Behrawan, 2008). Disasters, natural hazards and risks in Bangladesh are mostly considered geographical in nature (Hewitt, 1999; Cutter, 1993). Every year drought is experienced in some parts of the country, but mostly in the northwestern part which includes Rangpur and Rajshahi divisions. Most of the rivers could end drying-up due to the long term impacts of rainfall uncertainty and artificial barrage in the up-streams. Decreasing river flows in the dry season is negatively impacting on the ecosystem, river morphology and aquatic ecosystem in the western part of Bangladesh (Shahid and Behrawan, 2008). In the country, Brahmaputra River is the most vulnerable to the decreasing flows in the dry season, where a 26 million people is subjected to food insecurity (Immerzeel *et al.*, 2010). On the other hand, Ganges-Brahmaputra river basin is considered susceptible in the times of monsoon rainfall and extreme flooding (Mirza, 2002; Warrick *et al.*, 1996). Water in the Ganges River has decreased significantly by approximately 57% downstream beyond Farakka Barrage (Anonymous, 2007). Teesta is the main river in the northern part of Bangladesh. It enters Bangladesh through the Nilpharmary and Lalmonirhat Districts. The Government of Bangladesh has constructed a barrage on Teesta River at Dalia in Lalmonirhat district, which is a major source of water for irrigation in the northern part of Bangladesh. Its neighbor India has also constructed an embankment on Teesta River within its territory at Gazoldoba in Jolpaiguri (Anonymous, 2012; Higano and Fakrul, 2002). During the monsoon season, all doors of Gazoldoba barrage in India are opened causing flooding in the lowland areas of the northern part of Bangladesh but during dry season only few doors are opened, causing drought. Afroz and Rahman (2013) reported that the Teesta and Farakka barrages have reduced water flow by 80%.

Bangladesh is one of the most important agroeconomic based countries. Agricultural productivities which follow upward trends are prerequisites in the fight against food scarcity in the northern part of Bangladesh. Tomatillo is a species native to Mexico and Central America and it is, for the time being, one of the most important vegetable crops in Mexico (Cantwell *et al.,* 1992) ranking in the fourth place in planted area (47.472 ha) among commercially cultivated vegetables (Anonymous, 2011) introducing recently in Bangladesh. Considering the potentiality of this crop, there is plenty of scope for its improvement, especially under the drought situation for north-western region in Bangladesh. The concept of drought tolerance has been viewed differently by molecular biologist, biochemist, physiologists and agronomists. The major concern is to enhance the biomass and yield under limited input of water, which is a characteristic feature of rain fed agriculture. There are several physiological and biochemical traits contributing to the drought tolerance in crops. However, no tomatillo genotypes ever been screened for drought tolerance or exploited for their cultivation under drought situation in Bangladesh. To breed drought tolerant genotypes, it is necessary to identify physiological traits of the plants, which contributes to drought tolerance. Therefore, the present investigation was carried out to study the physiological and nutritional traits to facilitate the screening and selection of tomatillo genotypes for drought tolerance.

### 2.3 Genotypic variation

A genotype is the genetic blueprint of an individual. Genotypic variation is the variation in genotypes either between individuals of the same species or between different species that occurred during meiosis. Three ways of the variations can occur genetically are with mutations, gene flow, and meiosis. Accurate knowledge between the genetic diversity and the relationships among preserved germplasm collections of any crop is essential and important for establishing, managing and ensuring long term success of appropriate crop improvement programs through breeding (Gwag et al., 2010). The study on genetic diversity and population structure of germplasm collections has been useful in supporting conservation and genetic improvement strategies (Grandillo, 2014; Rao and Hodgkin, 2002).

### 2.3.1 Genotypic variation for agromorphogenic traits

Genetic variability among the traits is very important for selecting desirable types in breeding program. In the breeding of fruit trees, to characterize the season, number of buds and of flowers, and fruit set are critical data to identify populations with promising traits (Parra *et al.*, 2014). Determining the growth of reproductive structures enables the management of fruit supply according to the season and the adaptation of production technologies available in the region (Antunes *et al.*, 2008). An experiment consisted of six *Physalis* populations, was performed by Trevisani *et al.* (2016) arranged in a randomized block design to assess number of flower buds, number of flowers and number of fruits in 36, 43, 50, 57, 64 and 71 days after planting the seedlings in the field. They found significant effect of the population × time interaction, at 5% probability in analysis of variance.

The morphological description of the population is the first step towards selection of superior parents (Singh *et al.*, 2014). Breeding will only be successful in a selection program if the genetic variability in the traits of interest is high. Variations in genetic make-up between different populations can contribute to the formation of a genotypic constitution of *Physalis* adapted to the particular soil and climatic conditions of regions with high temperatures in Bangladesh. The use of cultivars with genetic variability in the trait production peak contributes to the uninterrupted supply of the fruit and, consequently, increases sales and thus the farmers' income (Segantini *et al.*, 2014).

Godina *et al.* (2013) evaluated yield and fruit quality in tomatillo autotetraploids (*Physalis ixocarpa*) and diploids under a completely randomized block design with four replications. They studied fruit yield, fruit weight, fruits per plant, equatorial and polar fruit diameter, total soluble fruit solids, fruit firmness, pH and Vitamin C content and they found equatorial diameter of fruit in diploids was 40.25 mm, the smaller diameter, 31.80 mm, while the wider was 46.50 mm for diploid; average polar diameter of fruits in diploids was 35.28 mm. The fruit equatorial diameter in autotetraploid was 40.45 mm. The polar diameter of autotetraploids showed an average of 31.44 mm and the values ranged from 30.32 to 32.34 mm. They also found diploids showed the following characteristics; fruit yield=1.809 kg plant$^{-1}$; number of fruits per plant=56.2 while the autotetraploid presented fruit yield=1.688 kg plant$^{-1}$, number of fruits per plant = 60.776. In the four diploid populations average fruit weight was 34.48 g/fruit with ranges from 6.16 g/fruit to 46.99 g/ fruit. In autotetraploids, the average fruit weight was 29.31 g/fruit with a range of 22.81 g/fruit to 34.99 g/fruit. As higher amount of biomass is produced the demand for nutrients is also higher, the plants are taller and need more days for flowering and harvest (Torres *et al.*, 2011); they also have a broader ecological tolerance, and larger cells (Cequea, 2000).

### 2.3.2 Genotypic variation for physiological traits

Water shortage conditions cause water losses within the plant and result in relative water content (RWC) reduction. Photosynthesis is particularly sensitive to the effects of water deficiency. Water relations are also altered and evaluated mainly by the relative water content and water potential in the leaves, with some studies already reporting that for the genus *Physalis* (Monroy *et al*., 2015; Souza and Amorim, 2009). Plants can quickly respond to water stress as a defense against water loss, with changes in stomatal conductance and reducing transpiration. However, this mechanism promotes an increase in leaf temperature and reduces gas exchange (Furlan *et al*., 2012; Silva *et al*., 2008). It is important to note that there are few studies that evaluate the mechanisms employed by *Physalis* species in water deficit conditions, especially for *P. angulata* (Ozaslan *et al*., 2016; Monroy *et al*., 2015; Souza *et al*., 2013). Thus, the characterization of the drought responses in physiological traits developed by the species may contribute to the cultivation techniques and future work on the selection of tolerant genotypes.

An experiment on effect of drought stress on water relation traits of four soybean genotypes was performed in a venyl house at the environmental stress site of Bangabandhu Sheikh Mujibur Rahman Agricultural University during September to December 2012 to investigate the internal water status under drought stress in soybean genotypes by Chowdhury *et al.* (2017). They found that drought (water) stress reduced the leaf water potential in all the genotypes though was more negative in tolerant genotypes than in susceptible ones. The lowest leaf water potential was obtained -1.58 MPa and the highest -1.2 MPa. Relative water content (RWC) decreased remarkably in all the genotypes and reduction was more in susceptible than tolerant genotypes. At 8.00 am, RWC of stressed plants decreased by 9.58, 9.02, 8.90 and 13.90% at vegetative stage. Water stress significantly reduced RWC at two sampling times (8:00 am and 1:00 pm) across the genotypes at different growth stages in all the four soybean genotypes studied. The reduction in RWC due to water stress was also reported by Omae *et al*. (2005) and Omae *et al.* (2007) in snap bean. Plants grown under water stress conditions showed a lower RWC than those grown under non stress conditions. Relative water content was higher in the morning, while decreased at noon. Several researchers reported that RWC of different crops was the highest in the morning and gradually decreased thereafter (Omae *et al.*, 2005). Upreti *et al.* (2000) reported that sensitive pea genotypes were more affected by a decline in relative water content than tolerant ones under drought stress condition.

Proline is another physiological indicator for screening of genotypes under drought stress and extensively studied molecule in the context of plant abiotic stress physiology. Proline is a common osmolyte in drought-stressed plants (Gupta, 2006). Proline accumulation has been associated with drought stress tolerance in several crop plants (Ashraf and Foolad, 2007). It defends plant tissues against drought stress preventing molecular denaturation, scavenges reactive oxygen species and interacts with phospholipids. There is now strong evidence that proline has surfeit of functions in both abiotic and biotic stress tolerance. Mwenye *et al.* (2016) reviewed the role of proline and root traits on selection for drought-stress tolerance in soybeans. They found that proline accumulates with increased drought stress and genotypic differences exist in proline accumulation among soybean cultivars of different sensitivities to drought stress. So, there is a positive correlation between stress-induced proline accumulation and drought tolerance.

### 2.3.3 Genotypic variation for antioxidant and nutritional traits

An experiment on selected nutritional and quality analyses of tomatillos accomplished by Margaret et al. (1995). They analyzed proximate composition, total dietary fiber and pH of tomatillos grown in Baja, California and found that moisture content averaged 92%. On a dry matter basis (DMB), tomatillos contained 11% protein, 18% fat, 13% ash and 5% total dietary fiber. On an as consumed basis (ACB), tomatillos contained 1% protein, 1.5% fat, 1% ash and 0.4% dietary fiber. Average calorie content was calculated to be about 31 kcals/100 g and average pH of was 3.76.

Presently, evidence is accumulating from different fields of science including human medicine and nutrition to support that antioxidants participate an important function in the prevention of human degenerative diseases (Kalt *et al.*, 1999). In this sense, *Physalis ixocarpa* is a special tomato which grows both as wild and cultivated plant that has many antioxidant properties. *P. ixocarpa* presented higher anthocyanin content when cultivated under full sun and red net. The relation between the red and far-red wavelengths is directly related to the synthesis of anthocyanin (Awad *et al.*, 2001). According to Awad *et al.* (2001), values of this relation lower than 1 reduce the cyanidin-3-galactoside (anthocyanin), quercetin 3-glucoside and total flavonoid contents, which results in deficient coloration in fruits. The higher anthocyanin content in *P. ixocarpa* in relation to other species occurs due to the fact that it is the only species which presents purple coloration. The values found for anthocyanin in *P. ixocarpa* were similar to the ones found by Mendoza *et al.*

(2010), with approximate variation between 4 and 9 mg pelar-3-gluc·100 $g^{-1}$. For *P. ixocarpa*, the soluble solid values reported in literature vary from 5.24 to 9.03% Brix (Álvarez *et al*., 2012). Similar result was found by Mendoza *et al*. (2011). Fruits of *P. peruviana* under red net presented low values of soluble solids (7.33% Brix), like the results found for *P. pubescens* in white net (8.33% Brix), as well as in full sun and *P. minima* stood out for presenting a high soluble solids content in all treatments, regarding the analyzed species. *P. pubescens* also presented a high content of soluble solids. *P. minima* produced under the subtropical edaphoclimatic conditions presented soluble solids values varying from 9.50 to 10.95% Brix, much higher than the 4.16% Brix reported by Patel *et al.* (2011). Silva *et al*. (2013) and Lima *et al*. (2013) found 11.26 and 14% Brix in fruits of *P. peruviana*.

Vitamin C is one of the main nutrients in fruits and vegetables that has an important role in human nutrition because it is an antioxidant that contributes to human health and is credited with strengthening the body in defense of cardiovascular diseases (Carr and Frei, 1999). Studies described *Physalis* species as fruits with considerable Vitamin-C content (Silva *et al*., 2013; Patel *et al*., 2011); however, in all these works, the values found were inferior to the ascorbic acid content of the fruits cultivated in subtropical area. Also in complete accordance with the present study, where *P. minima* and *P. ixocarpa* were, respectively, the species with higher and lower Vitamin C content, Álvarez *et al.* (2012) by working with *P. ixocarpa* demonstrated the low content of Vitamin C (8.21 and 2.61 mg/100 g), and Patel *et al.* (2011) found high content in *P. minima* (46.67 mg/100 g). The Vitamin C values presented by the studied *Physalis* species were superior to the ones of other small fruits traditionally known for having this Vitamin, such as blackberries and red raspberries (Guedes *et al*., 2013; Maro *et al.*, 2013). Lal *et al.* (2017) investigated a total ten selections of tomatillo on chlorophyll, ascorbic acid, phenolic, flavonoids, anthocyanin attributes and antioxidant activities (DPPH) characters. They found DPPH % inhibition varied between 42.54 and 84.65%;

however, total anthocyanin ranged between (1.23 and 5.65) mg 100 $g^{-1}$ fresh weight.

An experiment on characterization of different native american *Physalis* species (*Physalis peruviana* L., *Physalis pubescens* L., *Physalis angulata* L., *Physalis mínimos* L. and *Physalis ixocarpa* Brot.) and evaluation of their processing potential as jelly in combination with brie-type cheese was performed by Curi et al. (2018). They found that different *Physalis* species showed the soluble solids content ranged from 2.33 to 11.33% Brix and acidity ranged from 0.15 to 2.30 g of citric acid/100 g. The *Peruviana*, *Angulata* and *Pubences* species stood out for higher soluble solids and acidity. According to Vasco et al. (2008) classification all *Physalis* species can be classified as having a low phenol concentration (< 100 mg GAEs.100 g-1). Following the ascorbic acid classification proposed by Ramful et al. (2011) all *Physalis* species, except for *Ixocarpa*, are classified with high Vitamin C content (> 50 mg.100 g–1), showing that this fruit is a very good source of this Vitamin.

Yield and fruit quality in tomatillo between autotetraploids (*Physalis ixocarpa*) and diploids were evaluated and it was found that there were no differences among diploids and tetraploids in case of total soluble solute content in tomatillo fruit, therefore increasing the ploidy level did not change the plant's ability to synthesize soluble solids (Godina *et al.*, 2013). Godina *et al.* (2013) also found that for total soluble solid the diploids showed average value was 8.6% Brix and autotetraploid populations showed mean values of total soluble solids of 6.39 °Brix within a range of 6.17 to 6.63% Brix. In autoteraploids the Vitamin C content were higher than diploids, there were even some of them who doubled the value of the diploids, perhaps because polyploids are more capable of producing secondary metabolites (Cequea, 2000). So these results indicate that genome duplication in tomatillo could increase the Vitamin C content and results indicate that *Physalis ixocarpa* autotetraploids can be used to obtain improved genotypes with antioxidant properties.

## 2.4 Effect of drought on developmental stages of plant and crop production

Drought is a major abiotic stress limiting plant growth and productivity (Meng *et al*., 2016), especially in arid and semi-arid regions. Under abiotic stresses conditions, such as drought, some physiological processes are affected such as photosynthesis, protein synthesis and energy production (Chaves *et al*., 2009; Flexas *et al.*, 2004). Reproductive development at the time of flowering is especially sensitive to drought stress (Samarah *et al*., 2009c; Zinselmeier *et al.*, 1999, 1995). Therefore, an understanding of how a reproductive process becomes affected by drought is of particular interest for improving drought tolerance (Samarah *et al.*, 2009c).

Ozalsan *et al*. (2016) tested survival, growth, nutrient uptake and fecundity of two co-occurring, invasive *Physalis* species under water and salinity stresses, and different soil textures and they found that water stress hampered the growth and fecundity of the two *Physalis* species. *P*. *philadelphica* survived for longer period compared to *P*. *angulata* under severe water stress. Mortality was observed for some seedlings of *P*. *angulata* (25% mortality) under severe stress whereas, all seedlings of *P*. *philadelphica* were able to survive under all levels of water stress. Regarding interactions between water stress treatments and tested weeds, *P*. *philadelphica* observed minimum and maximum relative growth rate under no and severe water stress treatments, respectively. Similarly, *P*. *philadelphica* produced taller plants (91.31 cm) under stress free conditions while, the lowest plant height (19.96 and 18.13 cm) for both weeds was recorded under severe water stress. Reproductive output of both weeds was decreased with increasing water stress and the lowest reproductive output (19 seeds per plant) was noted under severe water stress. *P*. *philadelphica* produced higher number of seeds than *P*. *angulata* under drought. Previous studies also revealed that increasing water stress lowered fecundity of different weeds (Sarangi *et al.*, 2016; Chauhan, 2013; Chauhan and Johnson, 2010).

**2.5 Effect of different drought treatment on tomatillo plant**

Drought is an important natural phenomenon which affects morphological, physiological, biochemical and yield attributes of plants leading to death. During water stress many physiological and molecular processes are disturbed such as root-shoot growth, water relation, mineral absorption, leaf expansion and orientation, stomatal behavior, transpiration rate, photosynthesis and respiration rate, solute translocation, etc. Toxic elements such as reactive oxygen species (ROS) produced during stress period create oxidative damage to the cellular organization. Plants have its antioxidant system to scavenge such harmful element and accumulate osmoprotectants such as proline, glycien betaine, etc to maintain osmotic adjustment. *Physalis* can develop in a huge range of soil and climatic conditions and it is classified as a very tolerant species due to its adaptability to Mediterranean climates and to several soil types (Fischer, 2000). Farm producers of *Physalis*, in Colombia, are equally similar to farm producers from cold tempered climate regions (Rufato *et al.*, 2008). Altitude has strong influence on *Physalis* plants and fruits. In Colombia, *Physalis* is cultivated in high altitudes from 2000 to 2650 meters high (Fischer *et al.*, 2005). In Brazil, *Physalis* has good adaptation to the wide soil and climate conditions, being that excessive humidity, drought, cold and heat prejudice growing and development of the plants, as well the final quality of the product and decreasing productivity (Muniz *et al.*, 2011). As for the soil type, the ideal for cropping is sandy-clayey, good drainage, grained soil, preferentially those ones rich in organic material (greater than 4%) and pH between 5.5 and 6.8 (Fischer *et al.*, 2005). It is important to avoid waterlogged soil and those that, previously, were cultivated with other species of *Solanaceae* (Rufato *et al.*, 2008). In order to obtain a fruit with quality, *Physalis* needs around 1500 to 2000 hours of light a year (Rufato *et al.*, 2008). According to Fischer (2000), *Physalis* shows better growing and development in regions with annual temperatures between 13 to 18°C. Miranda (2004) stated the favorable temperature for growing and development of the plants is 18°C. Salazar (2006) found that 6.3°C is the physiologic-base temperature for *Physalis* growing. High temperatures (higher than 30°C) damage flowering and fruiting stages, promoting early ageing (Angulo, 2005). Low temperatures (nocturnal lower than 10°C) can obstruct the plant growing (Rufato *et al.*, 2008). Temperature and light have an important role in relation to size, color, nutritional content, taste and fruits ripening stage (Rufato, 2010). Rainwater should range between 1000 to 1800 millimeter and the average relative humidity from 70 to 80% is ideal during the growing season (Popova *et al.*, 2010). Water demand at least 800 millimeters during growing period. *Physalis* is very susceptible to drought and strong winds, so, its cultivation should be protected by windbreaks (Rufato *et al.*, 2008).

### 2.5.1 Effect of drought on agromorphogenic traits

Crop yield is affected by agronomic factors and various environmental variables such as water availability and temperature (Hatfield and Prueger, 2015; Awika, 2011). With a changing climate, droughts are predicted to become more intense and frequent in many regions (Trenberth *et al.*, 2014) and area increasing by 50% to 200% during the 21$^{st}$ century so far (Zhao and Dai, 2017). Therefore, a number of independent studies have investigated the individual effects of drought on different *Solanaceous* crops agronomic traits. The severity and duration of drought stress determine the extent of the yield loss by shortening the life cycle and duration of grain filling (Farooq *et al.*, 2014).

Leaves are an active border of exchanging energy, carbon and water in between plants, canopies and atmosphere. Leaf area index (LAI) is a dimensionless variable, represents the structural attribute of leaf components, estimated by area of leaf per unit area of soil surface (Cutini *et al.*, 1998). It also represents plants leaf photon interception, which highly influences biomass as well as yield production that is directly related with water consumption (Firouzabadi *et al.*, 2015). Thus, the assessment of LAI is essential and very significant in most of the physiological, horticultural and agronomic studies that involve crop growth corresponding to yield (Guo and Sun, 2001). Factors related to leaf area, such as photosynthesis and transpiration rate, directly affect the plant productivity, which makes the leaf area a key variable in physiological studies involving plant growth, light interception, photosynthetic efficiency, evapotranspiration, and answers to fertilizers and irrigation (Blanco and Folegatti, 2005). Hossain *et al.* (2017) assessed the leaf area index (LAI) of tomato and cucumber. The highest LAI obtained for tomato and cucumber was 5.21 and 3.21 $m^2/m^2$, respectively. For both crops, LAI was found significantly influenced at 50-days after transplanting. It also indicated that LAI significantly influenced (by 15%) deficit irrigation for both crops and methods that achieved the highest yield.

Drought stress during vegetative growth lowers the amount of carbohydrate accumulated in the stems and leaves, since the biomass produced in such organs from the vegetative to reproductive stages is needed to build up enough resources for translocation (Marcaida *et al.*, 2014); water limitation occurring during the reproductive stages directly constrain seeds development, causing a premature grain desiccation (Fábián *et al.*, 2011). Soil water deficits that follow during the reproductive growth are considered to have the most adverse influence on crop yield (Samarah *et al.*, 2009a, b; Costa-Franca *et al.*, 2000). Low seed set percentages are regularly related to several factors such as reducing pollen grain availability (Trueman and Wallace, 1999; Agren, 1996), increase ovary abortion (Boyer and Westgate, 2004), increase pollen grain sterility (Al-Ghzawi *et al.*, 2009; Schoper, 1986; Westgate and Boyer, 1986), slow stigma and style elongation, reducing time of pollination (Westgate and Boyer, 1986), lower pollen grain germination activity, pollen tube growth, and less development of fertilized seeds (Lee, 1988).

### 2.5.2 Effect of drought on physiological traits

Plants develop diverse morphological and physiological mechanisms to alleviate the negative effects of water and salinity stress (Farooq *et al*., 2012; Parida and Das, 2005; Zhu, 2001). Organic solutes accumulation under abiotic stresses conditions has been reported in several crops, such as cowpea (Sousa *et al*., 2015), pigeon pea (Monteiro *et al*., 2014), tomato (Ganbari and Sayyari, 2018) and citrus (Peris *et al*., 2017; Zou *et al*., 2013). An experiment was accomplished by Sivakumar (2014) to see the effect of drought with three treatments viz., control, Treatment-1 (for 30 days) and Treatment-2 (for 45 days) with three replications and reported that relative water content of plant decreased under drought stress than control. Srivastava *et al.* (2012) reported that relative water content and transpiration is important trait for assessment of drought tolerance, and is widely affected by environmental stress conditions.

Leite *et al*. (2018) assessed the physiological responses of *P. angulata* plants after 40 days under different water availability (100%, 80%, 60%, 40% and 20% of pot field capacity) and found the relative water content in the leaf remained constant up to 40% of the field capacity after that RWC reduced. The low soil water availability reduced the water potential of the plants, with no statistical difference, up to 40% of the field capacity, differing from the plants grown in 20% of the field capacity. The observed reduction in leaf water potential may be related to the sugars accumulation in leaves. Other species also showed reduced water potential when under conditions of low water availability (Mota and Cano, 2016; Ronchi *et al*., 2015). The contents of total soluble sugar were higher with the reduction of the amount of water in the soil. The accumulation of these solutes plays vital role in the tolerance to abiotic stresses (Ma *et al*., 2017).

Proline as an inert compatible osmolyte that protects sub-cellular structures and macromolecules under water stress conditions (Szabados and Savoure,

2009) and compatible osmo-protectant and osmolyte which accumulates largely under stress conditions (Seki *et al.*, 2007). Proline inhibits molecular denaturation, scavenges reactive oxygen species and interacts with phospholipids (Kavikishor and Sreenivasulu, 2014). Amino acids involving proline, choline, glycinebetaine are the important osmo-protectants against drought stress. Plants generally gather compatible solutes such as proline, betaine and polyols in the cytosol to raise osmotic pressure and thereby to maintain both turgor and driving gradient for water uptake (Rhodes and Samaras, 1994) and to protect membranes and proteins (Delauney and Verma, 1993). It has been shown that proline plays a vital role in the stabilization of cellular proteins and membranes in the presence of a high osmoticum concentration (Errabii *et al.*, 2006). Bandurska *et al.* (2017) performed an experiment on regulation of proline biosynthesis and resistance to drought stress in two barley (*Hordeum vulgare* L.) genotypes of different origin (the Syrian breeding line Cam/B1/CI and the German cultivar Maresi) to examine the effect of 10-day drought on tissue dehydration and proline biosynthesis in leaves as well as in roots of barley genotypes. He found that drought caused a gradual decrease of water content and an increase of proline and ABA content in roots and leaves of both genotypes.

### 2.5.3 Effect of drought on antioxidant and nutritional traits

The content of minerals in crops is linked to genotypes, environmental growing conditions, soil properties, and maturity of crops at harvesting time (Ballesta *et al.*, 2010). Medicinal and aromatic plants have assumed a growing consideration in many sectors, including agroalimentary, perfumes, pharmaceutical industries and natural cosmetics and the biosynthesis of secondary metabolites is heavily influenced by environmental stresses (Steinbauer *et al.*, 2015; McKiernan *et al.*, 2014). Water stress has been proven to induce an accumulation of total soluble solids (TSS) in many crop plants as an important adaptation mechanism (Babita *et al.*, 2010). Compatible soluble like sugars, glycerol, proline, or glycine betaine can also contribute to turgor maintenance as fundamental physiological means for lowering the negative effects of drought (Aldesuquy *et al*., 2013). Among the compatible soluble, soluble sugars can act: as osmotic agents and as osmoprotectors (Huan *et al*., 2014). In tomato fruits a decreased amount of irrigation water induces greater TSS contents (Patanè *et al*., 2011); in particular, has been found an average value of TSS, expressed as % Brix, of 5.78 for low water irrigation level and 4.30 for the largest water application, (Favati *et al*., 2009). At low moisture conditions, sugar levels increase in several crop species such as tomatoes (Wu and Kubota, 2008), cucumbers (Huang *et al*., 2009), and grapes (Deluc *et al*., 2009).

# CHAPTER III

# MATERIALS AND METHODS

In this chapter, information concerning methodology that was used in execution of the experiment was illustrated. It comprises a brief description of locations of experimental site, planting materials, climate and soil, seedbed preparation, layout and design of the experiment, pot preparation, fertilizing, transplanting of seedlings, intercultural operations, harvesting, data recording procedure, statistical and nutritional analysis etc., which are presented as follows:

## 3.1 Experimental site

The experiment was accomplished in near the net house of Genetics and Plant Breeding Department, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during the period from October 2017 to March 2018. Location of the experimental site is 23°74' N latitude and 90°35' E longitude with an elevation of eight meter from sea level (Anonymous, 2014b) in Agro-ecological zone of "Madhupur Tract" (AEZ-28) (Anonymous, 1988). The experimental site is shown in the map of AEZ of Bangladesh in Appendix I.

## 3.2 Planting materials

A total of four genotypes of tomatillo were collected personally from Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207 on September, 2017 and then they were used as planting materials for the experiment.

## 3.3 Treatments in the experiment

The two factorial experiment was conducted to evaluate the performance of four tomatillo genotypes under different drought treatments in the experiment.

### Factor A: Tomatillo genotypes

In this experiment, four tomatillo genotypes were used as factor A (Table 1).

**Table 1. Name and source of four tomatillo genotypes used in the study**

| Sl. No. | Genotypes No. | Name/ Accession No. | Source |
|---|---|---|---|
| 1 | $G_1$ | SAU Tomatillo-1 | Department of Genetics and Plant Breeding, SAU |
| 2 | $G_2$ | PI003 | |
| 3 | $G_3$ | SAU Tomatillo-2 | |
| 4 | $G_4$ | PI004 | |

SAU= Sher-e-Bangla Agricultural University

**Factor B: Different drought treatments**

Three drought treatments were employed in this experiment by witholding of water in the pots. Three treatments were $T_1$ (0 days witholding of water/Control) , $T_2$ (30 days witholding of water/moderate stress) and $T_3$ (45 days witholding of water/severe stress).

### 3.4 Design and layout of the experiment

The experiment was laid out and evaluated during Rabi season 2017-18 in CRD using two factors. Factor A included four genotypes and Factor B included three drought treatments. The experiment was conducted in five replications and total 60 plastic pots were used.

### 3.5 Climate and soil

Experimental site was located in the subtropical climatic zone, set imparted by plenty of sunshine and moderately low temperature prevails during October to March (Rabi season) which is suitable for tomatillo growing in Bangladesh. Weather information and physicochemical properties of the soil used in pot experiment are presented in Appendix II and Appendix III, respectively.

### 3.6 Seedbed preparation and raising of seedlings

Seed sowing was carried out on October 10, 2017 in the seedbed. Seeds were treated with Bavistin for five minutes before sowing. Seedlings of all genotypes were raised in seedbeds in the farm house, Sher-e-Bangla Agricultural

University, Dhaka-1207. Seeds were sown in rows spaced at 10 cm apart, beds were watered regularly to ensure maximum seedling growth. Seedlings were raised using regular nursery practices. Recommended cultural practices were taken up before and after sowing the seeds. When the seedlings became 45 days old then it was transplanted to the main plastic pot. Seedbed preparation, raising of seedling, formaldehyde treatment of soil, pot preparation and transplanting to the plastic pots were done in appropriate time with recommended operations. Seedbed preparation and raising of seedlings are shown in Plate 1 (A and B).

### 3.7 Manure and fertilizers application

Soil of pot was well pulverized and dried in the sun and only well decomposed cow dung was mixed with the soil before transplanting of the seedlings in plastic pots. Well decomposed cow dung was calculated for each pot considering the dose per hectare soil at the depth of 20 cm. On an average each plastic pot was filled with soil containing 100 g decomposed cow dung (10 tons/hectare).

### 3.8 Pot preparation and transplanting of seedlings

Weeds and stubbles were completely removed from soil which was used for transplanting. The soil was treated with Formaldehyde (45%) for 48 hours before filling the plastic pots to keep soil free from pathogen. Pots were filled up two days before transplanting (November 23, 2017). Each pot was filled with 7 kg soil. The pot size was 20 cm in height, 30 cm in top diameter and 20 cm in bottom diameter. Three pores were made in each plastic pot and then the pores were covered by gravels so excess water could easily drain out. When the seedlings became 45 days old, they were transplanted in the main plastic pot (one plant/pot). Plastic pot preparation, transplanting and tagging seedlings are shown in Plate 1 (C and D).

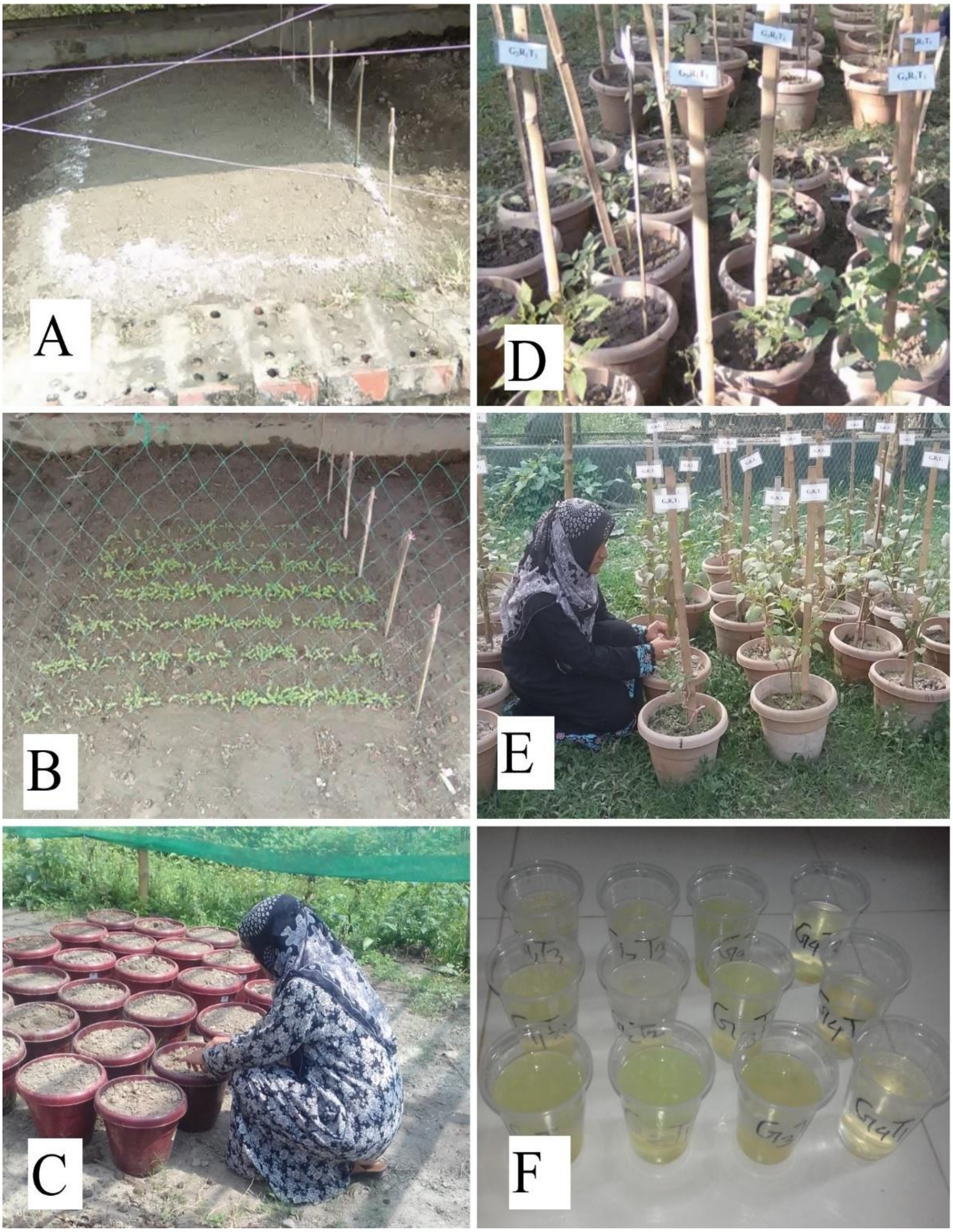


**Plate 1. Steps of seed sowing to seed extraction. A) Seedbed preparation and sowing of seeds, B) Raising of seedlings, C) Plastic pot preparation, D) Transplanting and tagging of seedlings in the pot, E) Weeding in the pot and F) Seed extraction of four tomatillo genotypes**

### 3.9 Application of drought treatments

Four tomatillo genotypes were evaluated under different drought treatments ($T_1$- Control condition or 0 Days withholding of water; $T_2$- 30 Days withholding of water and $T_3$- 45 Days withholding of water). Plants in control treatments ($T_1$) were always irrigated with fresh water. $T_2$ and $T_3$ drought treatments were employed on plants in the plastic pots seven days after transplanting from seedbed. For $T_2$ treatment the application of water was stopped for 30 days. After 30 days withholding of water, plants were re-watered for recovery. For $T_3$ treatment the water was withhold for 45 days, and then re-watered for recovery.

### 3.10 Intercultural operations

Necessary watering and intercultural operations were provided as and when required (Plate 1D and 1E) during the experimental period. Weeding was performed in all plastic pots as and when required to keep plants free from weeds. Diseases and pest is a limiting factor to tomatillo production. Experimental tomatillo plants were treated with Bavistin DF and Cupravit 50 WP to prevent unwanted diseases problem @ 1 g/l and 2 g/l respectively. Aphids, Colorado potato beetles, flea beetles, tomato hornworms are important pest of tomatillo during growing stage. They were controlled by using Malathion 250 EC @ 0.5 ml/l. Those fungicide and pesticide were sprayed two times, first at vegetative growth stage and next to early flowering stage to manage pest and diseases. When plants were well established, stalking was done to each plant by bamboo stick between 25-30 DAT to keep the plants erect. Proper tagging and labeling were done for each plant.

### 3.11 Harvesting and processing

Harvesting of fruits was done after maturity stage. Mature fruits were harvested when the papery husk surrounding the fruit turned from green to tan and begins to split; the fruit itself was bright green, purple, or yellow depending on the variety. The fruits per plant were allowed to ripe and then seeds were collected and stored at 4$^{o}$C for future use (Plate 1F). Harvesting was started from February 2, 2018 and completed by March 15, 2018.

### 3.12 Data recording

Data were recorded from each pot based on different biometric, physiological and nutritional traits. Different steps of data collection during the experiment are presented in Plate 2. Different steps of data collection are presented below. Data were recorded in respect of the following parameters:

#### 3.12.1 Agromorphogenic traits

Different biometric traits related to yield and its contributing characters were recorded viz., days to first flowering, plant height, leaf length, leaf width, leaf length × leaf width, leaf length/width, number of fruits per plant, days to maturity, average fruit length (mm), average fruit diameter (mm), average fruit weight per plant (g), number of seed per fruit and yield per plant (kg).

##### 3.12.1.1 Days to first flowering

The days to first flowering was counted from the date of tomatillo seedlings sowing to date of first flowering.

##### 3.12.1.2 Days to maturity

The days to maturity was counted and recorded from the date of tomatillo genotypes transplanting (DAT) to date of first harvesting in different genotypes under different drought stresses.

##### 3.12.1.3 Plant height (cm)

Plant height of each plant at mature stage was measured in cm using meter scale and mean was calculated.

##### 3.12.1.4 Number of fruits per plant

The total number of marketable fruits harvested from each plant was counted and recorded.

#### 3.12.1.5 Average fruit weight per plant (g)

Fruit weight was measured by electric precision balance. Average fruit weight per plant was recorded from randomly selecting 5 fruits per plant and mean value was calculated. Average fruit weight per plant was expressed in gram (g).

#### 3.12.1.6 Leaf length (cm)

Three leaves length from each plant at mature stage was measured in cm using meter scale and mean was calculated.

#### 3.12.1.7 Leaf width (cm)

Three leaves breadth from each plant at mature stage were measured in cm using meter scale and mean was calculated.

#### 3.12.1.8 Leaf length × width ($cm^2$)

Mean value of leaf length (cm) and width (cm) were multiplied and thus calculated leaf length × width in $cm^2$.

#### 3.12.1.9 Leaf length/width

Mean value of leaf length was divided by leaf breadth and thus calculated leaf length/width.

#### 3.12.1.10 Number of seeds per fruit

Seed extraction and drying was done from harvested fruits from tomatillo plants. Then number of seed per fruit was counted. Seeds were collected and preserved for future use.

#### 3.12.1.11 Average fruit length (cm)

Fruit length was measured using Digital Caliper-515 (DC-515) in millimeter (mm). Later it was converted to centimeter (cm). Mean was calculated for each treatment and genotype.

#### 3.12.1.12 Average fruit diameter (cm)

Fruit diameter were measured using Digital Caliper-515 (DC-515) in millimeter (mm). Later it was converted to centimeter (cm). Mean was calculated for each treatment and genotype.

#### 3.12.1.13 Yield per plant (kg)

Yield per plant was recorded from all harvests of each plant and expressed in kilogram (kg) per plant.

### 3.12.2 Physiological traits

Data related to different physiological traits such as relative water content (RWC) and proline content were recorded.

#### 3.12.2.1 Determination of relative water content

The relative water content (RWC) was measured in fully expanded leaves located in the middle third of the tomatillo plant. The relative water content (RWC) of tomatillo was assessed according to Barrs and Weatherly (1962). The fresh weight of the whole tomatillo plant was recorded by using a precision balance. Then the plant was floated in water under light until the weight stayed constant to attain full turgid and after that turgid weight was recorded. Then the plant was kept in hot air oven at 80°C for 48 hours and the dry weight was recorded. Finally, the relative water content (RWC) was calculated by using following formula,

$$\text{Relative water content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \text{ x } 100$$

#### 3.12.2.2 Determination of proline content

##### 3.12.2.2.1 Proline extraction

Proline accumulation was determined by the method as described by Sadasivam and Manickam (1996) in the experiment. Fresh leaves (0.5 g) were collected from tomatillo plants and grinded in mortar and pestle with 10 mL of 3% sulphosalicyclic acid and the homogenate was centrifuged at 18000×g. The homogenate was filtered and 2 mL of filtrate was added to the 2 mL of glacial acetic acid and 2 mL of acid ninhydrin. After that test tubes were kept for 1h at 100°C in water bath, followed by ice bath and the reaction mixture was vortexed with 4 mL of toluene. Toluene layer from each test tube was separated and absorbance was read at 520 nm. A standard curve of proline was used for calibration to determine proline content. Proline extraction step is shown in Plate 2 (A and B).

#### 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 during the experiment. By diluting this solution, 50 ppm, 100 ppm, 200 ppm, 400 ppm and 800 ppm solution were prepared in 20 mL each in different test tubes. Then the absorbances were measured with the help of Spectrophotometer at 520 nm and observations were recorded. By plotting the concentration of proline (ppm) in 'X' axis and obtained absorbance reading in 'Y' axis a standard curve was prepared (Appendix VII). From the absorbance reading obtained from samples, their respective proline content was estimated in ppm by using proline standard curve and then it was converted into micro gram per gram (µg/g) unit using the following formula:

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

### 3.12.3 Antioxidant and nutritional traits

Data were recorded on the basis of different antioxidant and nutritional traits using ripe fruits viz., Brix (%) and Vitamin C content (mg/100 g).

#### 3.12.3.1 Determination of Brix percentage

Brix percentages of tomatillo fruits were measured by Portable Refractometer

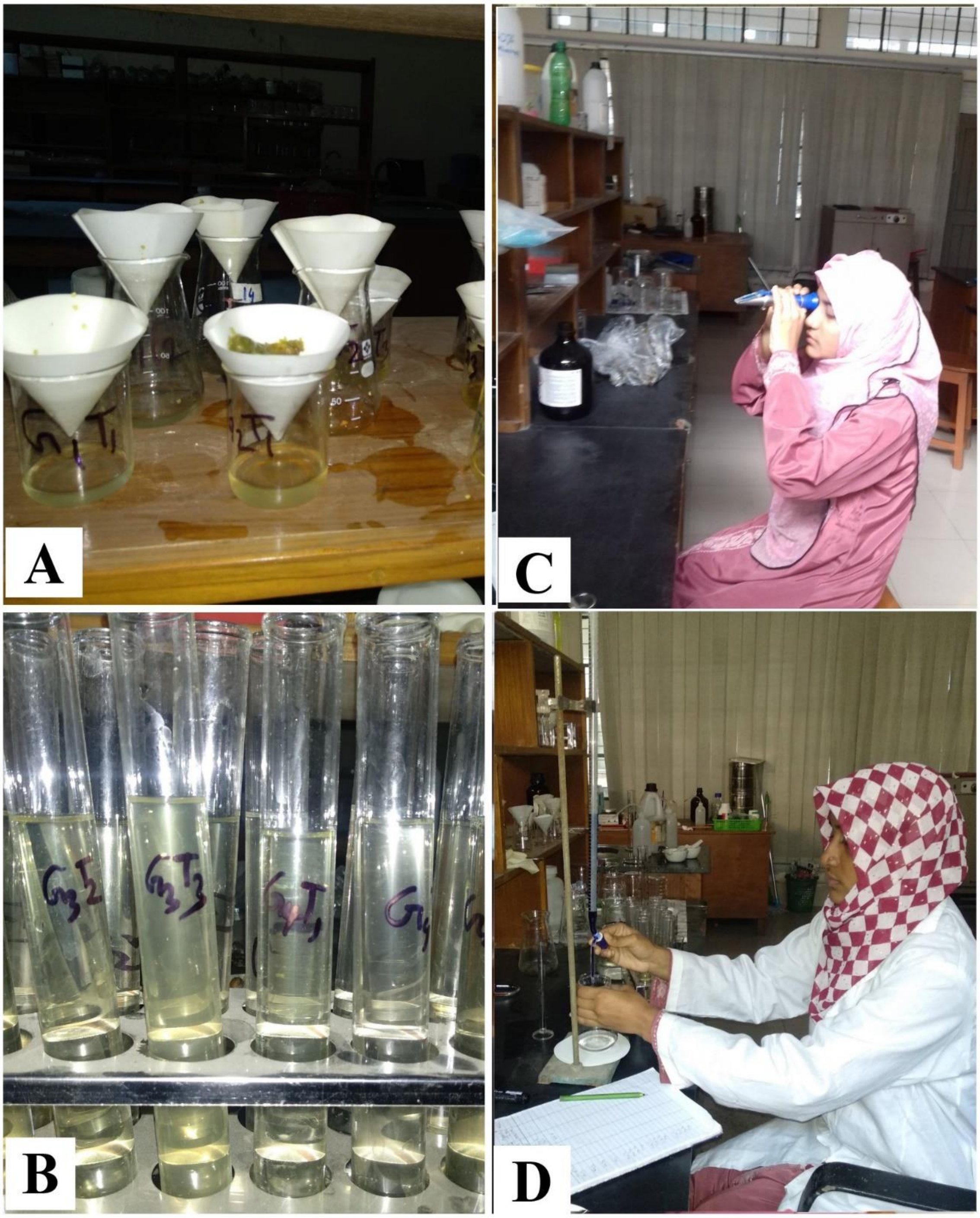


**Plate 2. Different data recording steps. A and B) Data recording steps in the Laboratory on proline content, C) Determination of Brix (%) by using Portable Refractometer in the Laboratory and D) Estimation of Vitamin C by Oxidation Reduction Titration Method in the Laboratory**

(ERMA, Tokyo, Japan) at room temperature. Single tomatillo fruit was blended and juice was collected to measure Brix percentage in the experiment. Measurement of Brix (%) is shown in Plate 2 (C).

#### 3.12.3.2 Determination of Vitamin C content

Vitamin C was measured by Oxidation Reduction Titration Method (Tee *et al*., 1988) in the experiment. Single fruit was blend and then tomatillo extract was filtrated by Whatman No.1 filter paper. The filtered liquid was then mixed with 3% metaphosphoric acid solution. The titration was conducted in presence of glacial acetic acid and metaphosphoric acid to inhibit aerobic oxidation with dye solution (2, 6-dichlorophenol indophenol) in the experiment. The solution was titrated with dye and slightly pink color from purple was observed carefully and then the observations were recorded. The observations mean gave the amount of dye required to oxidize definite amount of L-ascorbic acid solution of unknown concentration, using L-ascorbic acid as known sample and finally Vitamin-C content determined for each tomatillo genotypes against different drought treatments. The steps of Vitamin C determination are shown in Plate 2 (D).

### 3.13 Statistical analysis

Collected data were statistically analyzed using by Statistix 10 software. Mean for every treatment and genotypes was calculated and analysis of variance for each character was performed by F-test (Variance Ratio). Difference between treatments was assessed by Least Significant Difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984). Co-efficient of variation (CV %) were also estimated using MSTAT-C.

# CHAPTER IV

# RESULTS AND DISCUSSION

The experimental work was accomplished for the evaluation of four tomatillo genotypes to different drought treatments based on agromorphogenic, physiological and nutritional traits. In this chapter the findings of accomplished experimental work have been put forwarded and discussed. Data have been presented in table(s) for easy discussion, comprehension and understanding of the experiment. A summary of all the parameters have been shown in appendices for easy understanding. Results have been presented, discussed and promising interpretations are given in the following heads.

## 4.1 Agromorphogenic traits

### 4.1.1 Days to first flowering

From the result of the experiment it was observed that statistically significant variation was not found among the tomatillo genotypes in respect of days to first flowering (Appendix IV). Longest period was required (55.50 days) for the days to first flowering in $G_4$ while shortest period in $G_2$ (46.58 days) in Table 2.

Days to first flowering was significantly varied by different drought treatments (Appendix IV). Days taken to first flowering was earlier in $T_1$ (47.49 days) than $T_3$ (51.25 days) in Table 3.

Interaction of tomatillo genotypes and drought treatments affects non-significantly on days to first flowering (Appendix IV). $G_4T_3$ treatment required maximum period (60.50 days) whereas minimum from the $G_2T_1$ (45.50 days) which was statistically similar with $G_1T_1$ (46.00 days), $G_2T_2$ (46.25 days), $G_3T_1$ (47.25 days) and $G_1T_2$ (47.25 days) in Table 4.

The four tomatillo genotypes varied significantly under drought in days to first flowering. The reduction percentage of days to first flowering at treatment $T_2$

**Table 2. Mean performance of tomatillo genotypes on days to first flowering, days to maturity, plant height, no. of fruits/plant and average fruit weight/plant[Y]**

| Genotype [X] | Days to first flowering (DAS) | Days to maturity (DAT) | Plant height (cm) | No. of fruits/plant | Average fruit weight/plant (g) |
|---|---|---|---|---|---|
| **$G_1$** | 47.17 | 81.58 | 70.93 | 22.50 | 34.518 a |
| **$G_2$** | 46.58 | 81.33 | 71.52 | 18.92 | 27.478 b |
| **$G_3$** | 48.08 | 86.33 | 63.07 | 20.83 | 22.632 c |
| **$G_4$** | 55.50 | 88.17 | 64.01 | 25.00 | 13.895 d |
| **CV%** | 3.02 | 4.98 | 11.07 | 19.10 | 20.55 |
| **$LSD_{0.05}$** | ----- | ----- | ----- | ----- | 2.7925 |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$
[Y]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 3. Mean performance of genotypes under different drought treatments on days to first flowering, days to maturity, plant height, no. of fruits/plant, average fruit weight/plant[Y]**

| Treatment[X] | Days to first flowering (DAS) | Days to maturity (DAT) | Plant height (cm) | No. of fruits/plant | Average fruit weight/plant (g) |
|---|---|---|---|---|---|
| **$T_1$** | 47.44 c | 98.00 a | 77.08 | 28.13 a | 30.513 a |
| **$T_2$** | 49.31 b | 83.38 b | 73.11 | 23.44 b | 26.598 b |
| **$T_3$** | 51.25 a | 71.69 c | 51.95 | 13.88 c | 16.781 c |
| **CV%** | 3.02 | 4.98 | 11.07 | 19.10 | 20.55 |
| **$LSD_{0.05}$** | 1.0723 | 3.0234 | ----- | 2.9974 | 2.4184 |

[X]Three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water
[Y]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

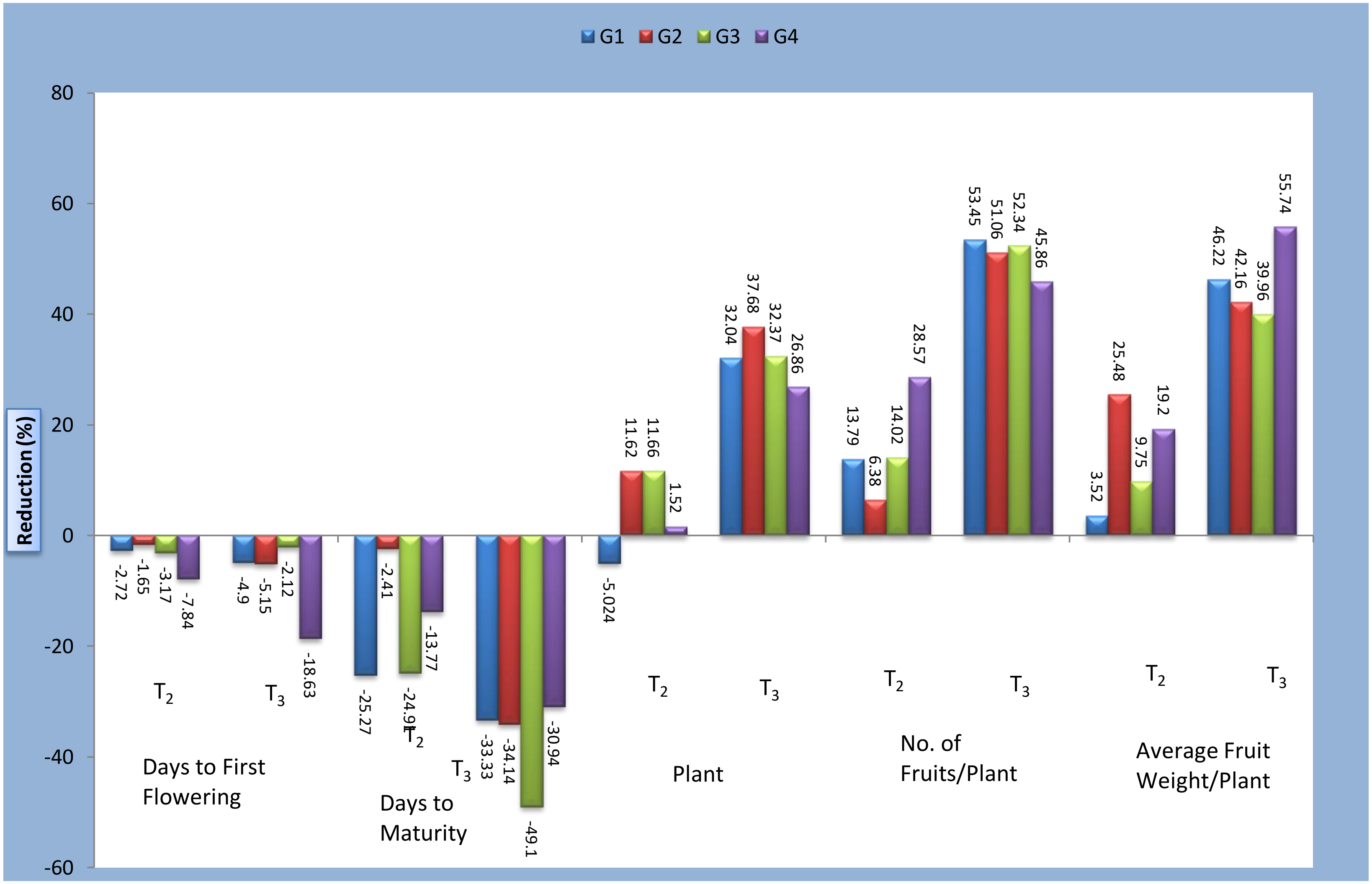


**Figure 1. Reduction percentage of days to flowering, days to maturity, plant height, no. of fruits/plant and average fruit weight/plant with increasing drought stress**

and $T_3$ is presented in Appendix VIII. Days to first flowering increased with drought treatments. Maximum reduction (late flowering) was observed in genotype $G_4$ (reduction percentage -7.84%) at moderate drought stress as well as severe drought stress (reduction percentage -18.63%) amongst all genotypes but minimum reduction (early flowering) was found in genotype $G_2$ (reduction percentage -1.65) at moderate stress and in genotype $G_3$ (reduction percentage -2.12%) at severe stress in Figure 1. So, $G_2$ is considered as a source of early flowering genotype in moderate stress and $G_3$ is considered in severe drought stress.

### 4.1.2 Days to maturity

From the result of the experiment it was observed that days to first fruit harvest from date of transplanting showed statistically non-significant variation among different tomatillo genotypes in Appendix IV. Longest period (88.17 days) was required for harvesting in $G_4$ genotype whereas shortest period (81.33 days) was required for $G_2$ genotype in Table 2.

Days to maturity were significantly affected by drought treatments (Appendix IV). Early harvesting was performed in treatment $T_3$ (71.69 days) treated tomatillo genotypes and delayed in $T_1$ (98.00 days). Treatment $T_2$ required 83.38 days in Table 3. Maturity time decreases with the increasing drought levels in tomatillo plants. Sibomana and Aguyoh (2013) found similar results in tomato and stated that maturity time decreases with increasing drought levels. Interaction effect of genotypes and drought treatments was found non-significant for days to maturity (Appendix IV). In this case earlier harvesting period (68.25 days) was observed in $G_1T_1$ followed by the $G_3T_1$ (69.25 days), $G_2T_1$ (72.50 days) and $G_2T_2$ (74.25 days) whereas delayed in $G_3T_3$ (103.25 days) followed by $G_4T_3$ (100.50 days) and $G_2T_3$ (97.25 days) in Table 4.

The four tomatillo genotypes varied significantly under drought in days to maturity. The reduction percentage of days to maturity at treatment $T_2$ and $T_3$

**Table 4. Interaction effect of tomatillo genotypes and drought treatments on days to first flowering, days to maturity, plant height, no. of fruits/plant and average fruit weight/plant (g)[Y]**

| Interaction[X] | Days to first flowering (DAS) | Days to maturity (DAT) | Plant height (cm) | No. of fruits/plant | Average fruit weight/plant (g) |
|---|---|---|---|---|---|
| $G_1 \times T_1$ | 46.00 | 68.25 | 77.95 | 29.00 | 41.378 |
| $G_1 \times T_2$ | 47.25 | 85.50 | 81.86 | 25.00 | 39.923 |
| $G_1 \times T_3$ | 48.25 | 91.00 | 52.97 | 13.50 | 22.253 |
| $G_2 \times T_1$ | 45.50 | 72.50 | 85.59 | 23.50 | 29.220 |
| $G_2 \times T_2$ | 46.25 | 74.25 | 75.64 | 22.00 | 21.775 |
| $G_2 \times T_3$ | 48.00 | 97.25 | 53.34 | 11.25 | 16.900 |
| $G_3 \times T_1$ | 47.25 | 69.25 | 74.09 | 26.75 | 32.935 |
| $G_3 \times T_2$ | 48.75 | 86.50 | 65.33 | 23.00 | 29.725 |
| $G_3 \times T_3$ | 48.25 | 103.25 | 49.78 | 12.75 | 19.775 |
| $G_4 \times T_1$ | 51.00 | 76.75 | 70.69 | 33.25 | 18.520 |
| $G_4 \times T_2$ | 55.00 | 87.25 | 69.62 | 23.75 | 14.968 |
| $G_4 \times T_3$ | 60.50 | 100.50 | 51.70 | 18.00 | 8.197 |
| **CV%** | 3.02 | 4.98 | 11.07 | 19.10 | 20.55 |
| **$LSD_{0.05}$** | ----- | ----- | ----- | ----- | ----- |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$ and three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water

[Y]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

is presented in Appendix VIII. Days to maturity was observed to increase with increasing drought treatments. Days to maturity increased maximum in genotype $G_1$ (reduction percentage -25.27%) at moderate drought stress and in genotype $G_3$ (reduction percentage -49.10%) in case of severe stress amongst all genotypes in Figure 1. Early maturity was observed in genotype $G_2$ (reduction percentage -2.41%) at moderate stress and in genotype $G_4$ (reduction percentage -30.94%) at severe stress. So, $G_2$ is considered as a source of early maturity genotype in moderate stress and $G_4$ is considered as an early maturity genotype in severe stress.

### 4.1.3 Plant height (cm)

From the result of the experiment it was observed that plant height showed statistically non-significant variation among four tomatillo genotypes (Appendix IV). Tallest plant was obtained from $G_2$ (71.52 cm) which was statistically similar with $G_1$ (70.93 cm) whereas shortest from $G_3$ (63.07 cm) which was statistically similar with $G_4$ (64.01 cm) in Table 2. The result showed that $G_2$ genotype gives the highest plant height.

The results also revealed that plant height was non-significantly influenced by drought stress (Appendix IV). Tallest plant was found at $T_1$ (77.08 cm) which is statistically similar with $T_2$ (73.11 cm) while shortest plant height from $T_3$ (51.95 cm) in Table 3. Drought stress caused a significant reduction in plant height. It also disturbs physiological processes and exposes plants to drought stress, which is reflected in low water absorption and transmission to different portions of the plant, as a result plant height gradually decreases. Similar consequences were stated by Wahb-Allah *et al*. (2001).

Plant height showed non-significant interaction effect between tomatillo genotypes and drought treatments (Appendix IV). Tallest plant was observed in $G_2T_1$ (85.59 cm) followed by $G_1T_2$ (81.86 cm), $G_1T_1$ (77.95 cm) and $G_2T_2$

(75.64 cm) whereas shortest plant was found from $G_3T_3$ (49.78 cm) followed by $G_4T_3$ (51.70 cm), $G_1T_3$ (52.97 cm) and $G_2T_3$ (53.34 cm) in Table 4.

The mean value of plant height of four genotypes showed significant variation under drought stresses. The reduction percentage of plant height at treatment $T_2$ and $T_3$ is presented in Appendix VIII. All genotypes showed shorter plant height than control with increasing drought stress except $G_1$. Increasing of plant height was observed (reduction percentage -5.02%) in genotype $G_1$ at moderate drought stress. Plants adapt different mechanisms to overcome the effect of drought. May be $G_1$ genotype overcome moderate drought effect by increasing its height. Decreasing of plant height was observed minimum in $G_4$ for both cases of moderate and severe drought stress amongst all genotypes (reduction percentage 1.52% and 26.86% respectively) in Figure 1.

### 4.1.4 Number of fruits per plant

Number of fruits/plant was not significantly varied statistically among different tomatillo genotypes (Appendix IV). Maximum number of fruits (25.00 / plant) was found from $G_4$ whereas minimum (18.92 / plant) was found in $G_2$ in Table 2. According to the present study $G_4$ afforded the maximum number of fruits per plant and $G_2$ was the minimum.

Number of fruits per plant was significantly varied statistically by drought treatments (Appendix IV). In Table 3 the highest fruit number (28.13 / plant) was found in $T_1$ whereas $T_3$ provide the lowest number of fruits (13.88 / plant). Maximum fruits per plant were found in control. Godina *et al*. (2013) found similar number of fruits in tomatillo. Number of fruits decrease in the plants, when they experienced drought stress during the early fruiting stage, would have been owing to reduced fruit size and fruit number. The fruits of plant treated with different drought level at this stage were smaller than those of the control in tomatillo plant. The reduction in the fruit number was due to dropping of immature fruits for applying different drought treatments in tomatillo. During the period of fruit enlargement, considerable amounts of carbohydrates and water are transported to the fruits as a process of fruit development. Therefore, size of the fruit largely depends on this phase in crop life cycle (Kozlowski, 1972).

Interaction of tomatillo genotypes and drought treatments non-significantly affects the number of fruits per plant (Appendix IV). Maximum number of the fruits (33.25 / plant) were obtained from $G_4T_1$ followed by $G_1T_1$ (29.00 / plant) whereas minimum number of fruits (11.25 / plant) was found in $G_2T_3$ in the Table 4.

The four tomatillo genotypes varied significantly under drought in number of fruits/plant. The reduction percentage of number of fruits per plant at treatment $T_2$ and $T_3$ is presented in Appendix VIII. Number of fruits per plant decreased minimum in genotype $G_2$ (reduction percentage 6.38%) at moderate stress while in genotype $G_4$ at severe drought stress (reduction percentage 45.86%) in Figure 1. So, $G_2$ and $G_4$ could be considered as the best genotype as they reduce fruit number less in moderate and severe stress respectively.

### 4.1.5 Average fruit weight per plant (g)

From the result of the experiment it was observed that average fruit weight per plant showed statistically significant variation among tomatillo genotypes in Appendix IV and Plate 4. $G_1$ tomatillo genotype had the maximum average fruit weight (34.52 g/plant) while minimum fruit weight (13.90 g/plant) was obtained in $G_4$ tomatillo genotype in Table 2. All means are significantly different from one another.

Average fruit weight per plant showed statistically significant variation with different drought treatments and they were significantly different from one another (Appendix IV). Maximum average fruit weight (30.51 g/plant) was obtained in $T_1$ whereas minimum average fruit weight (16.78 g/plant) was found in $T_3$ in Table 3. Naumova et al. (2018) found similar result in field grown tomatillo. May be less water flow in the fruit causes decrease fruit size and therefore reduces the fruit weight. Drought affects plant growth and development (Ferrara *et al*., 2011), and limits agricultural crop production (Rebey *et al*., 2012).

Interaction of tomatillo genotypes and drought treatments non-significantly affects the average fruit weight (Appendix IV). The highest average fruit weight (41.38 g/plant) was obtained from $G_1T_1$ followed by $G_1T_2$ (39.92 g/plant) while the lowest average fruit weight (8.20 g/plant) was found in $G_4T_3$ in Table 4.

The reduction percentage in average fruit weight per plant at treatment $T_2$ and $T_3$ is presented in Appendix VIII. Average fruit weight per plant decreased minimum in genotype $G_1$ at moderate drought stress (reduction percentage 3.52%) and in genotype $G_3$ at severe drought stress (reduction percentage 39.96%) in Figure 1. $G_1$ and $G_3$ could be considered as the best genotypes for moderate and severe stress respectively which can be used to transfer this trait to a potential yield contributing genotype.

### 4.1.6 Leaf length (cm)

From the result of the experiment it was observed that leaf length showed statistically significant variation among different tomatillo genotypes (Appendix V). Longest leaf length (10.38 cm) was observed in $G_1$ genotype whereas shortest leaf length (6.43 cm) was observed in $G_4$ genotype in the Table 5.

Leaf length was non-significantly affected by drought treatments (Appendix V). Longest leaf length was observed in treatment $T_1$ (9.21 cm) treated tomatillo genotypes while the shortest leaf length was found in $T_3$ (7.23 cm) in Table 6 and Plate 3. Hossain *et al*. (2017) indicated that LAI significantly influenced by deficit irrigation for both crops and methods that achieved the highest yield. A morphological comparison of leaf length between control and drought stressed plants are presented in Plate 3.

Interaction effect of genotypes and drought treatments was found non-significant for leaf length (Appendix V). Longest leaf length was observed in $G_1T_2$ (11.13 cm) followed by $G_1T_1$ (11.00 cm), $G_2T_2$ (9.65 cm), $G_2T_1$ (9.60 cm), $G_3T_1$ (9.33 cm) and $G_1T_3$ (9.00 cm) whereas shortest was observed in $G_4T_3$ (5.73 cm) in Table 7.

The four tomatillo genotypes varied non-significantly under drought in leaf length (cm). The reduction percentage of leaf length (cm) at treatment $T_2$ and $T_3$ is presented in Appendix IX. $G_3$ and $G_4$ genotypes showed decreasing of leaf length (reduction percentage 14.48% and 3.97% respectively) while increasing of leaf length was observed in genotype $G_1$ and $G_2$ (reduction percentage -1.14% and -0.52% respectively) at moderate drought stress. All genotypes showed decreasing leaf length (cm) in case of severe drought stress and minimum reduction was observed in $G_4$ (reduction percentage 17.33%) amongst all genotypes in Figure 2. May be $G_1$ and $G_2$ reduced the effects of drought by increasing leaf length (cm) at moderate stress which need further study in future to conclude. We can consider $G_4$ as the best variety as it showed minimum leaf length (cm) reduction because leaf indirectly influences yield as it is food producing part.

### 4.1.7 Leaf width (cm)

From the result of the experiment it was observed that leaf width (cm) showed statistically significant variation among different tomatillo genotypes (Appendix V). Longest leaf width (3.65 cm) was observed in $G_1$ genotype whereas shortest leaf width (2.03 cm) was observed in $G_4$ genotype which is statistically similar with $G_3$ (2.24 cm) in Table 5.

Leaf width (cm) was non-significantly affected by drought treatments in the Appendix V. Longest leaf width (cm) was observed in treatment $T_1$ (3.11 cm)

**Table 5. Mean performance of tomatillo genotypes on leaf length (cm), leaf width (cm), leaf L × W ($cm^2$) and leaf L/W[Y]**

| Genotype[X] | Leaf length (cm) | Leaf width (cm) | Leaf L × W ($cm^2$) | Leaf L/W |
|---|---|---|---|---|
| $G_1$ | 10.38 a | 3.65 a | 38.95 a | 2.91 b |
| $G_2$ | 9.00 b | 2.97 b | 28.50 b | 3.10 b |
| $G_3$ | 7.91 b | 2.24 c | 18.15 c | 3.56 a |
| $G_4$ | 6.43 c | 2.03 c | 13.41 c | 3.18 ab |
| CV% | 18.14 | 20.39 | 40.34 | 15.01 |
| $LSD_{0.05}$ | 1.2699 | 0.4611 | 8.2935 | 0.3971 |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$
[Y]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 6. Mean performance of genotypes under different drought treatments on leaf length (cm), leaf width (cm), leaf L×W ($cm^2$) and leaf L/W[Y]**

| Treatment[X] | Leaf length (cm) | Leaf width (cm) | Leaf L×W ($cm^2$) | Leaf L/W |
|---|---|---|---|---|
| $T_1$ | 9.21 | 3.11 | 30.01 | 3.09 |
| $T_2$ | 8.85 | 2.75 | 26.51 | 3.30 |
| $T_3$ | 7.23 | 2.31 | 17.74 | 3.17 |
| CV% | 18.14 | 20.39 | 40.34 | 15.01 |
| $LSD_{0.05}$ | ---- | ---- | ---- | ---- |

[X]Three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water
[Y]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: Interaction effect of tomatillo genotypes and drought treatments on leaf length (cm), leaf width (cm), leaf L × W ($cm^2$) and leaf L/W[Y]**

| Interaction[X] | Leaf length (cm) | Leaf width (cm) | Leaf L × W ($cm^2$) | Leaf L/W |
|---|---|---|---|---|
| $G_1 \times T_1$ | 11.00 | 4.05 | 45.02 | 2.71 |
| $G_1 \times T_2$ | 11.13 | 4.05 | 44.87 | 2.76 |
| $G_1 \times T_3$ | 9.00 | 2.85 | 26.97 | 3.25 |
| $G_2 \times T_1$ | 9.60 | 3.48 | 34.22 | 2.90 |
| $G_2 \times T_2$ | 9.65 | 2.80 | 30.39 | 3.44 |
| $G_2 \times T_3$ | 7.75 | 2.63 | 20.88 | 2.96 |
| $G_3 \times T_1$ | 9.33 | 2.60 | 24.35 | 3.75 |
| $G_3 \times T_2$ | 7.98 | 2.18 | 17.51 | 3.65 |
| $G_3 \times T_3$ | 6.43 | 1.95 | 12.59 | 3.29 |
| $G_4 \times T_1$ | 6.93 | 2.33 | 16.43 | 3.01 |
| $G_4 \times T_2$ | 6.65 | 1.98 | 13.27 | 3.35 |
| $G_4 \times T_3$ | 5.73 | 1.80 | 10.59 | 3.17 |
| **CV%** | 18.14 | 20.39 | 40.34 | 15.01 |
| **$LSD_{0.05}$** | ---- | ---- | ---- | ---- |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$ and three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water

[Y]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

treated tomatillo genotypes while the shortest leaf width was found in $T_3$ (2.31 cm) in Table 6. A morphological comparison of leaf width between control and drought stressed plants are presented in Plate 3.

Interaction effect of genotypes and drought treatments was found non-significant for leaf width (Appendix V). Longest leaf width was observed in $G_1T_1$ (4.05 cm) followed by $G_1T_2$ (4.05 cm) and $G_2T_1$ (3.48 cm) whereas shortest was observed in $G_4T_3$ (1.80 cm) in Table 7.

The four tomatillo genotypes varied non-significantly under drought in leaf width (cm). The reduction percentage of leaf width at treatment $T_2$ and $T_3$ is presented in Appendix IX. All genotypes showed decreasing of leaf width (cm) with increasing drought treatments. Decreasing of leaf width was observed minimum in genotype $G_1$ (reduction percentage 0%) at moderate drought stress and genotype $G_4$ showed minimum reduction (reduction percentage 22.58%) amongst all genotypes in case of severe drought stress in Figure 2.

### 4.1.8 Leaf length×width (cm$^2$)

It was observed from the experimental result that leaf L×W (cm$^2$) showed statistically significant variation among different tomatillo genotypes (Appendix V). Highest leaf L×W (38.95 cm$^2$) was observed in $G_1$ genotype whereas lowest leaf L×W (13.41 cm$^2$) was observed in $G_4$ genotype which is statistically similar with $G_3$ (18.15 cm$^2$) in Table 5.

Leaf L×W (cm$^2$) was non-significantly affected by drought treatments (Appendix V). Highest leaf L×W (cm$^2$) was observed in treatment $T_1$ (30.01 cm$^2$) treated tomatillo genotypes and lowest leaf L×W was found in $T_3$ (17.74 cm$^2$) in Table 6. Hossain *et al*. (2017) also found non-significant leaf area in tomato and cucumber by applying drought stress. The leaf area is used as an indicative of productivity and can be useful for cultural technical evaluations, as in seeding density, irrigation, fertilization, and other traits like agrochemicals application (Favarin *et al.*, 2002). So leaf area estimation is very important for tomatillo to determine seeding density, irrigation, fertilization.

Interaction effect of genotypes and drought treatments was found non-significant for leaf L×W ($cm^2$) in Appendix V. Highest leaf L×W ($cm^2$) was observed in $G_1T_1$ (45.02 $cm^2$) followed by $G_1T_2$ (44.87 $cm^2$) whereas lowest was observed in $G_4T_3$ (10.54 $cm^2$) in Table 7.

The reduction percentage of the leaf L×W ($cm^2$) at treatment $T_2$ and $T_3$ is presented in Appendix IX. All genotypes showed decreasing of leaf L×W ($cm^2$) with increasing drought treatments. Decreasing of leaf L×W ($cm^2$) was observed minimum in genotype $G_1$ (reduction percentage 0.33%) at moderate drought stress and in genotype $G_4$ (reduction percentage 35.87%) amongst all genotypes in case of severe drought stress in Figure 2. So, genotype $G_1$ and $G_4$ could be suggested for moderate and severe stress regions in Bangladesh respectively and they may be considered as potential yield contributing genotypes as leaf area indicates food producing unit for plant.

**4.1.9 Leaf length/width**

From the result of the experiment it was observed that leaf L×W ratio showed statistically significant variation among different tomatillo genotypes (Appendix V). Highest leaf L/W ratio (3.56) was observed in $G_3$ genotype whereas the lowest leaf L/W (2.91) was observed in $G_1$ genotype in Table 5.

Leaf L/W ratio was non-significantly affected by drought treatments (Appendix V). Highest leaf L/W ratio was observed in the treatment $T_2$ (3.30) treated genotypes and lowest $T_1$ (3.17). There were no significant pairwise differences among the means of leaf L/W ratio in Table 6.

Interaction effect of genotypes and drought treatments was found non-significant for leaf L/W ratio (Appendix V). Highest leaf L/W ratio was observed in $G_3T_1$ (3.75) whereas lowest was observed in $G_1T_1$ (2.71) in Table 7.

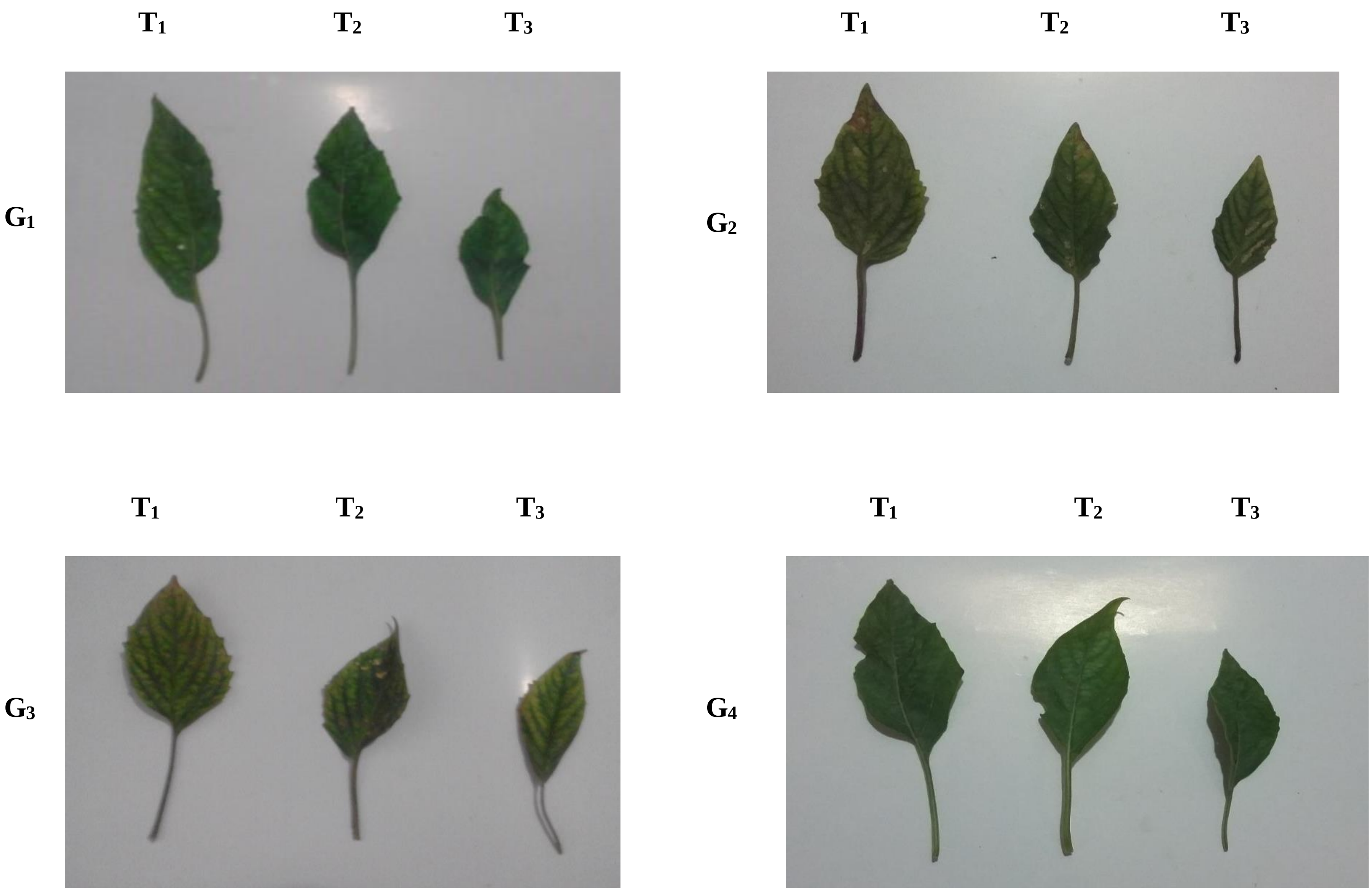


**Plate 3. Morphological comparison of leaves under control and stress conditions. $G_1$ (SAU Tomatillo-1), $G_2$ (PI003), $G_3$ (SAU Tomatillo-2), $G_4$ (PI004) and three drought treatments viz. $T_1$ (control), $T_2$ (30 days withholding of water), $T_3$ (45 days withholding of water)**

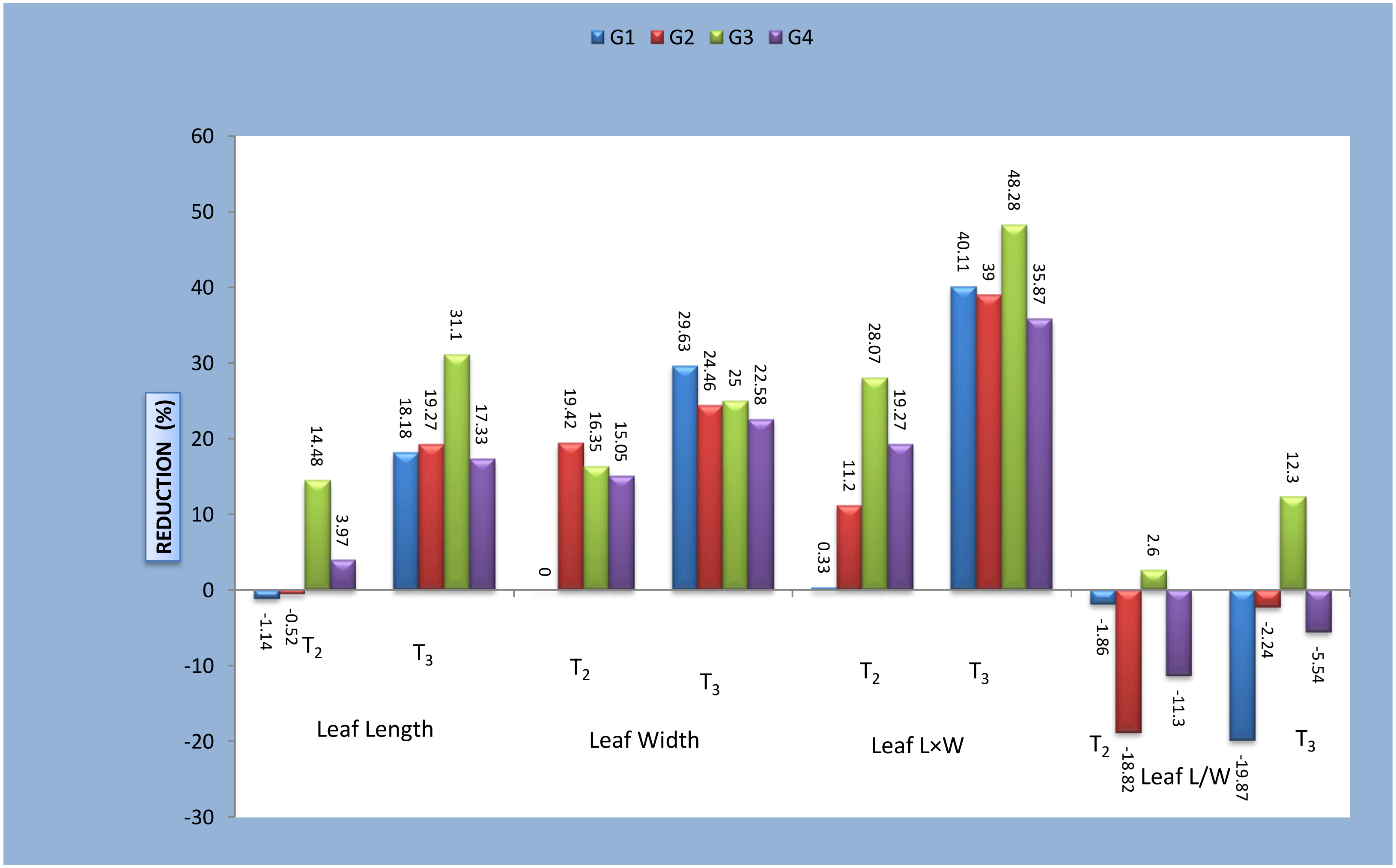


**Figure 2. Reduction percentage of leaf length (cm), leaf width (cm), leaf length×width ($cm^2$) and leaf length/width with increasing drought stress**

The leaf L/W ratio of four tomatillo genotypes varied non-significantly under drought. The reduction percentage of days to maturity at treatment $T_2$ and $T_3$ is presented in Appendix IX. All genotypes showed increasing leaf L/W ratio except $G_3$. Increasing of leaf L/W ratio was observed maximum in genotype $G_2$ (reduction percentage -18.82%) at moderate drought stress and genotype $G_1$ showed maximum reduction (reduction percentage -19.87%) amongst all genotypes in case of severe drought stress in Figure 2. $G_3$ showed minimum leaf L/W ratio at both moderate and severe stresses (reduction percentage 2.60% and 12.3% respectively) in Figure 2.

### 4.1.10 Number of seeds per fruit

Number of seeds per fruit was not significantly varied statistically among different tomatillo genotypes (Appendix V). Maximum number of seeds (353.58 / fruit) was found from $G_1$ followed by $G_2$ (332.58 / fruit) whereas minimum (219.17 / fruit) was found in $G_4$ in Table 8.

Number of seeds per fruit was significantly varied statistically by drought treatments (Appendix V). The highest seed number (342.75 /fruit) was found in $T_1$ whereas $T_3$ provide the lowest number of seeds (242.06 /fruit) in Table 9. Ozalsan *et al*. (2016) found number of seeds of *Physalis* weeds decreased with increasing water stress.

Interaction of tomatillo genotypes and drought treatments non-significantly affects the number of seeds per fruit (Appendix V). Maximum number of seeds (439.00 /fruit) were obtained from $G_1T_1$ whereas minimum number of seeds (184.25 /fruit) was found in $G_4T_3$ in Table 10.

The four tomatillo genotypes varied non-significantly under drought in number of seeds per fruit. The reduction percentage of number of fruits per plant at treatment $T_2$ and $T_3$ is presented in Appendix X. All the genotypes showed decreasing number of seeds per fruit with increasing drought stress. Number of seeds per fruit decreased minimum in genotype $G_4$ because the reduction percentage at 30 days was minimum (reduction percentage 4.24%) and in genotype $G_2$ at severe drought stress (reduction percentage 21.36%) in the Figure 3.

### 4.1.11 Average fruit length (cm)

Statistically non-significant variation was found for average fruit length among tomatillo genotypes (Appendix IV). Maximum fruit length (1.32 cm) was found from $G_1$ while the shortest one found from $G_4$ (0.95 cm) which is statistically similar with $G_3$ (0.98 cm) and $G_2$ (1.03 cm) in Table 8. Average fruit length of different genotypes is shown in Plate 4.

Average fruit length statistically varied significantly with different drought treatments (Appendix IV). Maximum fruit length (1.26 cm) was found in $T_1$ whereas the shortest (0.84 cm) in $T_3$ in Table 9 and Plate 4. Decrease in fruit length and diameter owing to the increase of drought levels was also found by Klepper *et al*. (1971). Results showed that the fruit length and diameter reflect variations in fruit tissue hydration. On the other hand, well-watered (control condition) plants had an increase in fruit length and diameter compared to moderate and severe stressed tomatillo plants.

Interaction between tomatillo genotypes and drought treatments non-significantly affects the fruit length (Appendix IV). Maximum fruit length (1.59 cm) was recorded from $G_1T_1$ whereas shortest (0.76 cm) from $G_2T_3$ combination followed by $G_4T_3$ (0.80 cm) and $G_3T_3$ (0.81 cm) in Table 10. Fruit size is reduced by drought stress mainly due to shorter fruit growth period in tomatillo.

The four tomatillo genotypes varied non-significantly under drought in average fruit length (cm) and all genotypes showed decreasing fruit length (cm) with increasing drought stresses. The reduction percentage of average fruit length (cm) at treatment $T_2$ and $T_3$ is presented in Appendix X. Average fruit length (cm) decreased minimum in genotype $G_2$ (reduction percentage 3.96%) at moderate drought stress and in genotype $G_4$ (reduction percentage 27.12%) at severe drought stress in Figure 3. So, $G_2$ could be considered as the best genotype for moderate stress while $G_4$ for severe drought stress because fruit length is very important trait as it influences total fruit production of any crop by determining fruit size.

### 4.1.12 Average fruit diameter (cm)

Statistically non-significant variation was recorded for fruit diameter (cm) among tomatillo genotypes. Maximum fruit diameter (1.4088 cm) was obtained from $G_1$ and minimum (1.02 cm) was measured from $G_4$ in Table 8 and in Plate 4.

Fruit diameter was significantly varied statistically with different drought treatments (Appendix VI). Maximum fruit diameter (1.36 cm) was recorded from $T_1$ whereas minimum (0.90 cm) from $T_3$ treatment in Table 9 and Plate 4. Reduction in fruit length and diameter due to the increase of drought levels was also found by Klepper *et al*. (1971). Results indicates that the fruit length and diameter changes due to the changes in fruit tissue hydration. On the other hand, well-watered plants had an increase in fruit length and diameter compared to the moderate and severe stressed plants in the experiment. Godina *et al*. (2013) found similar fruit diameter in tomatillo.

Interaction between tomatillo genotypes and drought treatments non-significantly affects the fruit diameter (Appendix VI). Maximum fruit diameter (1.76 cm) was obtained from $G_1T_1$ whereas minimum (0.85 cm) from $G_2T_3$ in Table 10.

The four tomatillo genotypes varied non-significantly under drought for average fruit diameter (cm) and all genotypes showed decreasing fruit diameter (cm) with increasing drought stresses. The reduction percentage of $T_2$ and $T_3$ is presented in Appendix X. Average fruit diameter decreased minimum in genotype $G_1$ and $G_2$ (reduction percentage 0% for both) at moderate drought stress and in genotype $G_4$ (reduction percentage 23.05%) at severe drought stress in Figure 3. So, $G_1$ and $G_2$ could be considered as the best genotypes for moderate stress while $G_4$ for severe drought stress because fruit diameter is very important trait as it influences total fruit production of any crop by determining fruit size.

### 4.1.13 Yield per plant (kg)

It was observed from the result of the experiment that the yield per plant was significantly varied statistically among tomatillo genotypes (Appendix IV). Maximum yield (0.8333 kg/plant) was found in $G_1$ whereas minimum yield (0.3697 kg/plant) was obtained in $G_4$ in Table 8. According to the present study $G_1$ genotype had the maximum yield and $G_4$ had the minimum yield. Perez *et al.* (2005) found similar yield in tomatillo in U.S.A.

The yield per plant was significantly influenced statistically by drought treatments (Appendix IV). The yield per plant was maximum (0.8435 kg/plant) in $T_1$ whereas minimum (0.2195 kg/plant) in $T_3$ in Table 9. Drought stress at flowering stage is very critical stage because it not only reduces flower formation but also increases flower shedding. Drought stress imposed on plants leads to decline yield through reducing seed set (Al-Ghzawi *et al.*, 2009; Westgate and Boyer, 1986). When plants expose to moisture stress at the flowering stage, a severe drop in flowering occurs and reduction in flower number decreases the amount of final yield of any crop. Hence, moisture stress during the flowering stage may have resulted in the highest reduction in yield in tomatillo.

Interaction between tomatillo genotypes and drought treatments significantly affected the yield per plant of tomatillo (Appendix IV). Maximum yield (1.1998 kg/plant) was obtained from $G_1T_1$ while minimum yield (0.1435 kg/plant) from $G_4T_3$ in Table 10.

$T_1$ $T_2$ $T_3$ $T_1$ $T_2$ $T_3$

$G_1$

$G_2$

$T_1$ $T_2$ $T_3$ $T_1$ $T_2$ $T_3$

$G_3$

$G_4$

**Plate 4. Morphological comparison of fruits under control and stress conditions. $G_1$ (SAU Tomatillo-1), $G_2$ (PI003), $G_3$ (SAU Tomatillo-2), $G_4$ (PI004) and three drought treatments viz. $T_1$ (control), $T_2$ (30 days withholding of water), $T_3$ (45 days withholding of water)**

**Table 8. Mean performance of tomatillo genotypes on average fruit length (cm), average fruit diameter (cm), no. of seeds/fruit and yield/plant (kg)[Y]**

| Genotype[X] | Average fruit length (cm) | Average fruit diameter (cm) | No. of seeds/fruit | Yield/plant (kg) |
|---|---|---|---|---|
| **$G_1$** | 1.32 | 1.41 | 353.58 | 0.8333 a |
| **$G_2$** | 1.03 | 1.17 | 332.58 | 0.5996 b |
| **$G_3$** | 0.98 | 1.11 | 285.00 | 0.4505 c |
| **$G_4$** | 0.95 | 1.02 | 219.17 | 0.3697 d |
| **CV%** | 9.32 | 8.87 | 10.02 | 25.34 |
| **$LSD_{0.05}$** | ---- | ---- | ---- | 0.0903 |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$

[Y]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 9. Mean performance of genotypes under different drought treatments on average fruit length (cm), average fruit diameter (cm), no. of seeds/fruit and yield/plant (kg)[Y]**

| Treatment[X] | Average fruit length (cm) | Average fruit diameter (cm) | No. of seeds/fruit | Yield/plant (kg) |
|---|---|---|---|---|
| **$T_1$** | 1.26 a | 1.36 a | 342.75 a | 0.8435 a |
| **$T_2$** | 1.11 b | 1.26 b | 307.94 b | 0.6268 b |
| **$T_3$** | 0.84 c | 0.90 c | 242.06 c | 0.2195 c |
| **CV%** | 9.32 | 8.87 | 10.02 | 25.34 |
| **$LSD_{0.05}$** | 0.0717 | 0.0750 | 21.443 | 0.0782 |

[X]Three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water

[Y]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

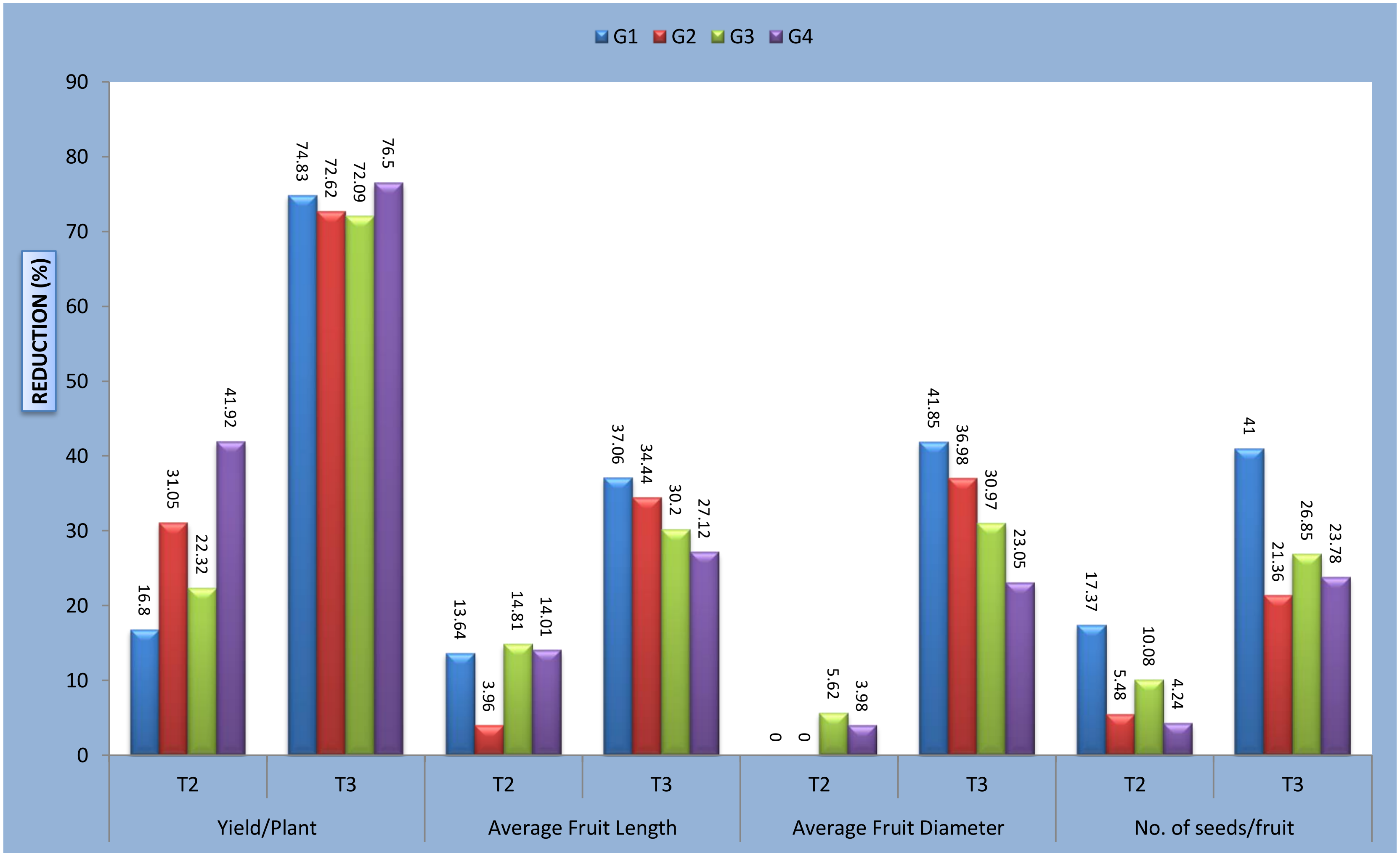


**Figure 3. Reduction percentage of yield/plant (kg), average fruit length (cm), average fruit diameter (cm) and no. of seeds/fruit with increasing drought stress**

**Table 10. Interaction effect of tomatillo genotypes and drought treatments on average fruit length (cm), average fruit diameter (cm), no. of seeds/fruit and yield/plant (kg)[Y]**

| Interaction[X] | Average fruit length (cm) | Average fruit diameter (cm) | No. of seeds/fruit | Yield/plant (kg) |
|---|---|---|---|---|
| **$G_1 \times T_1$** | 1.59 | 1.76 | 439.00 | 1.1998 a |
| **$G_1 \times T_2$** | 1.37 | 1.76 | 362.75 | 0.9982 b |
| **$G_1 \times T_3$** | 1.00 | 1.02 | 259.00 | 0.3020 h |
| **$G_2 \times T_1$** | 1.18 | 1.35 | 365.25 | 0.6885 d |
| **$G_2 \times T_2$** | 1.14 | 1.35 | 345.25 | 0.4747 e |
| **$G_2 \times T_3$** | 0.76 | 0.85 | 287.25 | 0.1885 g |
| **$G_3 \times T_1$** | 1.15 | 1.26 | 325.00 | 0.8750 c |
| **$G_3 \times T_2$** | 0.98 | 1.19 | 292.25 | 0.6797 bc |
| **$G_3 \times T_3$** | 0.81 | 0.87 | 237.75 | 0.2442 i |
| **$G_4 \times T_1$** | 1.10 | 1.12 | 241.75 | 0.6107 de |
| **$G_4 \times T_2$** | 0.95 | 1.07 | 231.50 | 0.3547 f |
| **$G_4 \times T_3$** | 0.80 | 0.86 | 184.25 | 0.1435 j |
| **CV%** | 9.32 | 8.87 | 10.02 | 25.34 |
| **$LSD_{0.05}$** | ---- | ---- | ---- | 0.1563 |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$ and three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water

[Y]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

The four tomatillo genotypes varied significantly under drought in yield per plant and all of them showed reduction in yield per plant with increasing droughts. The reduction percentage of yield per plant at treatment $T_2$ and $T_3$ is presented in Appendix X. Minimum reduction was found in genotype $G_1$ at moderate drought stress (reduction percentage 16.80%) and in genotype $G_3$ (reduction percentage 72.09%) at severe drought stress in Figure 3. $G_1$ can be considered as the best genotype for moderate stress regions while $G_3$ for prolonged and severe drought stress regions. Yield per plant is the most important trait for any crop as our final goal is to obtain the highest yield of any crop. So, $G_1$ and $G_3$ could be included for future breeding programs to improve yield contributing characters during drought stress.

### 4.2 Physiological traits

#### 4.2.1 Relative water content (RWC) (%)

It was observed from the result of the experiment that RWC (%) of leaves showed statistically significant variation among four tomatillo genotypes (Appendix VI). The highest Relative water content (89.02%) was found in $G_2$ whereas the lowest amount of RWC (79.70%) was found in $G_4$ in Table 11.

RWC (%) of leaves showed statistically significant variation among drought treatments in Appendix VI. The highest RWC (93.31%) was found in $T_1$ whereas lowest RWC (74.73%) in $T_3$ in Table 12. Schonfeld *et al.* (1988) reported that the cultivars that were resistant to drought had more RWC (%). Sivakumar (2014) stated that relative water content decreased with increasing drought stresses than control. Bandurska *et al*. (2017) also found gradual decrease of water content with increasing drought treatments.

RWC (%) of leaves was influenced non-significantly due to interaction between genotypes and drought treatments in Appendix VI. The highest relative water content (98.31%) was observed in $G_2T_1$ whereas the lowest relative water content (70.82%) was observed in $G_4T_3$ followed by $G_3T_3$ (71.57) in Table 13.

The four tomatillo genotypes varied significantly under drought in RWC (%) and all of them showed reduction of RWC (%) with increasing drought treatments. The reduction percentage of RWC (%) at treatment $T_2$ and $T_3$ is presented in Appendix XI. RWC (%) decreased minimum in genotype $G_1$ in case of both at moderate drought stress and at severe drought stress (reduction percentage 8.02% and 17.29% respectively) in Figure 4. So, $G_1$ is considered as a source of low RWC (%) for both moderate and severe stress.

### 4.2.2 Proline content (µg/g)

The result showed that proline content was varied significantly among the four tomatillo genotypes (Appendix VI). Maximum proline content (3573.8 µg/g) was found in $G_1$ whereas minimum (2382.1 µg/g) from $G_4$ in Table 11 and Plate 5. According to the study $G_1$ tomatillo genotypes have the highest proline content. Proline accumulation and root traits contribute to plants survival and productivity under soil water-limited stress (Manavalan *et al.*, 2009).

Proline content in tomatillo showed variation under different drought treatments (Appendix VI). Minimum proline content was obtained from $T_1$ (2050.1 µg/g) treated plant whereas the highest (4205.3 µg/g) was found in $T_3$ in Table 12 and Plate 5. Mwenye *et al.* (2016) found that proline accumulates with increased drought stress in soybean. Pan *et al.* (2006); estimated the amount of proline in grown tomatoes under drought stress and found increased proline concentrations.

Interaction of tomatillo genotypes and drought treatments significantly affects proline content in tomatillo (Appendix VI). Maximum proline content in tomatillo (4923.1 µg/g) was obtained from $G_1T_3$ while minimum (1620.8 µg/g) from $G_4T_1$ followed by $G_1T_1$ (1856.8 µg/g) in Table 13.

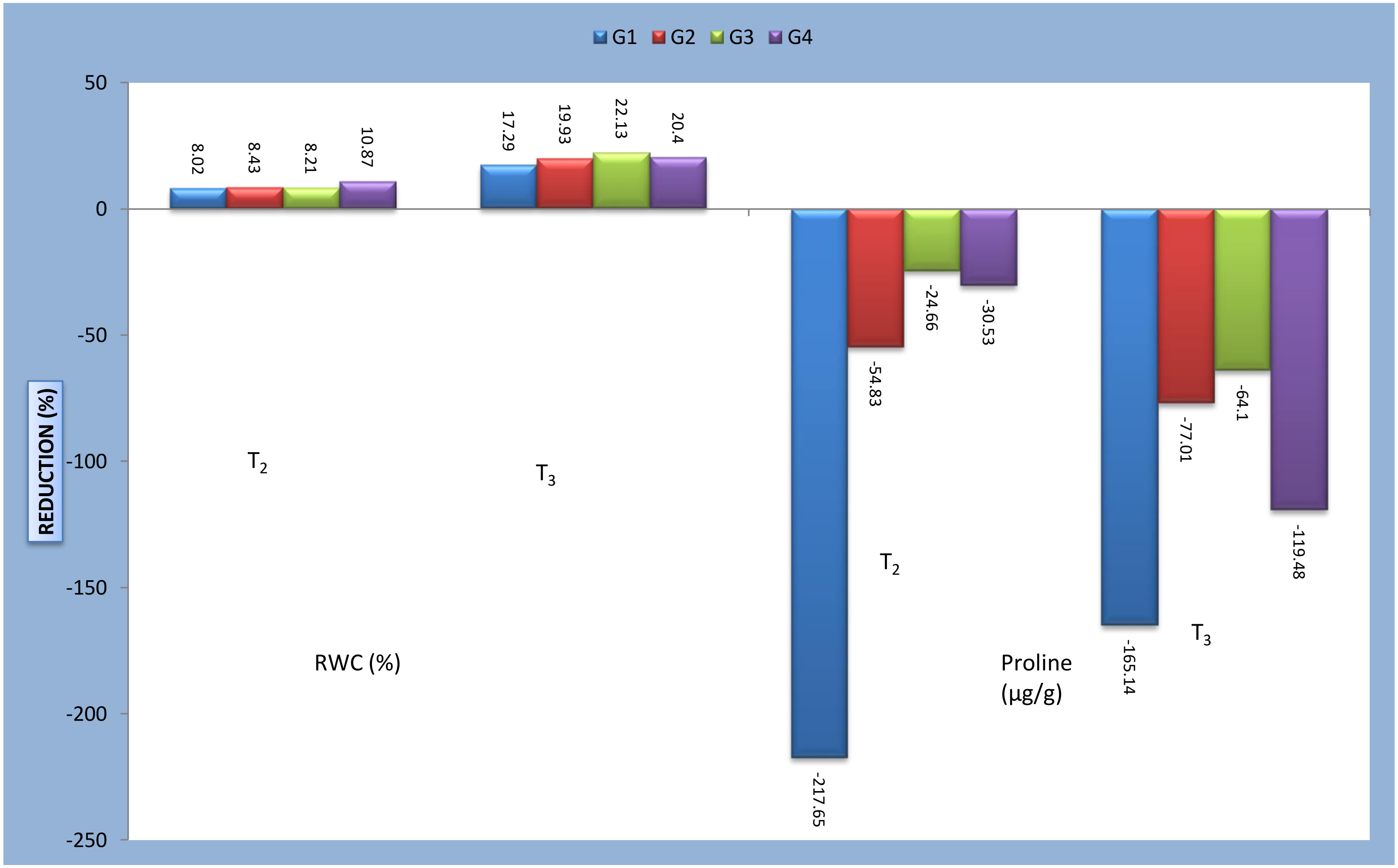


**Figure 4. Reduction percentage of RWC (%) and proline content (µg/g) with increasing drought stress**

**Table 11. Mean performance of tomatillo genotypes on relative water content (RWC) (%) and proline content (µg/g)[Y]**

| Genotype[X] | RWC (%) | Proline content (µg/g) |
|---|---|---|
| $G_1$ | 86.12 b | 3573.8 a |
| $G_2$ | 89.02 a | 3452.8 b |
| $G_3$ | 82.61 c | 3341.6 c |
| $G_4$ | 79.70 d | 2382.1 d |
| CV% | 2.25 | 4.21 |
| $LSD_{0.05}$ | 1.5776 | 218.85 |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$

[Y]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 12. Mean performance of genotypes under different drought treatments on relative water content (RWC) (%) and proline content (µg/g)[Y]**

| Treatment[X] | RWC (%) | Proline (µg/g) |
|---|---|---|
| $T_1$ | 93.31 a | 2050.1 c |
| $T_2$ | 85.05 b | 3307.3 b |
| $T_3$ | 74.73 c | 4205.3 a |
| CV% | 2.25 | 4.21 |
| $LSD_{0.05}$ | 1.3662 | 96.580 |

[X]Three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water

[Y]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. Interaction effect of tomatillo genotypes and drought treatments on relative water content (RWC) (%) and proline content (μg/g)[Y]**

| Interaction[X] | RWC (%) | Proline (μg/g) |
|---|---|---|
| **$G_1 \times T_1$** | 94.06 | 1856.8 hi |
| **$G_1 \times T_2$** | 86.51 | 4041.3 c |
| **$G_1 \times T_3$** | 77.80 | 4923.1 a |
| **$G_2 \times T_1$** | 98.31 | 2387.1 f |
| **$G_2 \times T_2$** | 90.02 | 3696.0 cd |
| **$G_2 \times T_3$** | 78.72 | 4225.3 b |
| **$G_3 \times T_1$** | 91.91 | 2507.9 f |
| **$G_3 \times T_2$** | 84.36 | 3126.4 e |
| **$G_3 \times T_3$** | 71.57 | 4115.5 b |
| **$G_4 \times T_1$** | 88.97 | 1620.8 i |
| **$G_4 \times T_2$** | 79.30 | 2115.6 gh |
| **$G_4 \times T_3$** | 70.82 | 3557.3 d |
| **CV%** | 2.25 | 4.21 |
| **$LSD_{0.05}$** | ---- | 379.06 |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$ and three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water

[Y]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

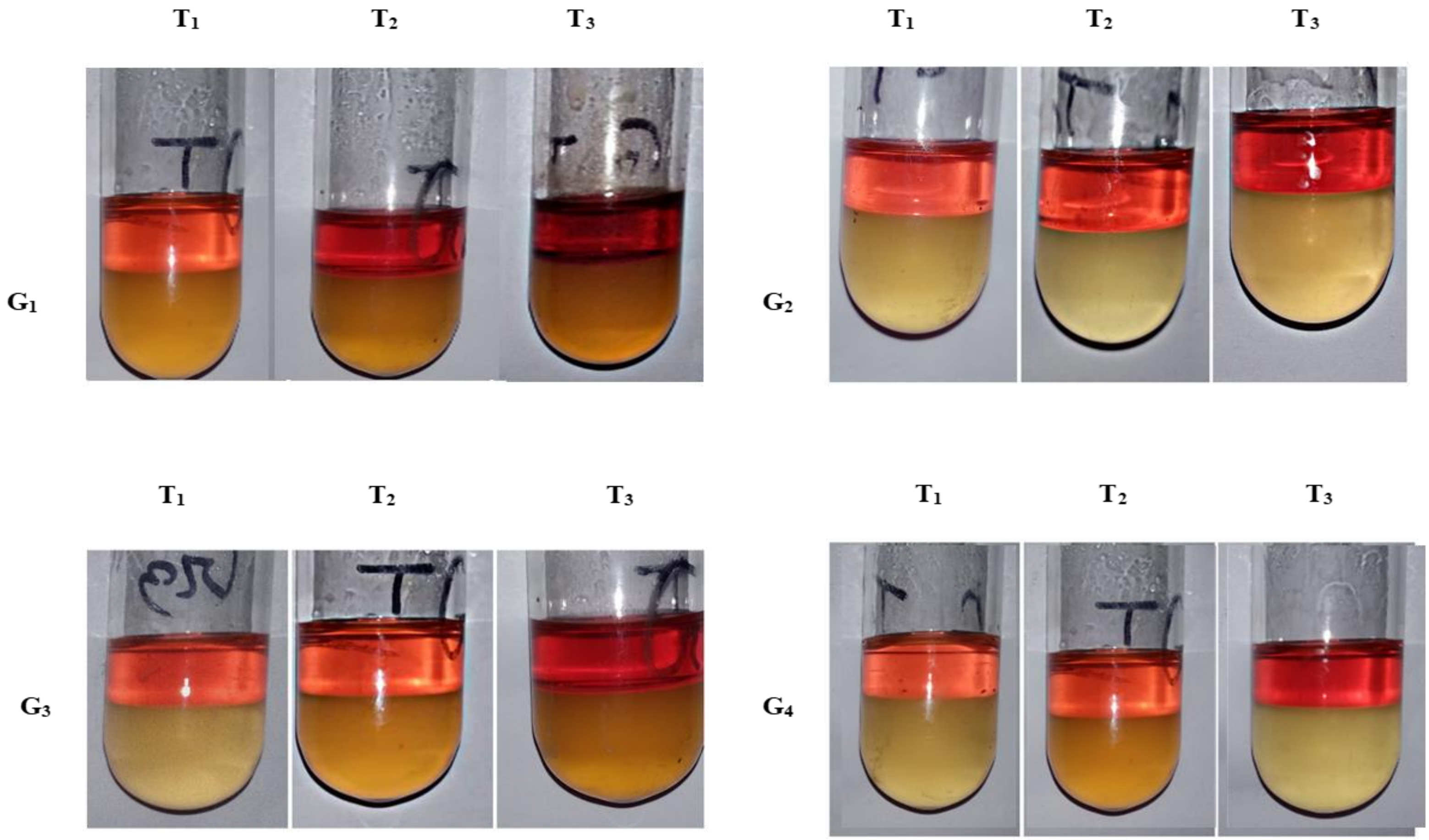


**Plate 5. Comparison of proline content under control and stress conditions. $G_1$ (SAU Tomatillo-1), $G_2$ (PI003), $G_3$ (SAU Tomatillo-2), $G_4$ (PI004) and three drought treatments viz. $T_1$ (control), $T_2$ (30 days withholding of water), $T_3$ (45 days withholding of water)**

The four tomatillo genotypes varied significantly under drought for proline content**.** All genotypes showed increasing of proline content with increasing drought treatments. The increasing percentage of proline content at treatment $T_2$ and $T_3$ is presented in Appendix XI. Increasing of proline content was found the highest in genotype $G_1$ both at moderate drought stress and at severe drought stress (reduction percentage -217.65% and -165.14% respectively) in Figure 4. So, $G_1$ is considered as a source of high proline content genotype in both moderate stress and severe stress. Increasing proline content with increasing drought stress is good qualitative trait for any crop as proline has positive correlation with stress.

### 4.3 Antioxidant and Nutritional traits

#### 4.3.1 Brix (%)

The result of the experiment it was observed that Brix (%) was varied significantly among the four tomatillo genotypes (Appendix VI). Maximum Brix (7.01%) was found in $G_4$ whereas minimum (5.31%) from $G_3$ in Table 14. According to the study, $G_4$ tomatillo genotypes have the highest Brix (%). Curi *et al*. (2018) and Mendoza *et al.* (2011) also found similar result in case of Brix (%) in tomatillo.

Brix (%) in tomatillo showed variation in drought treatments (Appendix VI). Maximum Brix (%) was obtained from $T_3$ (7.32%) treated plant whereas lowest (5.02%) was found in $T_1$ in Table 15. Better water supply instigated lower Brix in tomatillo compared to control. The soluble solid content of fruits was frequently very high without irrigation in tomatillo. In spite of this, the level of Brix yield per hectare remarkably increased as a result of significantly higher yield quantity as well as quality. Favati *et al*. (2009) found that Brix (%) increased in tomato fruits with decreasing water content.

Interaction of tomatillo genotypes and drought treatments significantly affects Brix (%) in tomatillo (Appendix VI). Maximum Brix in tomatillo (8.18%) was obtained from $G_1T_3$ while minimum (4.13%) from $G_3T_1$ followed by $G_2T_1$ (4.70%) in Table 16.

The four genotypes varied significantly under drought in Brix (%) of tomatillo fruit. Brix (%) of all genotypes increased with increasing drought treatments. The increasing percentage of Brix at treatment $T_2$ and $T_3$ is presented in Appendix XII. Increase of Brix (%) was found highest in genotype $G_1$ both at moderate drought stress and at severe drought stress (reduction percentage -39.71% and -61.18% respectively) in Figure 5. $G_3$ also showed increasing Brix (%) for both at moderate stress and severe stress (reduction percentage -32.73% and -59.33% respectively). So, $G_1$ and $G_3$ could be cultivated in moderate and severe drought stress conditions. $G_1$ and $G_3$ could be selected for future qualitative character improvement breeding.

### 4.3.2 Vitamin C content

From the result of the experiment it was observed that Vitamin C content varied significantly among the four tomatillo genotypes (Appendix VI). Maximum Vitamin C content (9.536 mg/100 g) was found in $G_3$ whereas minimum (3.996 mg/100 g) from $G_1$ in Table 14. According to the study $G_3$ tomatillo genotypes have the highest Vitamin C content. Curi *et al.* (2018) found similar results in *Physalis*. Ramful *et al*. (2011) also found similar results in tomatillo and proposed that this fruit is a very good source of Vitamin C.

Vitamin C content in tomatillo showed variation by the drought treatments (Appendix VI). Maximum Vitamin C content was obtained from $T_1$ (8.793 mg/100 g) treated plant whereas the lowest (5.314 mg/100 g) was found in $T_3$ in Table 16. Mahendran and Bandara (2000); found that the Vitamin C content reduced with decreasing water content of chilli fruits. But opposing to this study, Torrecillas *et al.* (1995) observed that the concentration of Vitamin C increased with increasing water stresses.

Interaction of tomatillo genotypes and drought treatments significantly affects Vitamin C content (Appendix VI). Maximum Vitamin C (11.677 mg/100 g) content was obtained from $G_3T_1$ while minimum (3.215 mg/100 g) from $G_1T_3$ in Table 15.

The four genotypes varied significantly under drought in Vitamin C content. Reduction of Vitamin C content was observed in all genotypes with increasing drought treatments. The reduction percentage of Vitamin C content at treatment $T_2$ and $T_3$ is presented in Appendix XII. Vitamin C content decreased minimum in genotype $G_1$ both at moderate drought stress and at severe drought stress (reduction percentage 12.57% and 31.42% respectively) in Table 16. After $G_1$, $G_3$ showed minimum reduction in both moderate and severe drought conditions (reduction percentage 23.94% and 37.25% respectively). Vitamin C is important qualitative character of any crop. So, $G_1$ followed by $G_3$ could be suggested for cultivation in both moderate and severe drought stress conditions. These varieties can also be used in future qualitative improvement breeding program.

**Table 14. Mean performance of tomatillo genotypes on Brix (%) and Vitamin C content (mg/100 g)[Y]**

| Genotype[X] | Brix (%) | Vitamin C (mg/100 g) |
|---|---|---|
| **$G_1$** | 6.17 b | 3.996 d |
| **$G_2$** | 5.86 c | 6.126 c |
| **$G_3$** | 5.31 d | 9.536 a |
| **$G_4$** | 7.01 a | 8.519 b |
| **CV%** | 4.84 | 3.94 |
| **$LSD_{0.05}$** | 0.2480 | 0.2306 |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$
[Y]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 15. Mean performance of genotypes under different drought treatments on Brix (%) and Vitamin C content (mg/100 g)[Y]**

| Treatment[X] | Brix (%) | Vitamin C (mg/100 g) |
|---|---|---|
| **$T_1$** | 5.02 c | 8.793 a |
| **$T_2$** | 5.93 b | 7.025 b |
| **$T_3$** | 7.32 a | 5.314 c |
| **CV%** | 4.84 | 3.94 |
| **$LSD_{0.05}$** | 0.2148 | 0.1997 |

[X]Three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water
[Y]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. Interaction effect of tomatillo genotypes and drought treatments on Brix (%) and Vitamin C content (mg/100 g)[Y]**

| Interaction[X] | Brix (%) | Vitamin C (mg/100 g) |
|---|---|---|
| $G_1 \times T_1$ | 5.08 fg | 4.687 g |
| $G_1 \times T_2$ | 7.09 bc | 4.098 gh |
| $G_1 \times T_3$ | 8.18 a | 3.215 i |
| $G_2 \times T_1$ | 4.70 gh | 10.236 b |
| $G_2 \times T_2$ | 6.06 e | 6.907 e |
| $G_2 \times T_3$ | 7.31 b | 3.674 hi |
| $G_3 \times T_1$ | 4.13 h | 11.677 a |
| $G_3 \times T_2$ | 5.48 f | 8.881 c |
| $G_3 \times T_3$ | 6.58 c | 7.327 d |
| $G_4 \times T_1$ | 4.85 g | 10.954 b |
| $G_4 \times T_2$ | 6.15 d | 7.879 d |
| $G_4 \times T_3$ | 7.45 b | 6.177 f |
| **CV%** | 4.84 | 3.94 |
| **$LSD_{0.05}$** | 0.6475 | 0.8483 |

[X]Four tomatillo genotypes coded from $G_1$ to $G_4$ and three drought treatments viz. $T_1$, Control; $T_2$, 30 days withholding of water; $T_3$, 45 days withholding of water

[Y]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

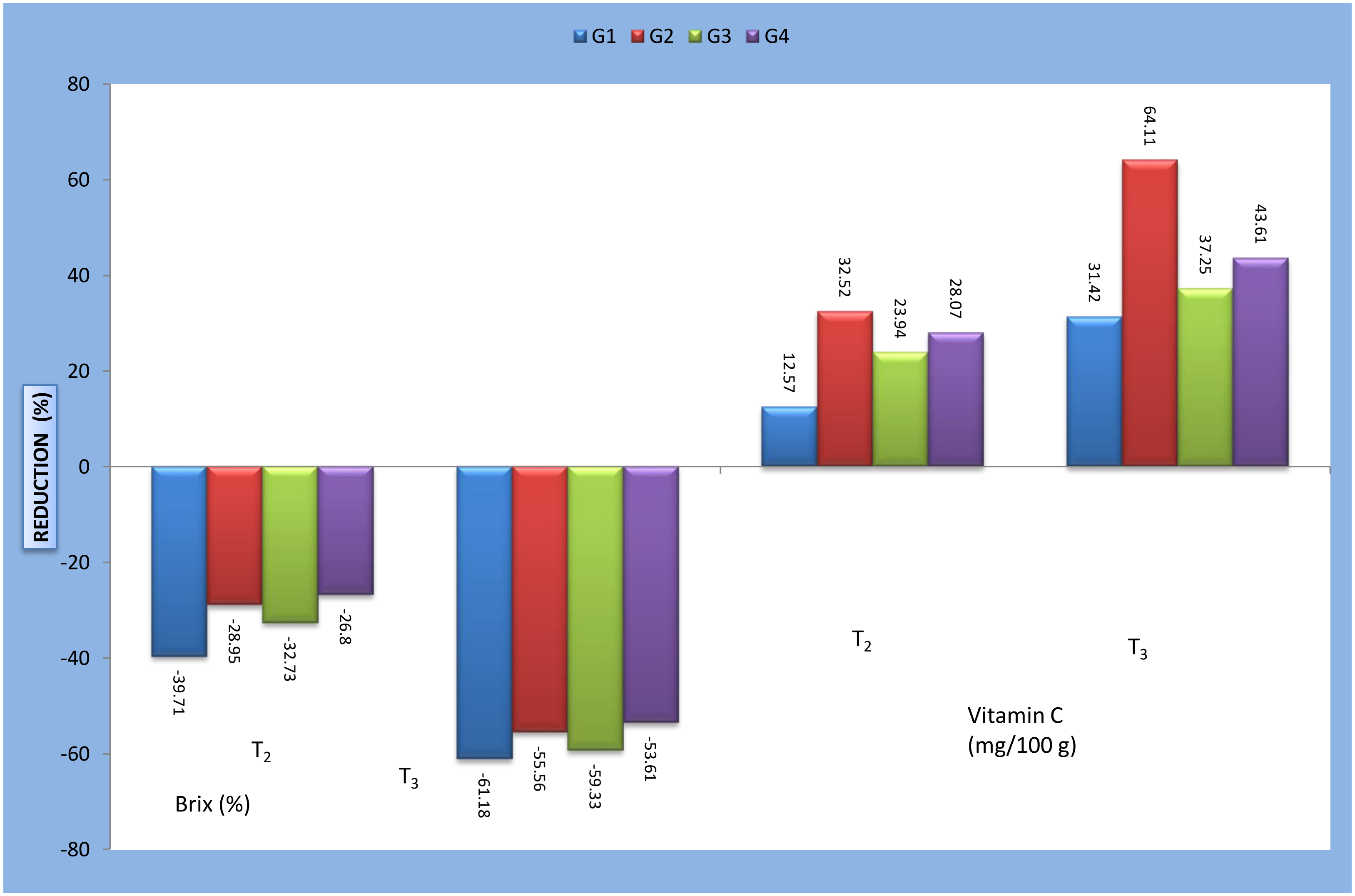


**Figure 5. Reduction percentage of Brix (%) and Vitamin C content (mg/100 g) with increasing drought stress**

# CHAPTER V

# SUMMARY AND CONCLUSION

Tomatillo (*Physalis ixocarpa* Brot./*Physalis philadelphica*), the odd-looking distant cousins of the beloved tomato belongs to the Solanaceae family is a new vegetable crop in Bangladesh introduced from Mexico and total production is low as compared to increasing total demand. It is also known as a husk tomato, due to the dry cover that surrounds the fruit. Tomatillos grow in the summer garden just like their relatives: tomatoes, eggplants, and peppers. In fact, the leaves look a little like the foliage of eggplant, but the fruit is somewhat different. Large amounts of land in northern section of Bangladesh remain uncultivable owing to high level of drought. The affected areas of Bangladesh are increasing rapidly day by day due to many reasons especially climate change for global warming. To overcome the drought problem in our country, we have to develop drought-tolerant plants to cope with the global warming effects. Thus development of drought tolerant crops is a key global agricultural goal for our country as well as the world. As per our knowledge, not any single research has been done yet for the evaluation of the tomatillo against drought in our country as it is a new crop. Evaluation followed by screening can be an easier process to determine drought tolerant genotypes of tomatillo.

A pot experiment was conducted to observe the performances of four tomatillo genotypes under three different drought treatments. The experiment was done at near the net house of Genetics and Plant Breeding Department, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh, during the months of October 2017 to March 2018. Two factorial experiment included 4 tomatillo genotypes viz. $G_1$ (SAU Tomatillo-1), $G_2$ (PI003), $G_3$ (SAU Tomatillo-2) and $G_4$ (PI004) and three drought treatments viz. $T_1$ (Control), $T_2$ (30 days withholding of water) and $T_3$ (45 days withholding of water) were outlined in Completely Randomized Design (CRD) with five replications.

Collected data were statistically analyzed for the evaluation of four tomatillo genotypes under different drought treatments. In combination of tomatillo genotypes and drought levels, early flowering was observed in $G_2T_1$ (45.5 days) interactions and late flowering was observed in $G_4T_3$ interaction (60.50 days). Early harvesting period (68.25 days) was observed in $G_1T_1$ whereas delayed harvesting was observed in $G_3T_3$ interaction (103.25 days). Among interactions of tomatillo genotypes and drought treatments, in case of plant height, the tallest plant (85.588 cm) was observed in $G_2T_1$ whereas the shortest plant (49.780 cm) was found from $G_3T_3$ at mature stage. In interaction of tomatillo genotypes and drought treatments maximum number of fruits (33.250 /plant) were obtained from $G_4T_1$ interaction whereas minimum number of fruits (11.250 /plant) was found in $G_2T_3$ interaction. The highest average fruit weight (41.378 g/plant) was obtained from $G_1T_1$ interaction while the lowest average fruit weight (8.197 g/plant) was found in $G_4T_3$ interaction. Considering yield per plant, Maximum yield (1.1998 kg/plant) was obtained from $G_1T_1$ interaction while minimum yield (0.1435 kg/plant) from $G_4T_3$ interaction. Maximum fruit length (1.5900 cm) was recorded from $G_1T_1$ combination whereas shortest (0.7630 cm) from $G_2T_3$ combination. In case of diameter of fruit, maximum fruit diameter (1.7623 cm) was obtained from $G_1T_1$ interaction whereas minimum (0.8485 cm) was obtained from $G_2T_3$ interaction.

Drought stress adversely affects the physiology of tomatillo at all stages of growth and development. Observation of physiological characters played important role for the selection of suitable genotype for future breeding purpose for tomatillo. Genotypes showed significant variation in physiological characters such as, relative water content and proline content. In case of relative water content, the highest relative water content (98.313%) was observed in $G_2T_1$ interaction whereas the lowest relative water content (70.822%) was observed in $G_4T_3$ interaction. Maximum proline content in tomatillo (4923.1 µg/g) was obtained from $G_1T_3$ while minimum (1620.8 µg/g) from $G_4T_1$ interaction.

Not only the yield characters but also the antioxidant and nutritional characters were adversely affected by high drought treatment. The genotypes varied significantly in their antioxidant and nutritional characters as maximum Brix (%) in tomatillo (8.1800%) was obtained from $G_1T_3$ interaction while minimum (4.1250%) from $G_3T_1$ interaction. The highest Vitamin-C content in tomatillo (11.677 mg/100 g) was obtained from $G_3T_1$ while minimum (3.215 mg/100 g) from $G_1T_3$ interaction.

Analyzing the data of this study it could be concluded for genotype $G_1$ that the traits such as, plant height, average fruit weight per plant, leaf width, leaf L×W, yield per plant, average fruit diameter, RWC (%) and Vitamin C content decreased minimum while leaf L/W ratio, proline content and Brix (%) increased maximum at moderate drought stress but leaf L/W ratio, proline content and Brix (%) increased maximum while RWC (%) and Vitamin C content decreased minimum at severe drought stress. Genotype $G_2$ could be considered as early flowering and early maturing genotype at moderate stress that showed minimum reduction for the traits as no. of fruits/plant, average fruit length, average fruit diameter and no. of seeds/fruit but showed maximum reduction in the traits as average fruit weight/plant, leaf width and Vitamin C content at moderate stress whereas plant height and Vitamin C content decreased maximum but no. of seeds/fruit decreased minimum at severe drought stress. Genotype $G_3$ showed maximum decreasing in case of plant height, leaf length, leaf L×W, leaf L/W ratio, average fruit length and average fruit diameter but minimum decreasing in proline content at moderate drought stress while maximum increase in maturity time but maximum decrease in leaf length, leaf L×W, leaf L/W ratio and RWC (%) and minimum reduction in average fruit weight/plant and proline content at severe stress. Genotype $G_4$ could be considered as late flowering and late maturing genotype which showed minimum reduction in plant height, leaf length, no. of seeds/fruit and Brix (%) but maximum reduction in no. of fruits/plant, yield/plant and RWC (%) at moderate stress while showed maximum reduction in no. of fruits/plant, average fruit weight/plant and yield/plant but minimum reduction in plant height, no. of fruits/plant, leaf length, leaf width, leaf L×W, average fruit length, average fruit diameter and Brix (%) at severe drought stress. From the findings of the present study, the following conclusions could be drawn:

i. $G_1$ could be cultivated at moderate drought condition for early harvesting, the highest average fruit weight, maximum yield, maximum proline content and maximum Brix (%) content.
ii. Regarding antioxidant and nutritional traits such as for Brix (%) and for Vitamin C content, $G_1$ followed by $G_3$ could be recommended for moderate as well as severe drought stress regions in Bangladesh.

The above genotypes could be recommended as parent material for future hybridization or genetic transformation program. However, final recommendation should be made by repetition of the present experiment.

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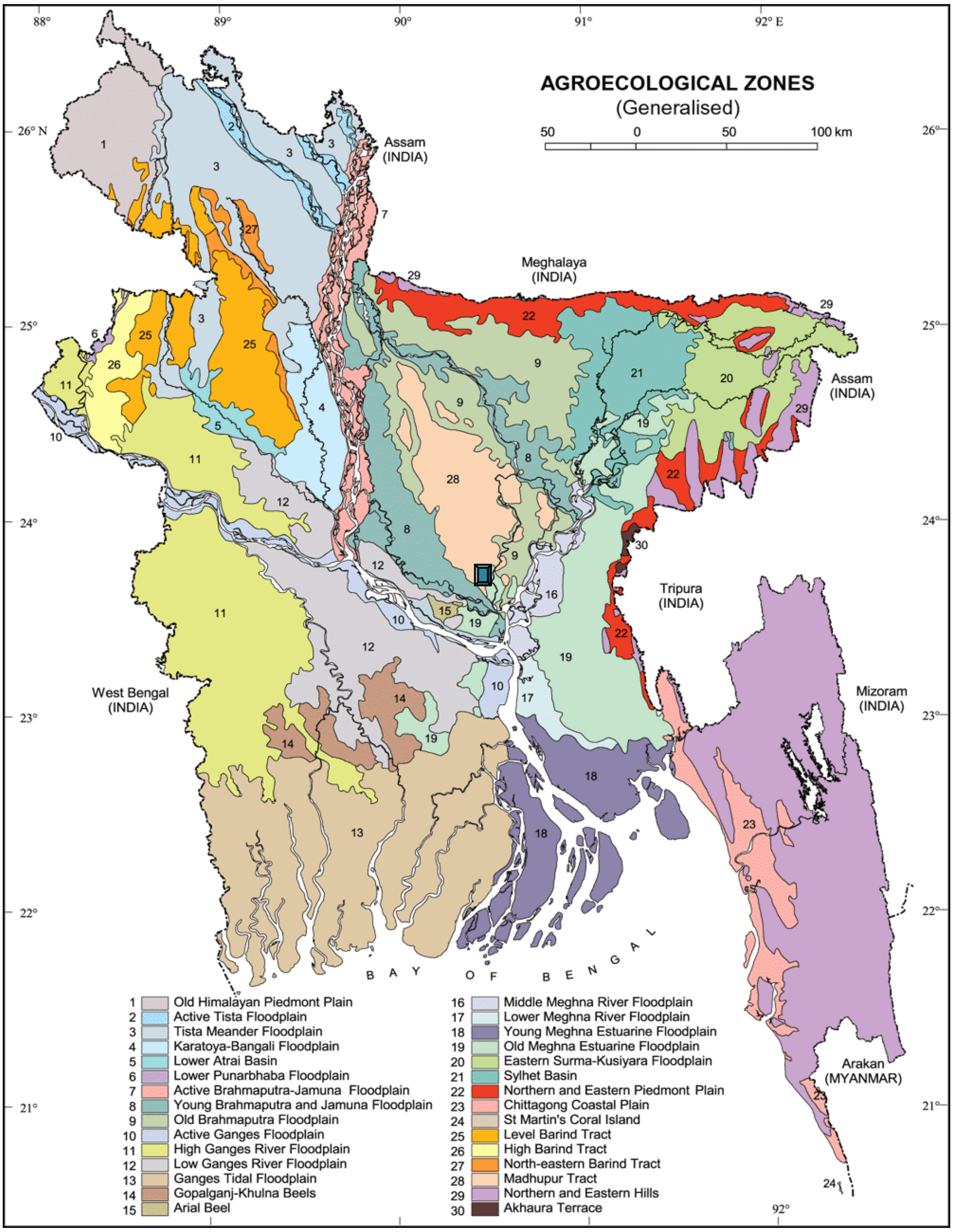
# APPENDICES

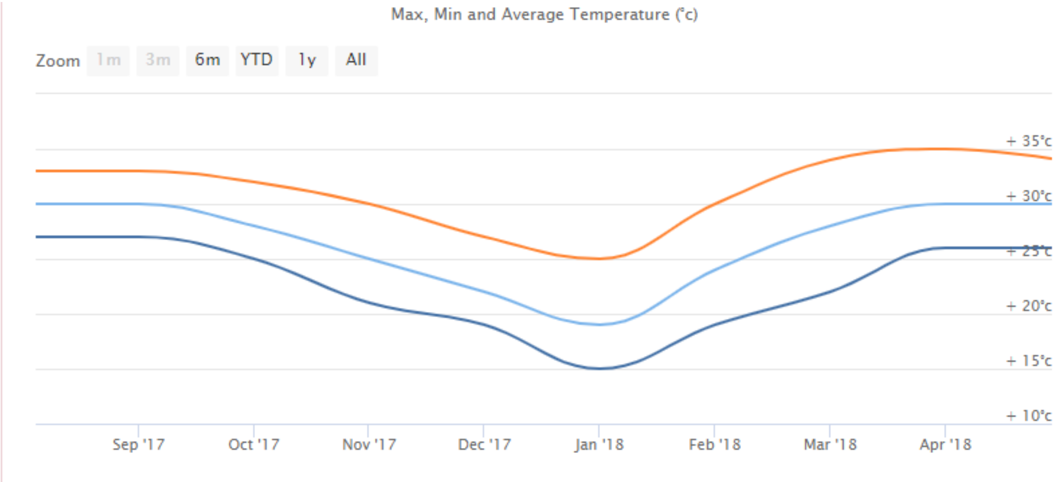
## Appendix I. Map showing the experimental site under the study



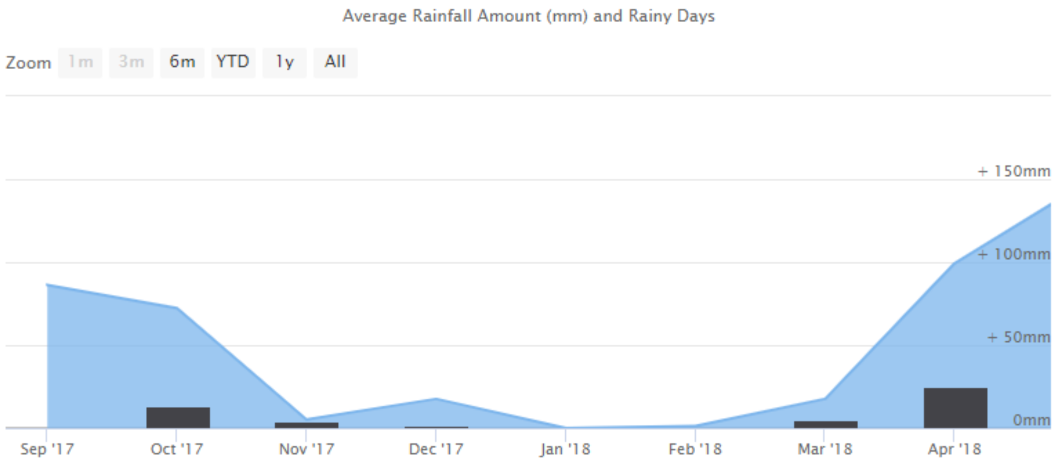

The experimental site under study

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

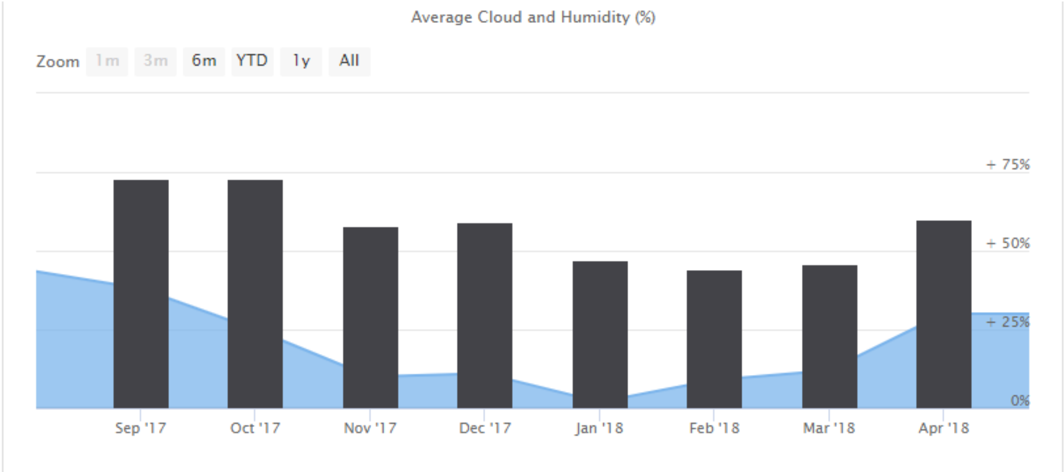
## Air temperature




## Average rainfall amount (mm) and rainy days



**Average cloud and humidity (%)**




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

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

**Mechanical composition:**

| **Particle size** | **constitution** |
|---|---|
| Sand | 40% |
| Silt | 40% |
| Clay | 20% |
| Texture | Loamy |

**Chemical composition:**

| **Soil characters** | **Value** |
|---|---|
| Organic matter | 1.44 % |
| Potassium | 0.15 meq/100 g soil |
| Calcium | 3.60 meq/100 g soil |
| Magnesium | 1.00 meq/100 g soil |
| Total nitrogen | 0.072 |
| Phosphorus | 22.08 µg/g soil |
| Sulphur | 25.98 µg/g soil |
| Boron | 0.48 µg/g soil |
| Copper | 3.54 µg/g soil |
| Iron | 262.6 µg/g soil |
| Manganese | 164 µg/g soil |
| Zinc | 3.32 µg/g soil |

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

**Appendix IV. Analysis of variance of the data on days to first flowering, days to maturity, plant height, number of fruits per plant, average fruit weight per plant, yield per plant and average fruit length**

| Source of variation | Degrees of freedom (df) | Mean Square of | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Days to first flowering | Days to maturity | Plant height | No. of fruits/plant | Average fruit weight/plant | Yield/plant | Average fruit length |
| Factor A (genotype) | 3 | 622.167$^{NS}$ | 141.02$^{NS}$ | 238.990$^{NS}$ | 79.910$^{NS}$ | 900.437* | 0.498* | 0.350$^{NS}$ |
| Factor B (Drought) | 2 | 116.292* | 2780.9* | 2920.730$^{NS}$ | 843.937* | 800.681* | 1.606* | 0.703* |
| A×B | 6 | 93.708 $^{NS}$ | 89.48 $^{NS}$ | 71.410 $^{NS}$ | 13.660 $^{NS}$ | 35.015$^{NS}$ | 0.057* | 0.024$^{NS}$ |
| Error | 33 | 73.333 | 17.670 | 55.640 | 17.364 | 11.304 | 0.011 | 0.010 |

*Significant at 0.01 level of probability; $^{NS}$ Non-significant

**Appendix V. Analysis of variance of the data on number of seeds per fruit, leaf length, leaf width, leaf length × width and leaf length/width**

| Source of variation | Degrees of freedom (df) | Mean Square of | | | | |
|---|---|---|---|---|---|---|
| | | No. of seeds/fruit | Leaf length | leaf width | leaf L×W | Leaf L/W |
| Factor A (genotype) | 3 | $42674.10^{NS}$ | 33.467* | 6.504* | 1551.300* | 0.903* |
| Factor B (Drought) | 2 | 41838.40* | $17.926^{NS}$ | $2.609^{NS}$ | $638.580^{NS}$ | $0.181^{NS}$ |
| A×B | 6 | $2970.60^{NS}$ | $0.819^{NS}$ | $0.281^{NS}$ | $51.870^{NS}$ | $0.293^{NS}$ |
| Error | 33 | 888.600 | 2.338 | 0.308 | 99.700 | 0.229 |

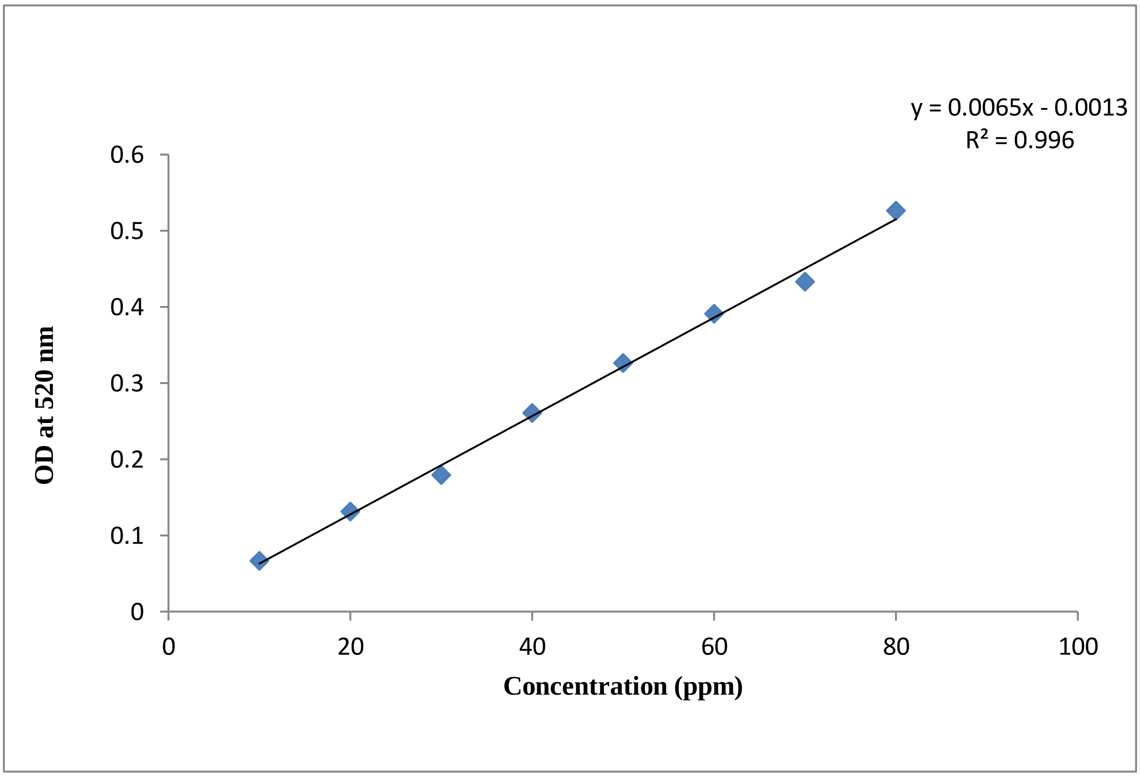
* Significant at 0.01 level of probability; NS Non-significant

**Appendix VI. Analysis of variance of the data on average fruit diameter, relative water content (RWC) (%), Brix (%), Vitamin C and proline content**

| Source of variation | Degrees of freedom (df) | Mean Square of | | | | |
|---|---|---|---|---|---|---|
| | | Average fruit diameter | RWC (%) | Brix (%) | Vitamin C | Proline |
| Factor A (genotype) | 3 | 0.338$^{NS}$ | 198.390* | 6.027* | 74.064* | 3567845* |
| Factor B (Drought) | 2 | 0.948* | 1387.340* | 21.404* | 48.427* | 18800000* |
| A×B | 6 | 0.053 $^{NS}$ | 5.730 $^{NS}$ | 0.616* | 2.508* | 1037661* |
| Error | 33 | 0.011 | 3.610 | 0.091 | 0.077 | 18027.700 |

* Significant at 0.01 level of probability; $^{NS}$ Non-significant

## Appendix VII. Proline standard curve



**Appendix VIII. Reduction percentage of days to first flowering, days to maturity, plant height, no. of fruits/plant and average fruit weight/plant with increasing drought stress**

| | Days to first flowering (DAS) | | Days to maturity (DAT) | | Plant height (cm) | | No. of fruits/plant | | Average fruit weight/ plant (g) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** |
| **$G_1$** | -2.72 | -4.9 | -25.27 | -33.33 | -5.02 | 32.04 | 13.79 | 53.45 | 3.52 | 46.22 |
| **$G_2$** | -1.65 | -5.15 | -2.41 | -34.14 | 11.62 | 37.68 | 6.38 | 51.06 | 25.48 | 42.16 |
| **$G_3$** | -3.17 | -2.12 | -24.91 | -49.10 | 11.66 | 32.37 | 14.02 | 52.34 | 9.75 | 39.96 |
| **$G_4$** | -7.84 | -18.63 | -13.77 | -30.94 | 1.52 | 26.86 | 28.57 | 45.86 | 19.90 | 55.76 |

**$T_2$**: 30 days witholding of water; **$T_3$**: 45 days witholding of water

**Appendix IX. Reduction percentage of leaf length, leaf width, leaf L×W and leaf L/W with increasing drought stress**

| | Leaf length (cm) | | Leaf width (cm) | | Leaf L×W ($cm^2$) | | Leaf L/W | |
|---|---|---|---|---|---|---|---|---|
| **Genotype** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** |
| **$G_1$** | -1.14 | 18.18 | 0 | 29.63 | 0.33 | 40.11 | -1.86 | -19.87 |
| **$G_2$** | -0.52 | 19.27 | 19.42 | 24.46 | 11.20 | 39.00 | -18.82 | -2.24 |
| **$G_3$** | 14.48 | 31.10 | 16.35 | 25.00 | 28.07 | 48.28 | 2.60 | 12.30 |
| **$G_4$** | 3.97 | 17.33 | 15.05 | 22.58 | 19.27 | 35.87 | -11.30 | -5.54 |

**$T_2$**: 30 days witholding of water; **$T_3$**: 45 days witholding of water

**Appendix X. Reduction percentage of yield/plant (kg), average fruit length (cm), average fruit diameter (cm) and no. of seeds/fruit with increasing drought stress**

| | Yield/plant (kg) | | Average fruit length (cm) | | Average fruit diameter (cm) | | No. of seeds/fruit | |
|---|---|---|---|---|---|---|---|---|
| **Genotype** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** |
| **$G_1$** | 16.80 | 74.83 | 13.64 | 37.06 | 0 | 41.85 | 17.37 | 41.00 |
| **$G_2$** | 31.05 | 72.62 | 3.96 | 34.44 | 0 | 36.98 | 5.48 | 21.36 |
| **$G_3$** | 22.32 | 72.09 | 14.81 | 30.20 | 5.62 | 30.97 | 10.08 | 26.85 |
| **$G_4$** | 41.92 | 76.50 | 14.01 | 27.12 | 3.98 | 23.05 | 4.24 | 23.78 |

**$T_2$**: 30 days witholding of water; **$T_3$**: 45 days witholding of water

**Appendix XI. Reduction percentage of RWC (%) and proline content (μg/g) with increasing drought stress**

| | RWC (%) | | Proline (μg/g) | |
|---|---|---|---|---|
| **Genotype** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** |
| **$G_1$** | 8.02 | 17.29 | -217.65 | -165.14 |
| **$G_2$** | 8.43 | 19.93 | -54.83 | -77.01 |
| **$G_3$** | 8.21 | 22.13 | -24.66 | -64.10 |
| **$G_4$** | 10.87 | 20.40 | -30.53 | -119.48 |

**$T_2$**: 30 days witholding of water; **$T_3$**: 45 days witholding of water

**Appendix XII. Reduction percentage of Brix (%) and Vitamin C (mg/100 g) with increasing drought stress**

| | Brix (%) | | Vitamin C (mg/100 g) | |
|---|---|---|---|---|
| **Genotype** | **(%) $T_2$** | **(%) $T_3$** | **(%) $T_2$** | **(%) $T_3$** |
| **$G_1$** | -39.71 | -61.18 | 12.57 | 31.42 |
| **$G_2$** | -28.95 | -55.56 | 32.52 | 64.11 |
| **$G_3$** | -32.73 | -59.33 | 23.94 | 37.25 |
| **$G_4$** | -26.80 | -53.61 | 28.07 | 43.61 |

**$T_2$**: 30 days witholding of water; **$T_3$**: 45 days witholding of water