VARIABILITY OF SHELF LIFE AND SOME QUALITY TRAITS OF TOMATILLO FRUIT (*Physalis ixocarpa* Brot.) ESTIMATED FROM F_2 GENERATION

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CERTIFICATE

This is to certify that the thesis entitled, *" Variability of Shelf Life And Some Quality Traits of Tomatillo Fruit (Physalis ixocarpa Brot.) Estimated From F_2 Generation" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in GENETICS AND PLANT BREEDING*** *embodies the result of a piece of bona fide research work carried out by MD. Mosabbirul Islam, Registration No. 18-09247 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 acknowledged.

Dated: December, 2020
Place: Dhaka, Bangladesh

Prof. Dr. Naheed Zeba
Supervisor

Dedicated to

My beloved parents and wife

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SOME COMMONLY USED ABBREVIATIONS

Full word	Abbreviations
Agricultural	*Agril.*
Agriculture	*Agric.*
And others	*et al.*
Canonical Variate Analysis	CVA
Centimeter	cm.
Coefficient of variation	CV
Degree Celsius	^oC
Department of Genetics and Plant Breeding	GEPB
Etcetera	etc.
Food and Agricultural Organization	FAO
Genetic advance	GA
Genotypic coefficient of variation	GCV
Gram	g
Hectare	ha.
Horticulture	*Hort.*
International	*Intl*.
Journal	*J.*
Kilogram	kg
Least Significant Difference	lsd
Milligram	mg
Muriate of Potash	MOP
Phenotypic coefficient of variation	PCV
Principle Component Analysis	PCA
Randomized Complete Block Design	RCBD
Sher-e-Bangla Agricultural University	SAU
Ton/hectare	ton/ha
Triple Super Phosphate	TSP
Vitamin-C	Vit-C

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ABSTRACT

An experiment was conducted at experimental field, Sher-e-Bangla Agricultural University, Dhaka. Bangladesh during the period from October 2018 to April 2019 to analyze the diversity of tomatillo genotypes in their qualitative traits. Eighteen genotypes were used for the study. Genotype G_5 showed the highest vit-C among all genotypes whereas highest days of shelf life was found in G_7. G_{10} gave the best result on lycopene content followed by rest of the genotypes and highest brix percentage was found in G_{10}. G_8 showed highest amount of non-reducing sugar and G_1 showed highest amount of reducing sugar. High to moderate GCV and PCV values were shown by all the characters except total sugar (%) that showed low GCV and PCV values. Vit-C had significant and positive correlation with shelf life (r=0.523**) and brix (r=0.355*). This indicates that if Vit-C will increase, shelf life and brix will increase as same degree. Considering higher heritability and higher genetic advance selection of genotype based on pH value, Vit-C, brix, lycopene at 472nm, total sugar and non-reducing sugar would be more satisfactory. The highest positive direct effect was observed in shelf life can be used as either direct or indirect selection criteria to improve brix percentage and other traits those are positively associated with it. Selecting plants with higher shelf life of fruits will increase other associated qualitative traits specially fruit brix. There was moderate level of variation present among eighteen genotypes of tomatillo due to low inter genotypic distance. Considering the quality performance of tomatillo genotypes G_5 could be selected for higher Vit-C, G_7 could be selected for higher shelf life and G_8 could be selected for higher non-reducing sugar. Highest Brix percentage as well as Lycopene contents belongs to G_{10} genotype.

CHAPTER I

INTRODUCTION

Throughout history humans have used some 3000 plant species for food. The recent tendency has been to exploit fewer and fewer species and today, only around 20 species supply most of the world's food. Many beneficial plant species have been underused or have not been developed to their full potential (Vietmeyer 1986). Useful plant species have often been overlooked because they are native to the tropics, regions neglected by the world's research institutions which are oriented toward crop production in temperate zones. There are several Solanaceous species with edible fruit that are popular in Latin America in addition to tomato and chilies. *Physalis ixocarpa* Brot.; commonly known as the husk tomato and by the Spanish names of *tomate de cascara, tomate verde, tomate de fresadilla, tomatillo, and miltomate*, is an important vegetable crop in the diets of Mexicans and Central Americans. In Mexico, the fruits are used in the making of chili sauce and dressings for popular dishes such as tacos and enchiladas. *P. ixocarpa* is gaining ground as a new crop in California due to the increased popularity of Mexican food in the United States (Quiros 1984) and has production potential in the southern United States. In Louisiana, tomatillo imported from Mexico is sold as a fresh fruit in a few grocery stores. There is a potential market for fresh produce and the Louisiana sauce industry may be interested in opening a new ethnic market for their products. Developing a new crop is a difficult and complex process. One way is to import an exotic crop from areas where the crop is already grown or consumed and adapt it to local conditions (Laidig 1983). It is a source of vitamins A and C, and it has been suggested that chemicals present in tomatillos, e.g. ixocarpalactone A, may have cancer chemopreventive properties (Choi 2006). The husk tomato is an annual species, with a height from 1 to 1.5 m, adapted to humid tropical and subtropical conditions. It is a source of vitamins A and C, and it has been suggested that chemicals present in tomatillos, e.g. ixocarpalactone A, may have cancer chemo-preventive properties (Choi 2006). The husk tomato is an annual species, with a height from 1 to 1.5 m, adapted to humid tropical and subtropical conditions.

The fruit is enclosed in a husk that usually breaks because of fruit expansion and has to be removed before consumption (Escobar 2009). Flower budding occurs approximately 42 days after sowing, whereas flowering takes place at 48 days. Fruit development starts one week after flowering, and harvesting is carried out between 70 and 100 days after sowing (Mulato 1987). Both, the cultivated and most wild types of tomatillo, are diploid 2n = 2x = 24, although some other species of the same genus are polyploids (Menzel 1951, Peña 2002, Santiaguillo 1994). It is an obligated outcrossing species that shows gametophytic self-incompatibility, which prevents the inbred line formation through self-pollination to obtain hybrids (Sahagún 1999). This species is native to Mexico and Central America, and it is presently one of the most important crops in Mexico (Cantwell 1992), being the fourth vegetable in production surface with an area of 47,473 ha in 2009. This is due to its high consumption

The genus *Physalis,* established by Linneaus in 1753, contains about 100 species of annual and perennial herbs (Willis 1966). The genus is characterized by the presence of pendant flowers and an inflated fruiting calyx which encloses the berry (Sullivan 1984). Four species are cultivated in different parts of the world for their fruit: *P. peruviana* L. (cape gooseberry, uchuba) and *P. pruinosa* L. (ground cherry, husk tomato) are used as jam fruits; *P. alkekengi* L. (Chinese lantern) is used as an ornamental; and *P. ixocarpa* Brot. (tomatillo, tomate de cascara) is used as a vegetable or for sauces. Several species of *Physalis* are widespread in America as endemic weed species. Six important *Physalis* spp. are prevalent in the phytogeographic region of Mesoamerica (Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, and Panama, and the Mexican states of Chiapas, Yucatan, and Quintana Roo): *P. angulata* L., *P. cordata* Mill., *P. gracilis* Miers, *P.*

ignota Britt., *P. lagascae* R. & S., and *P. pubescens* L. (Gentry *et al* 1986). These *Physalis* spp. can be intercrossed, but incompatibility has been found (Pandey 1957 and Quiros 1984). The basic chromosome number of the genus is N=12 and most species are diploid; *P. peruviana* is a tetraploid (Menzel 1951). It is an important vegetable item in the diet of Mexicans and Central Americans (Waterfall, 1958; Cartujano-Escobar *et al.*, 1985). The fruits are used as vegetable, salad and making sauces (Waterfall, 1967; Small, 2011). 100gm of edible tomatillo contain energy 32 Kcal, carbohydrates 5.84 g, protein 0.96 g, total fat 1.02 g, dietary fiber 1.9 g, vitamins (Folates 7 µg, Niacin 1.850 mg, Pyridoxine 0.056 mg, Thiamin 0.044 mg, vitamin-A 114 IU, vitamin-C 11.7 mg, Vitamin E 0.38 mg, vitamin K 10.1 µg), Sodium 1 mg, Potassium 268 mg, Calcium 7 mg, Copper 0.079 mg, Iron 0.62 mg, Magnesium 20 mg, Manganese 0.153 mg, Phosphorus 39 mg, Selenium 0.5 µg, Zinc 0.22 mg, Carotene-ß 63 µg, Carotene-α 10 µg, Lutein-zeaxanthin 467 µg (Yamaguchi, 1983). It is good source of antioxidant phyto-chemicals known as Withanolides. Ixocarpalactone-A is one such Withanolides present in tomatillo which has been found to have anti-bacterial and anti-cancer properties. It is rich in flavonoids helps to protect from lung and oral cavity cancers (Hamm, 1985; Quiros, 1984).

This experiment was conducted to find out the Brix, pH, Vitamin-C, Lycopene, Reducing Sugar, Non-Reducing Sugar, Total Sugar, Shelf Life by using established protocol to check it out which germplasm or variety possesses countable amount of above traits. In this research eighteen tomatillos genotypes have used. The main objective of this work was to genotypic analysis of tomatillo genotypes based on their nutritional traits through chemical manipulation, as well as to perform cytological and phenotypic characterization of the products and their progenies. At the same time replications were taken under consideration. With conceiving the above scheme in mind, the present research work has been undertaken in order to fulfill the following objectives:

- To analyze the status of Vit-C, lycopene, reducing sugar, non-reducing sugar, total sugar, pH and brix percentage of different tomatillo genotypes
- To find out the best genotype depending on antioxidant status and nutritional traits

CHAPTER II

REVIEW OF LITERATURE

In northeast Nebraska, P. lanceolata was known by the Omaha and Ponca as makan bashahon-shon, "crooked medicine," apparently referring to the crooked roots of the plant. The root of this species (actually *P. longifolia, P. virginiana, or P. ixocarpa*; was used as medicine by the Omaha, Ponca, and Winnebago to treat headache and stomach trouble, and as a dressing for wounds. The Omaha Buffalo doctors used *P. viscosa* (Gilmore reported that this was actually *P. lanceolata*) roots to dress wounds. The Lakota were reported to eat *P. heterophylla* fruits to increase the appetite (Rogers 1980). The Iroquois used the dried leaves and roots of *P. heterophylla* as a wash to treat venereal disease sores and also internally as a tea to treat stomachache after one was sick. The dried leaves and root of *P. pubescens* were used by the Navaho as a "life medicine" (Vestal 1952). The Meskwaki made an infusion of the whole plant of *P. virginiana* to treat dizziness. Many other *Physalis* species throughout the world have been used traditionally for medicine. One anti-cancer finding relates directly to the work discussed below. It is the use of a paste, extracted from the leaves and stems of *P. mimima* L. (a weedy species, most likely native to the New World tropics), which traditionally has been used in Thailand and Malaysia as a medicine to treat cancer (Lee *et al* 2005).Tomatillo has been known to botanists for nearly 400 years as *P. philadelphica* Lam.Two varieties from numerous plant types called tomate by the Aztecs. Botanists have suggested that the small-fruited miltomate is a wild-type plant, whereas, the tomatillo is a domesticated plant that derives from plants similar, if not identical, to miltomate (Hudson 1986). The specific boundaries in *Physalis* are poorly defined with some duplication of names and many changes in the nomenclature during the last 50 years. The complexity of the genus is caused mainly by the wide range of genetic variability present presumably resulting from interspecific hybridization (Menzel

1951, 1957; Waterfall 1958) and also by the ambiguity of the earlier taxonomic descriptions (Raja-Rao 1979). For example, *P. aequata* Jacq. and *P. capscicifolia* Rydb are considered synonymous with *P. ixocarpa.* To clarify the taxonomic classification of *Physalis*, Menzel (1951, 1957) and Waterfall (1967) made extensive cytologic and taxonomic studies of the genus. Menzel reduced *P. philadelphica* to synonymy under the variable *P. ixocarpa* Brot. a name that had to come to be widely used for the domesticated tomatillo (Hudson 1986). The only apparent difference between the two species was the length of the peduncle, with the peduncle of *P. ixocarpa* shorter than that of *P. philadelphica*. Waterfall (1958) accepted this nomenclature when studying the species of North Mexico, but he reversed himself when he analyzed *Physalis* spp. from Mexico and Central America (Waterfall 1967). He incorporated the small-flowered *P. ixocarpa* within the broader limits of *P. philadelphica*. Fernandes (1974) made a thorough investigation of this nomenclatural problem and concluded that *P. ixocarpa* is a distinct species, different from *P. philadelphica* based on previous cytological evidence, the distinctive sigma, and the small flowers of the type. Chromosome morphology has recently been used to understand the interspecific relationships in the genus.The morphology of chromosomes during the pachytene stage with most important *Physalis* spp. and demonstrated cytological differences between the species. Nevertheless, the taxonomic complexity of the genus is not yet clarified, especially between *P. ixocarpa* and *P. philadelphica.*

Plants in the genus *Physalis* have herbaceous stems. Some have short to elongated rhizomes, the leaves are usually broadly ovate to linear and generally alternate. The flowers are solitary in the axis of the leaves, sometime pendant in the axillary branches causing them to appear to be axillary between the two branches. The pendant blossoms are often hidden by the foliage and many of the flowers hang just above the ground (Sullivan 1986). The flowers have corollas campanulate to rotate with the petal borders reflexed. Petals are usually yellow with a dark purple spot near the base of each petal. The calyx is united, with lobes more than one half its length. The androecium has five stamens with the filaments attached to the base of the corolla tube. The anthers are ovate-oblong and dehiscent by lateral slits. The fruit is a two carpet, many seeded-berry (Waterfall 1958). There are several reports concerned with the development and growth of tomatillo plants (Mulato-Brito 1985; Cartujano-Escobar 1985a, b), and we have 2 years of experience with tomatillo growing in Louisiana.

Tomatillo seedlings form a single shoot which has three to five internodes above the cotyledons. The last internode ends with a flower, one leaf and two lateral ramifications. Each ramification has one node which terminates in the same pattern, one end flower, one leaf and two branches. This pattern continues until senescence, "with the exception that when two leaves are formed there is no further branching. One characteristic of the main branches is that the internodes differ in length and have many adventitious roots. When these roots contact soil, they grow into the soil and are independent of the main root system. The number of fruits set is variable, but generally fruit are set until the 10th and 11th weeks after emergence. Tomatillo is similar to tomato plants, in that the biggest fruit are from the first flowers on the main branches. The lateral and sublateral branches produce more flower buds but they abscise and do not produce harvestable fruit. Mulato-Brito (1985) reported that the greater total number of nodes on the lateral and sublateral branches produce more fruits than the main branches, but that those fruits rarely reach commercial size. Maximum fruit production is reached by 11 weeks after emergence. The high number of fruits present at this time compete with each other for the available nutrient supply Most of the fruit on lateral branches are dropped or they do not reach commercial size (Cartujano-Escobar 1985b). A breeding program should select for a determinant plant type suitable for mechanical harvesting of tomatillo. The elimination of sublateral branch production and reduction of internode number in lateral branches would be very important for restricting fruit production to a short period of time. The seed germinates in 7-10 days, followed by elongation of the primary shoot for 4 weeks. The first flower bud is formed before the elongation of the primary shoot end, which is around the 3rd or 4th week after emergence, and flowering continues until senescence of the plant. The first flower appears 4 to 5 weeks after emergence and the first fruit appears one week later, reaching 3 cm in diameter at 8 weeks. The symptoms of senescence are visible after 12 weeks, with the plant reaching total senescence at 14 or 15 weeks after emergence. The growing period for tomatillo is short (3 to 4 months) and several overlapping crops could be produced in Louisiana. The only limit to plant growth is low temperatures. The growth of tomatillo is poor at temperatures of 16-18°.C or less. Plants grown in Louisiana during the hottest part of the summer produced marketable size fruits (up to 7 cm in diameter). Among unexploited tropical fruits husk tomato or tomatillo (*Physalis ixocarpa* Brot.), is very promising. *Physalis* is an important genus of the Solanaceae family. It is native from tropical and subtropical America and is widely distributed throughout the world. It is fairly adaptable to a wide variety of soils light (sandy), loamy and clay and requires well-drained soils. It could grow in semi-shade or no shade and dry or moist soil. *Physalis* fruit and juice are nutritious, containing particularly high levels of niacin, carotenoids and minerals. Moreover, many medicinal properties have been attributed to *Physalis*. A decoction is used in the treatment of abscesses, cough, fevers or sore throat. A single plant may yield up to 0.5 kg fruits and carefully tended plants can provide 20–33 tons/hectare. Fruits are long lasting, can be stored in a sealed container and kept in a dry atmosphere for several months and also freeze well.

The fruit is a berry (1.25– 2 cm wide), with smooth, waxy, orange-yellowish kernels. The part of the *Physalis* that can be use discomposed of husk(6%)and berry(94%).They are protected by papery husks with many minute seeds in a juicy pulp, which is sweet and tangy , resembling Chinese lanterns . Nowadays, *Physalis* is included in the priority list of many governments' horticulture and fruit export plan. It is relatively unknown in importing markets and remains an exotic fruit. It is exported from several countries including Colombia, Egypt, Zimbabwe and South Africa, but Colombia stands out as one of the largest producers, consumers and exporters. In addition, no data about *Physalis* fruit juice are yet available. In this work we are reporting for the first time, on the chemical composition and some physicochemical parameters of *Physalis* fruit juice. The data obtained will present here are an important indication of the potentially nutraceutical and economic potential of *Physalis* as a new source of fruit juices.

The crop has received much attention by the researchers on various aspects of its production under different adverse condition. Many studies on the genetic variability have been carried out in many countries of the world. The work so far done in Bangladesh is not adequate and conclusive. Nevertheless, some of the important and informative works and research findings so far been done at home and abroad on this aspect.

2.1 Medicinal Chemistry, Anti-Cancer Studies, and Recent Discoveries

Physalis species are known to contain carbohydrates, lipids, minerals, vitamins, and phytosterols (Puente 2011). They also are a major contributor of withanolide-type structures. Withanolides are classically defined as a group of C28 ergostane-type steroids with a C-22,26 δ-lactone group, first isolated from the genus Withania (Lavie 1965). They are restricted to the Solanaceae family (subfamily Solanoideae), which includes the genera Datura, Physalis, and Withania (Zhang 2012; Misico 2011; Chen 2011; Eich 2008; and Veleiro *et al* 2005).

Withanolides have attracted substantial recent interest due to their exhibition of significant biological activities, specifically antimicrobial, antitumor, anti-inflammatory, immunomodulatory, and insect-antifeedant activities (Misico 2011; Chen 2011). The typical withanolide, withaferin A, has been shown in vitro and in vivo to suppress the growth of an array of tumor cells, including breast, pancreatic, prostate, lung, leukemia, and head and neck squamous cell carcinoma, by inducing programmed cell death (Samadi *et al* 2010)—therefore possessing potential to inhibit tumorous cell growth. Most research on these compounds has been studies of ashwagandha, Withania somnifera, an important plant in Ayurvedic medicine for more than 3,000 years. It has been used traditionally as a liver tonic, anti-inflammatory agent, aphrodisiac, treatment for arthritis and rheumatism, and as an adaptogen to promote overall health and longevity. As part of an ongoing study of withanolides by our group, the phytochemistry and biological activity of P. longifolia was evaluated for the first time (Zhang 2011). Fourteen new withanolides (named withalongolides A-N), four acetylated derivatives and eight known compounds were isolated and identified from the aerial parts of this species (Zhang 2011). The classically defined withanolide-type steroids isolated from P. longifolia have a diversity of oxygenation patterns. Eight known compounds were also identified from P. longifolia, including seven withanolides (sitoindoside IX, withaferin A, 2,3-dihydro-3βmethoxywithaferin A, viscosalactone B, 2,3-dihydro-3β-O-sulfate withaferin A, 2,3-dihydrowithaferin A, and 3α,6α-epoxy-4β,5β,27-trihydroxy-1- oxowitha-24-enolideJ) and a flavonoid glucoside, rutin (Zhang 2011). A voucher specimen for this material was collected in Ellsworth County, Kansas, by Hillary Loring (collection number 3583) and deposited in the R.L. McGregor Herbarium at the University of Kansas. When tested against human head and neck squamous cell carcinoma and against melanoma cell lines for their cytotoxicity, eight withanolides (including withalongolides A,B, C, D, E,G,H, sitoindoside IX, withaferin A, and 2,3-dihydro3β-O-sulfate withaferin A) and four acetylated derivatives showed potent cytotoxic effects against the cancer cells compared to normal fetal (MRC-5) fibroblast control cells tested with IC50 values in the range

0.067–9.3 μM, while the other withanolides were inactive (Zhang 2011). In addition, none of the withanolides demonstrated cytotoxicity in normal fibroblast cells at or below the concentrations where cancer cells demonstrated toxicity. For these reasons, there is considerable interest in these new compounds. Other recent anti-cancer discoveries include those found in P. angulata, which demonstrated anti-metastatic and anti-angiogenic activity (Hsua 2011). This species also was found to contain anti-proliferative withanolides, cyto-toxic against prostate cancer cells (Jin 2012), and as well as Physalin B, which has anti-melanoma activity (Hsu 2012). In addition, P. minima has been shown to have significant cytotoxic activity on human lung cancer cells (Leong 2011).

2.2 Nutritional Analysis

Tomatillo is becoming very popular vegetable crop with the days gone where tomato already popular and has versatile use. Tomatillo found to have anti-bacterial and anti-cancer properties. It is rich in flavonoids helps to protect from lung and oral cavity cancers (Quiros, 1984; Hamm, 1985). Lycopene, a precursor of beta-carotene with well-known antioxidant activity and powerful health properties .Research for new anticancer drugs focuses more on the natural compounds such as physicochemical constituent from the regular human diet. Because of the lack of severe side effects yet efficiently can act on a wide range of receptors or molecular targets involved in carcinogenesis and cardiovascular diseases. In vivo, in vitro and clinical studies conducted in recent years have revealed an inverse association between the dietary intakes of lycopene with the risk of prostate cancer (PCa). L-Ascorbic acid (AsA), which is an essential nutrient component for human health and plant metabolism that plays key roles in diverse biological processes such as cell cycle, cell expansion, stress resistance, hormone synthesis, and signaling. Many scientists have studied quality character as well as anti-carcinogenic properties of tomatillo on human and many animals. Among them most relevant recent publications are reviewed below is good source of antioxidant phytochemicals known as Withanolides. Ixocarpalactone-A is one such Withanolides present in tomatillo.

2.3 Variability

The fundamental key to achieving the genetic improvement of a crop through a proper breeding program is to judge the amount and nature of variation of plant characters in the breeding population. It helps the breeder for improving the selection efficiency. For this reason, many researchers studied the variation of various characters in tomatillo. Some of those are presented below:

The success of any crop improvement program depends on the presence of genetic variability and the extent to which the desired trait is heritable. Genetic diversity can be estimate using both morphological and molecular markers. The presence of genetic variability in the breeding material has been emphasized by previous researchers (Naz *et al.*, 2013; Reddy *et al.*, 2013; Singh, 2009; Shuaib *et al.*, 2007).

2.3.1 Lycopene

Tomatillo has scanty amount of lycopene and it hardly found in tomatillo.). Standard methods for determining the carotenoid content require the extraction of the analyte as well as other cleanup step. Lycopene (LYC) is the red pigment. Lycopene's antioxidant capacity is roughly twice that of β-carotene. Numerous epidermiological and intervention studies have demonstrated that dietary intake of LYC-rich foods result in decreased incidence of certain cancers, including the prostate, lung, mouth, and colon cancer, coronary heart diseases, cataracts and possibly macular degeneration. Lycopene is an important intermediate in the biosynthesis of many carotenoids, including beta carotene, responsible for yellow, orange or red pigmentation, photosynthesis, and photo-protection. Like all carotenoids, lycopene is a polyunsaturated hydrocarbon (an un-substituted alkene). Some of the previous reports on Lycopene experiment are discussed here (Moigrădean *et al.*, 2007; Alda *et al.*, 2009; Dong *et al.*, 2010; Cucu and Loco, 2011; Datta *et al.*, 2013) Lycopene may lower the incidence of prostate cancer. Someone conducted an experiment in human. Oxidative stress is recognized as one of the major contributors to the increased risk of cancer and lycopene being a potent antioxidant has been found to inhibit proliferation of several types of human cancer cells, including endometrial, prostate, breast, upper aero digestive tract and lung. Lycopene has tumor suppressor activity.

2.3.2 Sugar Content

The tomatillo or husk tomato (*Physalis ixocarpa* Brot.) is a solanaceous fruit vegetable used to prepare the green sauces of Mexican cooking. Developmental changes were studied from anthesis to yellowing in fruits of cultivar 'Rendidora'. During an 8 week growth period both chlorophyll and carotene contents decreased. Sugar content increased to 7.0% due to accumulation of non-reducing sugars. Titratable acidity also increased with fruit development, reaching 1.3% at 8 weeks. Total pectin content reached a maximum (1.1%) at 6 weeks of development. The ascorbic acid content of tomatillos was low (3–4 mg 100 g^{-1}) and did not change during fruit development. Tomatillos may be harvested commercially from about 5 to 8 weeks of development, or when the fruits have filled the calyx (husk). The respiration rate of tomatillos was low (18–25 l CO_2 g^{-1} h^{-1} at 20 °C) and decreased with time in storage. Respiration rates were higher for immature than for mature and ripening fruits. Rates of ethylene production were also low (0.2–2.0 nl g^{-1} h^{-1} at 20°C) for immature and mature fruits. Ripening and ripe fruits (horticulturally over-mature) showed much higher rates of ethylene production (20–40 nl g^{-1} h^{-1}) than developing fruits. Storage of fruits at 10°C and 20°C resulted in greater weight loss and color change than storage at lower temperatures. The freshness of the husks was also maintained at lower temperatures. Storage at 2.5°C for 3 weeks resulted in higher decay levels and surface pitting than storage at 5°C. Decay incidence was decreased by a chlorinated water wash before storage Marita (1992).

2.3.3 Shelf Life

Tomatillos can be forced-air or room cooled. The main reason to cool rapidly is to retain the fresh appearance of the husk. Tomatillos can be stored under a wide range of conditions. At ambient temperatures, the husks will dry, but the fruit will remain in good condition for about 1 week. For longer storage life temperatures of 5°C to 10°C (41°F to 50°F) with moderate humidity levels (80-90% RH) are recommended to retain the freshness of the fruit and the husk. At 5°C (41°F) chilling injury will occur after about 3 weeks. To improve their postharvest shelf life and quality, tomatillos should be cooled by forced-air or room cooling after packing but before shipment. A key to tomatillo quality is the freshness and appearance of the calyx. To maintain a green calyx, store fruit at 41° to 45°F (5° to 7.2°C). At higher temperatures the calyx will dry out, but fruit quality will still be acceptable. Tomatillos are sensitive to chilling injury if stored below 45°F (7.2°C) for longer than two weeks. Chilling injury takes the form of fruit surface pitting and elevated levels of decay. Typical transit and storage conditions are 45° to 55°F (7.2° to 12.8°C) with high relative humidity (90 to 95%). Yellowing of tomatillos is hastened by exposure to ethylene, so storage with ethylene producing fruit is not recommended. With proper storage conditions, you can expect a shelf life of 2 to 3 weeks. Cartons are palletized and then shipped, primarily by truck to terminal.

2.3.4 pH

pH of tomatillo (*Physalis ixocarpa* Brot.) grown in Sher-e Bangla Agricultural University, Dhaka was analyzed. Fruit’s p^H differed significantly in all the genotypes ranging from 2.71 to 4.30 (Table 2). Tomatillo somewhat sour in taste at row condition that’s why at row stage tomatillo content little bit higher pH then ripping stage. Average kcal content was calculated to be about 31 kcals/100 g. The average pH of tomatillos was 3.76.(US Dept. of Agril.,1982; Sapers, G.M., *et al.*,1984; Prosky, L., 1984; Ramos, D.1991 and McKee, L.H., 1992)Unlike tomatoes, they have a paper like husk which must be removed before consumption (Axelius, B. 1992.). Also unique to tomatillos is the waxy coat and sticky like substance noted on the surface.

The unique, slightly acidic flavor of the tomatillo has made it a popular item in the sauces of various Mexican dishes such as enchiladas and salsas. It can also be found in gourmet sauces, stews and relishes. It is often used in lieu of tomatoes because of its green color and/or its acidity. Due to the increase in popularity of tomatillos in the USA, a number of people throughout the 128 country have contacted the New Mexico Cooperative Extension Service located at New Mexico State University (NMSU) for advice on the proper canning methods for tomatillos as well as general nutritional information.

Therefore, the objectives of this study were to determine the pH levels, proximate composition like brix, vitamin C, Lycopene, Moisture content and dry matter content of various samples of tomatillos; to calculate the carbohydrate content of these samples and to calculate the kilo-calories of the samples involved. As indicated previously, tomatillos are often used as an acid source in lieu of tomatoes in various home preserved sauces and salsas. In part, this is a function of the green color which when combined with green chili pepper results in a green sauce which is desired for dishes like green enchiladas. Because sauces and salsas are used year round in Mexican foods and because having a green sauce or salsa is desirable for certain dishes, such sauces or satsas are often preserved for use at other times of year. In the southwestern part of the U.S., such sauces and salsas are typically preserved using hot packing or water-bath canning techniques. Unfortunately most of the ingredients (chilli peppers and onions) for such sauces and salsas are not at a pH safe for preservation using this technique. Addition of an acid source like vinegar results in products which are only marginally acceptable or are unacceptable. Therefore, based on this research, the tomatillo appears to have promise as a potential ingredient for sauces and salsas preserved by the water-bath canning method while having a nutritional profile which is low in calories and similar to that of the tomato for which it is being substituted. In addition, because of the high Brix content of tomatillos noted in this research, this unusual food appears to be a potential source of trace elements which indicates the need for further research in this arena. Also of interest is the constituents which make up the 1.5 percent fat. However, it is important to remember that these data were derived from tomatillos grown in one location under 54 plots in same land during a single season in a single year. Of note, however, are subsequent studies being conducted at Sher-e Bangla Agricultural University, Dhaka, Bangladesh which indicates that the pH of other batches of tomatillos is near about 4.0 and appears to be stable using various canning and storing time.

2.3.5 Vitamin-C

As purple tomatillo genotypes produce juice violet in color that's why possibility of having vitamin-C is very rare. On the other hand tomatillo genotypes which are greener and produce juice as like tomatoes do and show vitamin-C presence but very scanty amount. Some wild relatives of tomatillo shows higher amount of vit-C. Varieties cultivated in Philadelphia and New Hampshire shows higher vit-C. Tomatoes are excellent sources of vitamin C, with some varieties containing concentrations comparable to those found in oranges. Although all tomatoes contribute to our vitamin C intake, there are different amounts of vitamin C in different genotypes. For example, raw green tomatoes contain 23.4 milligrams, orange tomatoes contain 16 milligrams and yellow tomatoes contain 9 milligrams per 100 grams, which is slightly more than half of a large, 3-inch tomato. Sun-dried tomatoes are much richer in vitamin C, containing 39.2 milligrams per 100 grams. Crushed, canned tomatoes and tomato juice contain smaller amounts, respectively contributing 9.2 and 18.3 milligrams of vitamin C to our daily intake (Lee and

Media, 2014). Lee *et al.*, (2005) were analyzed tomatoes regarding ascorbic acid (Vit. C), lycopene content and antioxidant activity. Organic tomatoes presented higher content of ascorbic acid and total phenolics (641.39 and 4466.66 mg/100 g EAG on dry wt. basis) than did the conventional tomatoes (510.16 and 3477.50 mg/100 g EAG on dry wt. basis, respectively). There was no difference in lycopene concentrations between the organic and conventional. Schwarz *et al.*, (2013) evaluated ten tomato hybrids (Supera, Granadero, AP-529, AP-533, Katia, Laura, Fascinio, Tinto, Red Spring and Venus) for their quality, viz. soluble solids, ascorbic acid, lycopene and reducing sugars. The best performing hybrid for traits and for both segments was Granadero, but this hybrid showed low genotypic stability. So Venus and Tinto, despite lower yields, could be recommended because they presented good quality and stability.

Five tomato cultivars: four large-fruit (Rumba, Juhas, Kmicic, Gigant) and one cherry cultivar (Koralik) were selected for study by Hallmann *et al.*, (2007). The organic tomato fruits contained more dry matter, total and reducing sugars, vitamin C, total flavones and beta-carotene, but less lycopene in comparison to conventionally grown tomatoes. The study done by Schulzova *et al.*, (2007) to investigate the effects of tomato cultivation systems on the content of both health promoting and of toxic components represented by carotenoids (lycopene, beta -carotene), vitamin C and glycol-alkaloids (alpha-tomatine, dehydrotomatine). The levels of biologically active compounds were shown to be strongly affected by the degree of fruit maturity. A study was conducted by Ramirez (2005) to test whether tomato fruits from a genotype with elevated levels of natural antioxidants produce seeds with a functionally greater total antioxidant capacity. The tomato genotype which produces elevated levels of lycopene and ascorbic acid, and the recurrent parent 'Flora-Dade' were grown in the field and greenhouse under standard agronomic practices. Harer *et al.*, (2002) grew 37 tomato genotypes in a field experiment. Correlation studies showed that genotypic correlation was higher than phenotypic correlation for all characters examined. Among them the ascorbic acid content had negative direct effects and association with fruit yield.

2.3.6 Brix (%)

Brix percentage is the sugar content of an aqueous solution. One percent Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. If the solution contains dissolved solids other than pure sucrose, then the % Brix only approximates the dissolved solid content. Various reports are available on variation of Brix % for different genotypes of tomatillo and tomato.

Nalla *et al.*, (2014) done a field experiment using 27 tomato genotypes and reported fruit yield per plant (20.51), total soluble solids (17.38), and equatorial diameter (15.38) contributed high for divergence. For total fruit number, total soluble solids content, fruit firmness, length and pH, in a general way and for the majority of the genotypes, there were no statistical differences between the averages of the F_1 and F_2 generations. There was a significant ($p<0.01$) difference among genotypes and environments for all quality traits, Genotype x Environment interaction was significant ($p<0.01$) for all quality traits except for TSS found by Panthee *et al.*, (2013). panthee *et al.*, (2012) found high estimates of genotypic coefficient of variation, heritability and genetic advance for acidity, total soluble solids, ascorbic acid content, and shelf life.

A study evaluated the components of production and total soluble solids (Brix) of tomato cultivar Carolina. The fruits were harvested when they began the color change from green to red; on the occasion were evaluated content of soluble solids, number, weight, length and diameter. Petersen *et al.*, (1998) found highest fruit yield (27.79 t/ha), total soluble solid content (6.11%), acidity (0.93%) and lycopene content (7.64 mg/100 g of juice). Seven tomato lines studied by Chen (2009) and found general heritability for vitamin C and total soluble solid content was high.

Lines belonging to L. esculentum var. cerasiforme were better breeding materials in terms of vitamin C, organic acid and total soluble solid content.

Cheema *et al.*, (2003) studies on combining ability for 10 important characters and significant general (GCA) and specific combining ability (SCA) variances were observed for different characters except for total soluble solids indicating the importance of both additive and non-additive gene effects in the expression of these characters. Four commercial brands of tomato juices and ketchups were studied. Results showed that Brix is higher in ketchup (25-33 degrees Brix) than in tomato juices (4.8-5.5 degrees Brix). Pearson correlations showed statistically significant ($P<0.05$) correlations between Brix and HMF, lycopene, dry matter (negative correlation) and juice (negative); HMF and lycopene and dry matter (negative correlation); lycopene and dry matter (negative), pulp and juice; dry matter and pulp (negative) and juice; and pulp and juice (negative correlation).

Harer *et al.*, (2002) were grown 37 tomato genotypes in a field experiment and correlation studies showed that genotypic correlation was higher than phenotypic correlation for all characters examined. Among them the total soluble solid content had positive but low direct effects and positive association with fruit yield.

Dhaliwal *et al.,* (2002) conducted an experiment with twelve parents and their 66 F_1 hybrids to study the genetics of traits that are important for processing and bulk handling of tomatoes viz. TSS%, pericarp thickness and number of locules. The analysis of variance for combining ability exhibited the significance of both general combining ability and specific combining ability effects for all characters studied.

The chemical contituents are concerned in the quality of tomato fruit in respect to color, texture, flavor, nutritive value, and wholesomeness. In general, high sugar contents, redness of color, and firm texture are associated with prominence of rich flavor. Biochemical changes as influenced by growth, maturation, and environment of tomato fruit are discussed.

2.4 Erroneous Hallucinogenic Information

The Louisiana State Act 159 (2006) treats P. subglabrata (now recognized as a variety of P. longifolia) as a hallucinogenic plant. This potential hallucinogen is listed at two popular culture web sites: Wikipedia (2011), and Erowid (2011), apparently because it is listed in this state act. We conducted extensive searches of the internet and academic search engines but could not find any information to support this claim. We contacted the state representative (now the Louisiana Commissioner of Agriculture) who sponsored the bill, and he informed us that “The Plant Kingdom and Hallucinogens” by Dr. Richard Evans Schultes (1970) was the source of information for Act 159. This law restricts cultivation and possession of 39 listed “hallucinogenic plants,” including the fungi Amanita muscaria and Psilocybe spp., entire genera such as Erythrina spp. and also Salvia divinorum, Ipomoea violacea, and Solanum carolinense. However, Schultes made no mention of the genus Physalis. We have no alternative but to believe that the inclusion of P. subglabrata (P. longifolia var. subglabrata) as a hallucinogenic plants in Louisiana State Act 159 is erroneous.

2.5 Toxicity

There is little evidence of substantial toxicity in Physalis species. The fruits of species north of Mexico have been documented to be eaten as food. Two cases of livestock poisoning involving P. angulata foliage in hay or forage have been reported for California (Fuller and McClintock 1986). However, the toxicity has not been confirmed. Overall there is little reason to consider the plants toxic, other than that they may contain solanine-type glycoalkaloids (Burrows and Tyrl 2001) present throughout most of the Solanaceae. Solanine, a chemical destroyed by heat, is reported to be in the green fruit. This may explain why the young bud clusters of the plants and some sauces were prepared by cooking. Like other members of the Solanaceae, including tomatoes and peppers, only the fruits of Physalis are generally consumed as food, while the foliage is considered somewhat poisonous.

2.6 Heritability and genetic advance

Selection of plants with phenotypic characteristics is the most important task for all plant-breeding practices. The effectiveness of selection for yield depends upon heritability. A character with high heritability gives a better response to selection. Heritability and genetic advance are the most important parameters to judge the breeding potentiality of a population for future development through selection. Many researchers have studied heritability and the genetic advance of yield and many yield contributing characters of tomato a close relative of tomatillo. The literature very relevant to the present study are reviewed below:

According to Saleem (2013) a study of the quantitative genetics of yield and some yield-related traits. The highest estimates of genotypic and phenotypic coefficients of variability (GCV and PCV) were recorded for a number of fruits per plant while fruit width was the most heritable trait.

2.7 Correlation and path coefficient analysis

2.7.1 Correlation between the characters

Correlation between the characters is an estimate to evaluate the inter-relationships between the characters, which will help the breeders to choose selection techniques. In most cases, the correlation between qualitative characters was studied because quality is one of the main targets of most of the breeders. The quality contributing characters are also interrelated among themselves. Therefore, an association of characteristics with quality is important for planning effective selective breeding program for maximization of yield. Such correlation studies may vary due to agro-climatological variations from year to year. If any component of quality has higher heritability than yield itself and there is a positive correlation between these, then there may be some possibility to increase the total yield by selecting that component. However, negative correlation coefficient among yield components was observed indicating selection for any component might not bring improvement for yield. Many authors have studied the correlation between yield and yield contributing characters of tomatillo.

2.7.2 Path coefficient analysis between qualitative characters

The study of correlation does not provide an exact picture of the relative importance of the direct and indirect influence of each of the component character towards the desired character. Therefore, this can be overcome by following path coefficient analysis technique by further partitioning the correlation coefficient into direct and indirect effects. Path coefficient is a standard tool, which measures the direct influence of one character upon another and permits the separation of correlation coefficient into components of direct and indirect effects. Path coefficient between quality contributing characters provides an exact picture of the relative importance of direct and indirect influences of each other component characters on fruit yield. It also provides valuable additional information for improving fruit quality via selection for its qualitative components.

CHAPTER III

MATERIALS AND METHODS

This chapter provides information regarding methodology that was used in execution of this experiment. It is about a brief description of locations of experimental site, planting materials, climate and soil, seed bed preparation, layout and design of the experiment, land preparation, manuring and fertilizing, transplanting of seedlings, intercultural operations, harvesting, data collection procedure, statistical and biochemical analysis procedure etc., which are described and some are illustrated as follows:

3.1 Experimental site

The experiment was conducted at plot no.38 Agronomy experimental field, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during the period from October 2018 to April 2018. Location of the site is 23°74' N latitude and 90°35' E longitude with an elevation of 8 meter from sea level (Anon., 2004) in Agro-ecological zone of "Madhupur Tract" (AEZ-28) (Anon.1988). The experimental site is shown in the map of AEZ of Bangladesh in (Appendix I).

3.2 Planting materials

A total of eighteen genotypes (Table 1) among them four tomatillo originated from Maxico, one is from USA and rest genotypes were collected from the Department of Genetics and Plant Breeding, SAU.

Table 1. Name and origin of tomatillo genotypes used in the present study

Sl. No.	Genotypes No.	Source
1	G1	
2	G2	
3	G3	
4	G4	
5	G5	
6	G6	
7	G7	
8	G8	
9	G9	
10	G10	GEPB, SAU
11	G11	
12	G12	
13	G13	
14	G14	
15	G15	
16	G16	
17	G17	
18	G18	

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

3.3 Climate and soil

Experimental site was located in the subtropical climatic zone, set aparted by plenty of sunshine and moderately low temperature prevails during October to March (Rabi season) which is suitable for tomato as well as tomatillo growing in Bangladesh. The soil was sandy loam in texture having pH 5.46- 5.62. Weather information and physicochemical properties of the soil are presented in (Appendix II and Appendix III respectively).

3.4 Seed bed preparation and raising of seedling

The seed sowing was carried out on October 28, 2018 in the seedbed. Before sowing, seeds were treated with tilt-200 EC thoroughly for 5 minutes. Seedlings of all genotypes were raised in seedbeds besides the electric pole adjacent to the gate of agronomy field in the Sher-e-Bangla Agricultural University, Dhaka-1207. Seeds were sown in rows spaced at 10 cm apart, beds were watered in a regular manner. Seedlings were raised using regular nursery practices. Recommended cultural practices were taken up before and after sowing the seeds. When the seedlings become 20 days old, those were transplanted in the main field.

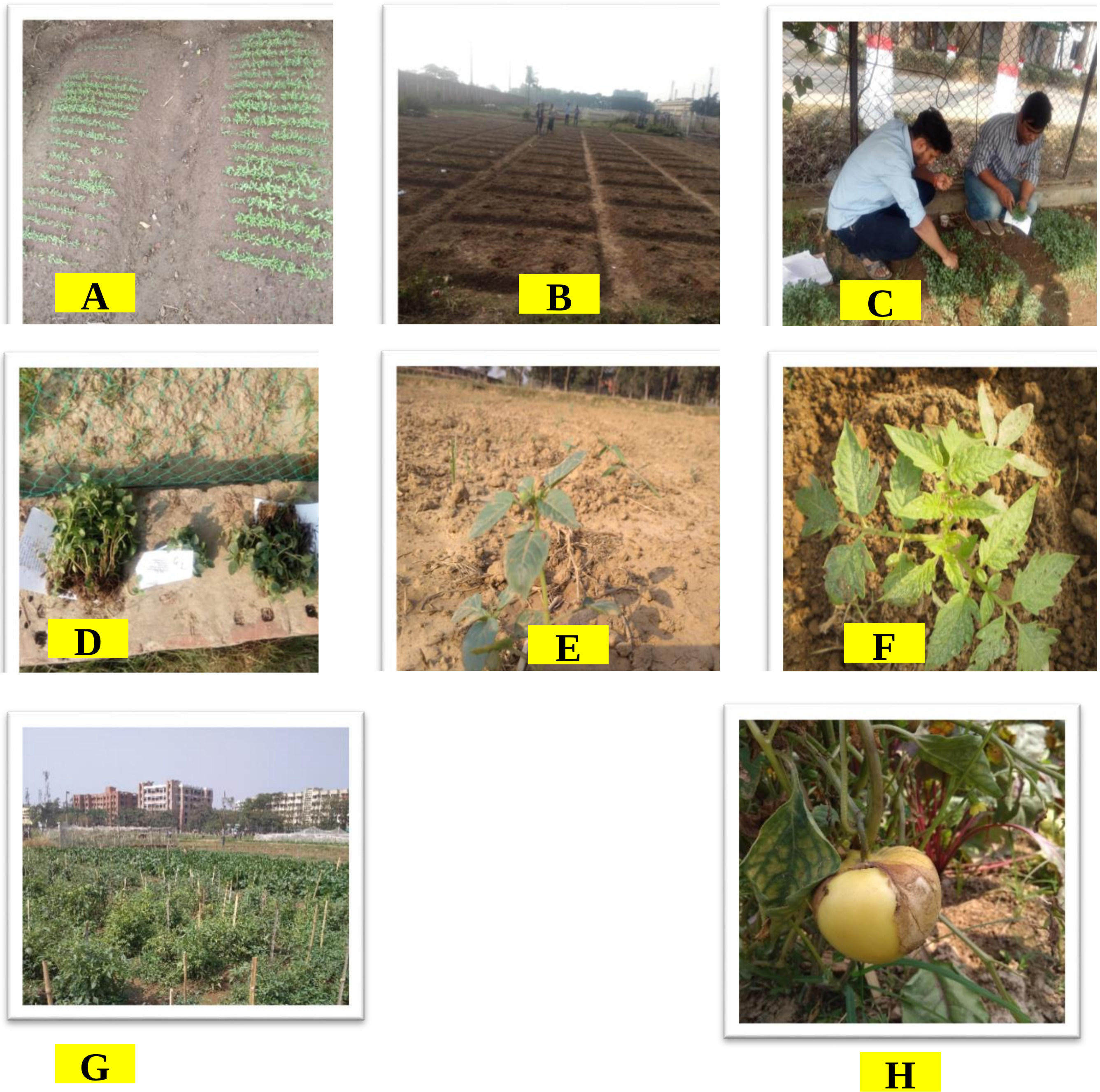

Plate 1.

A. Newly born seedlings B. Final Land Preparation and Plot designing C. Uprooting of seedlings to transplant in main field D. Seedlings were ready to transplant E. Vigorous seedling on main field F. Irrigation was done G. Flowering stage of Tomatillo H. Mature fruit were ready to harvest

A.Lycopene Test

B.Vit-C

C. Brix Test

D. Brix Test

Plate 2.

A) Lycopene test
B) Vit-C test
C) Brix test
D) Brix test

Plate 3: Fruits of G_1-G_7 Genotypes

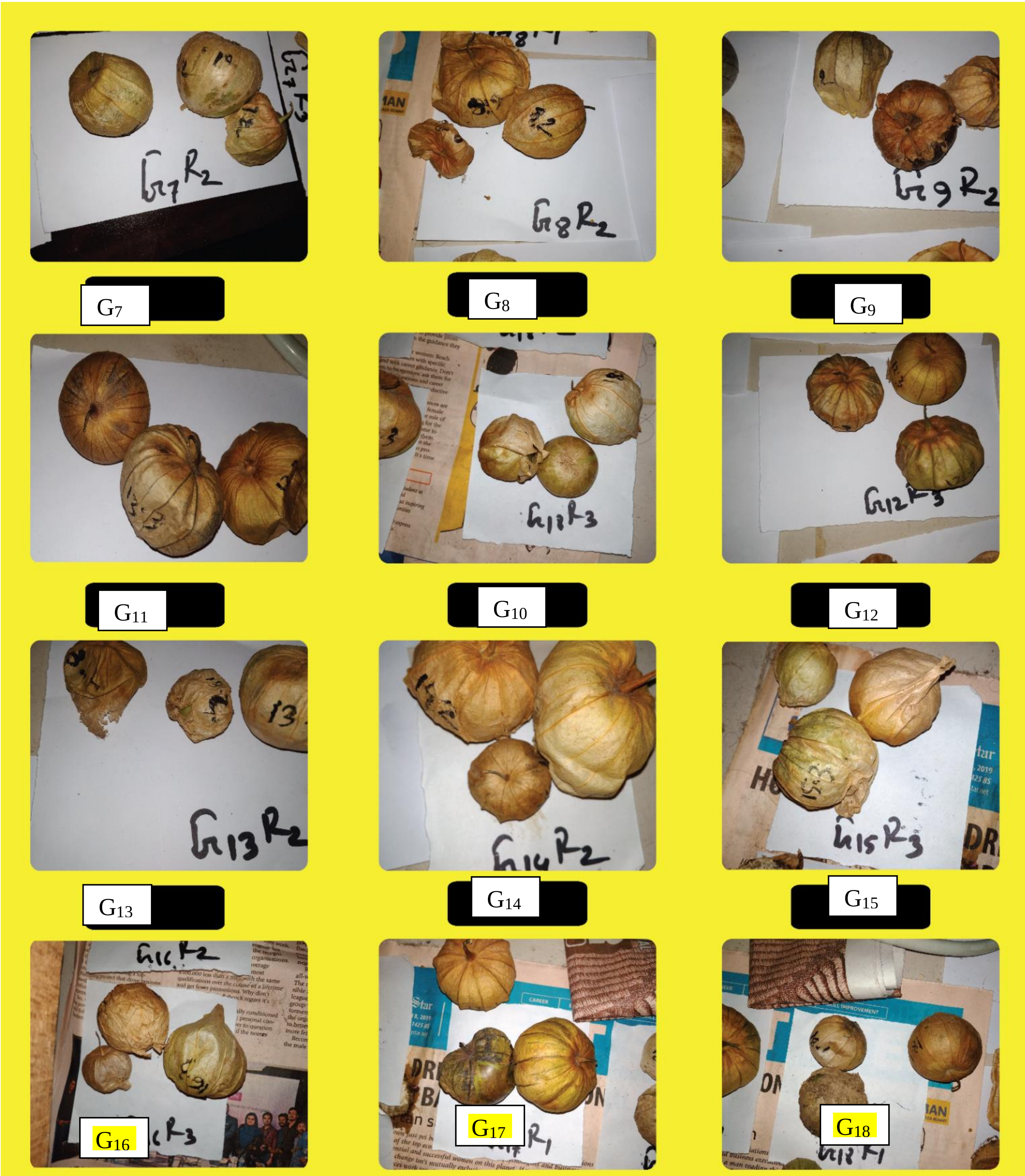

Plate 3: Fruits of G_7-G_{18} Genotypes

3.5 Design and layout of the experiment

The experiment was laid out and evaluated under field condition during Rabi 2015-16 in Randomized Complete Block Design (RCBD).

Genotype	:	18
Replications	:	3
Spacing	:	40 cm × 60 cm
Plot size	:	60× 90 cm
Date of transplanting	:	1st December 2018

3.6 Land preparation

The experimental plot was in crumble structured as cultivated previously, ploughed and brought into a fine tilth and raised the nursery bed, applied the recommended dose of fertilizers and farm yard manures (FYM) whatever it needed. Weeds and other stubbles were removed carefully from the experimental plot and leveled properly. The final land preparation was done on November 28 November, 2018 leaving the whole land into the plot form.

3.7 Transplanting of seedlings

The seedlings were raised in the seedbed in usual way and 20 days old seedlings were transplanted in the main field on 1 December, 2018. The transplanted seedlings were watered regularly to make a firm relation with roots and soil to stand along.

3.8 Manure and fertilizers application

Manuring and fertilization of tomato and tomatillo almost same .Total cow dung and Triple Super Phosphate (TSP) were applied in the field during final land preparation. Half Urea and half Muriate of Potash (MOP) were applied in the plot after three weeks of transplanting. Remaining Urea and Muriate of Potash (MOP) were applied after five weeks of transplanting. Doses of manure and fertilizers used in the study are presented in Table 2.

Table 2. Doses of manures and fertilizers used in the study

Sl. No.	Fertilizers/ Manures	Dose	
		Applied in the plot	**Quantity/ha**
1.	Urea	10.5 kg	550 kg
2.	TSP	08 kg	450 kg
3.	MOP	4.5 kg	250 kg
4.	Cow dung	200 kg	10 ton

3.9 Intercultural operations

When the seedlings were well established, first weeding was done uniformly in all the plots. Second weeding was done after 20 days of the first one. Mechanical support was provided to the growing plants by bamboo sticks to keep them erect. During early stages of growth, pruning was done by removing some of the lateral branches to allow and plants to get more sunlight and to reduce the self-shading and incidence of increased insect infestation. Thinning and gap filling, staking, pesticide application, irrigation and after-care were also done as per requirement.

3.10 Harvesting and processing

All of the tomatillo varieties used in this experiment was indeterminate types. So, harvesting continued for about one and half month because fruits of different lines matured progressively at different dates and over long time. The fruits per entry were allowed to ripe and then seeds were collected and stored at 4^{o}C for future use. Harvesting was started from March 1, 2018 and completed by April 23, 2018. During Raising of seedlings, experimental field in growing condition of plants, intercultural operation, growth stage of a single tomatillo and tomato plant closed eyes were applied there.

3.11 Antioxidant and nutritional traits

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

3.12 Determination of Brix percentage

Brix percentages were measured by Portable Refractometer (ERMA, Tokyo, Japan) at room temperature. Single fruit was blend and juice was collected to measure brix percentage.

3.13 Determination of Vitamin-C

Vitamin-C was measured by Oxidation Reduction Titration Method (Tee *et al.*, 1988). Single fruit was blend and tomato extract was filtrated by Whatman No.1 filter paper. It 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). The solution was titrated with dye. 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.

3.14 Determination of Lycopene content

Absorption determination for lycopene content was estimated following the method of Alda (2009) by using T60 UV-Visible Spectrophotometer. Lycopene in the tomato was extracted using hexane:ethanol:acetone (2:1:1) (v/v) mixture. One gram juice of the each sample were homogenized with 25 ml of hexane:ethanol:acetone, which were then placed on the orbital shaker for 30 min., adding 10 ml distilled water and was continued agitation for another two min. The solution was then left to separate into distinct polar and non- polar layers. The absorbance was measured at 472 nm and 502 nm, using hexane as a blank. The lycopene concentration was calculated using its specific extinction coefficient (E 1%, 1cm) of 3450 in hexane at 472 nm and 3150 at 502 nm. The lycopene concentration was expressed as mg/100g product.

At λ = 472nm: lycopene content (mg/100g) =

At λ = 502 nm: lycopene content (mg /100g) =

Where,

m = the weight of the product (g)

E = extinction coefficient

3.15 Determination of pH of the flesh

Sample of 5gm each of the fresh mesocarp were homogenous in 5ml of boil distill water and deionize water (pH 7) and the pH of the homogenate was measured with a pH meter.

3.16 Determination of Non-Reducing Sugar & Total Sugar by Lane-Eynon Method

The Lane-Eynon method is an example of a tritration method of determining the concentration of reducing sugars in a sample. A burette is used to add the carbohydrate solution being analyzed to a flask containing a known amount of boiling copper sulfate solution and a methylene blue indicator. The reducing sugars in the carbohydrate solution react with the copper sulfate present in the flask. Once all the copper sulfate in solution has reacted, any further addition of reducing sugars causes the indicator to change from blue to white. The volume of sugar solution required to reach the end point is recorded. The reaction is not stoichemetric, which means that it is necessary to prepare a calibration curve by carrying out the experiment with a series of standard solutions of known carbohydrate concentration.

3.17 Determination of Reducing Sugar

Deducting Non-reducing Sugar from Total sugar, we have got amount of reducing sugar.

3.18 Determination of Shelf life

Shelf life of 18 Genotype's of tomatillo was determined at central Research center, SAU.

3.19 Statistical analysis

Collected data were statistically analyzed using MSTAT-C computer package program. Mean for every treatments were 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).

3.19.1 Estimation of genotypic and phenotypic variances

Genotypic and phenotypic variances were estimated according to the formula given by Johnson (1955).

Genotypic variance, $\sigma^2_g = \dfrac{GMS - EMS}{r}$

Where,

GMS = Genotypic mean sum of squares

EMS = Error mean sum of square

r = number of replications

Phenotypic variance, $\sigma^2_{ph} = \sigma^2_g + EMS$

Where,

σ^2_g = Genotypic variance

EMS = Error mean sum of square

Environmental variance ($\sigma^2 e$) = EMS

Where,

EMS = Mean Square Error

3.19.2 Estimation of genotypic and phenotypic coefficient of variation

Genotypic and phenotypic coefficient of variation was calculated by the formula suggested by Burton (1952)

Genotypic co-efficient of variation, GCV % = $\frac{\sqrt{\sigma^2 g}}{\bar{x}} \times 100$

Where,

σ^2_g = Genotypic variance

$\bar{x}$ = Population mean

Similarly,

The phenotypic co-efficient of variation was calculated from the following formula.

Phenotypic co-efficient variation, PCV = $\frac{\sqrt{\sigma^2 ph}}{\bar{x}} \times 100$

Where,

σ^2_{ph} = Phenotypic variance

$\bar{x}$ = Population mean

3.19.3 Estimation of heritability

Broad-sense heritability was estimated (Lush, 1943) by the following formula, suggested by Johnson (1955).

Heritability, $h^2{}_b\% = \frac{\sigma^2{}_g}{\sigma^2{}_{ph}} \times 100$

Where,

$h^2{}_b$ = Heritability in broad sense

$\sigma^2{}_g$ = Genotypic variance

$\sigma^2{}_{ph}$ = Phenotypic variance

3.19.4 Estimation of genetic advance

The expected genetic advance for different characters under selection was estimated using the formula suggested by Lush (1943) and Johnson (1955).

Genetic advance, GA = K. h^2. σ_p

Or Genetic advance, GA = K. $\frac{\sigma^2{}_g}{\sigma^2{}_{ph}}.\sigma_{ph}$

Where,

K = Selection intensity, the value which is 2.06 at 5% selection intensity

σ_{ph} = Phenotypic standard deviation

$h^2{}_b$= Heritability in broad sense

$\sigma^2{}_g$ = Genotypic variance

$\sigma^2{}_{ph}$ = Phenotypic variance

3.19.5 Estimation of genetic advance mean's percentage

Genetic advance as a percentage of the mean was calculated from the following formula as proposed by Comstock and Robinson (1952):

$$\text{Genetic advance (\% of mean)} = \frac{\text{Genetic Advance}}{\text{Population mean } (\bar{x})} \times 100$$

3.19.6 Estimation of simple correlation coefficient:

Simple correlation coefficients (r) was estimated with the following formula (Clarke, 1973; Singh and Chaudhary, 1985).

$$r = \frac{\sum xy - \frac{\sum x . \sum y}{N}}{\sqrt{[\{\sum x2 - \frac{(\sum x)2}{N}\}\{\sum y2 - \frac{(\sum y)2}{N}\}]}}$$

Where,

$\sum$ = Summation

x and y are the two variables correlated

N = Number of observation

3.19.7 Estimation of genotypic and phenotypic correlation coefficient

For calculating the genotypic and phenotypic correlation coefficient for all possible combinations the formula suggested by Miller (1958), Johnson (1955) and Hanson (1956) were adopted. The genotypic co-variance component between two traits and have the phenotypic co-variance component were derived in the same way as for the corresponding variance components. The covariance components were used to compute the genotypic and phenotypic correlation between the pairs of characters as follows:

$$\text{Genotypic correlation, } r_{gxy} = \frac{GCOVxy}{\sqrt{GVx.GVy}} = \frac{\sigma_{gxy}}{\sqrt{(\sigma^2_{gx} . \sigma^2_{gy})}}$$

Where,

σ_{gxy} = Genotypic co-variance between the traits x and y

σ^2_{gx} = Genotypic variance of the trait x

σ^2_{gy} = Genotypic variance of the trait y

Phenotypic correlation (r_{pxy}) = $\frac{PCOVxy}{\sqrt{PVx.PVy}}$ = $\frac{\sigma}{\sqrt{(\sigma^2_{px}}}$

Where,

σ_{pxy} = Phenotypic covariance between the t[illegible]

σ^2_{px} = Phenotypic variance of the trait x

σ^2_{py} = Phenotypic variance of the trait y

CHAPTER IV

RESULTS AND DISCUSSION

The result of this present investigation of genetic analysis that means Genetic Variability, Genetic Diversity Analysis, association of Characters and path co-efficient analysis of Tomatillo (*Physalis ixocarpa* Brot.) fruit of F_2 Generation illustrated and discussed here under following sections:

4.1 Mean, Range and Analysis of Variance

4.1.1 Fruit p^H

Fruit p^H differed significantly in all the genotypes ranging from 2.74 to 4.33 (Table 3). The CV value of fruit p^H was observed 6.03%. The maximum p^H was observed in genotype G17 (4.33) and the lowest p^H found in the genotype G11 (2.74) (Table 3)

4.1.2 Fruit's Vitamin-C

Vitamin C was observed from the result of the experiment that vitamin C contents of tomatillo fruit were varied significantly among the eighteen tomatillo genotypes. The average value of vitamin C of fruit varied from 5.19 mg to 170.94 mg/100g (Table 3). Maximum vitamin C (170.94 mg/100g) was found in G5 whereas minimum (5.94 mg/100g) from G_{16} (Table 4). According to the study maximum concentration of vitamin C was found in G5 tomatillo genotypes. High vitamin C in tomatillo not only improves the nutrition, it also aids in better retention of natural colour and flavour of the products (Thamburaj, 1998).

Table 3. Range, mean CV (%) and standard deviation of eighteen tomatillo genotypes

Parameters	Range		Mean	CV (%)	SD	SE
	Min	Max				
Reducing sugar	1.16	1.58	1.37	7.02	0.14	0.06
Total sugar	2.56	3.27	2.81	1.11	0.16	0.02
Non-reducing sugar	1.10	1.79	1.46	2.40	0.14	0.02
pH	2.74	4.33	3.31	6.03	0.47	0.12
Lycopene	0.25	0.84	0.55	7.14	0.19	0.02
Vit-C	5.19	170.94	22.49	3.01	37.00	0.39
Shelf Life(days)	10.33	36.67	25.11	16.75	7.44	2.43
Brix Percentage	2.00	6.87	4.44	6.83	1.34	0.18

Table 4. Mean performance of eighteen genotypes of tomatillo in respect of eight important characters

Genotype	Reducing sugar	Total sugar	Non-reducing sugar	pH	Lycopene	Vit-C	Shelf Life(days)	Brix Percentage
G1	1.58 a	2.97 b	1.55 cd	3.50 bc	0.81 ab	18.94 d	27.66 bcd	3.46 f
G2	1.23 efg	2.67 j	1.44 gh	3.06 efgh	0.52 fg	15.07 f	10.33 f	2.00 g
G3	1.43 abcd	2.86 de	1.43 h	3.43 bcd	0.77 bc	7.87 j	24.33 cd	2.16 g
G4	1.47 abc	2.91 cd	1.45 fgh	3.22 cdef	0.53 efg	16.79 e	28.00 bc	5.00 cd
G5	1.36 bcde	2.85 ef	1.49 efg	3.02 efgh	0.56 ef	170.94 a	14.00 f	6.10 b
G6	1.47 abc	2.56 k	1.09 k	3.12 defg	0.57 def	9.01 i	29.00 bc	4.00 e
G7	1.29 defg	2.79 gh	1.50 def	3.32 bcde	0.30 ij	6.48 k	36.66 a	3.40 f
G8	1.52 ab	3.27 a	1.79 a	3.62 b	0.58 de	11.99 g	29.33 bc	5.16 c
G9	1.32 cdef	2.67 j	1.35 j	2.95 fgh	0.77 bc	10.86 h	21.00 de	3.10 f
G10	1.15 g	2.72 ij	1.60 bc	3.12 defg	0.84 a	9.67 i	28.66 bc	6.86 a
G11	1.44 abcd	2.80 fg	1.37 ij	2.74 h	0.50 gh	11.82 gh	29.00 bc	5.13 c
G12	1.16 fg	2.67 j	1.51 de	2.86 gh	0.73 c	36.17 b	15.00 ef	4.83 cd
G13	1.32 cdef	2.74 hi	1.42 hi	2.78 h	0.63 d	20.17 c	24.00 cd	4.50 de
G14	1.43 abcd	2.92 c	1.49 efg	3.46 bc	0.31 ij	14.81 f	26.00 cd	5.96 b
G15	1.36 cde	2.79 gh	1.43 h	4.07 a	0.35 i	11.91 gh	26.33 cd	5.23 c
G16	1.40 bcd	2.74 hi	1.34 j	3.34 bcde	0.32 i	5.19 l	25.00 cd	4.58 d
G17	1.36 cde	2.98 b	1.64 b	4.33 a	0.25 j	9.10 i	24.33 cd	3.18 f
G18	1.38 bcde	2.74 hi	1.39 hij	3.62 b	0.45 h	17.97 d	33.33 ab	5.26 c
LSD	0.15	0.05	0.05	0.33	0.06	1.125	6.97	0.50

Values with same letter(s) are statistically identical at 5% level of probability

4.1.3 Brix (%)

From the result of the experiment it was observed that brix (%) of tomatillo fruit was varied significantly among the eighteen tomatillo genotypes. The ranges of brix from 2.00% to 6.87% with the mean value of 4.44% (Table 3). Maximum brix (6.87%) was found in G10 whereas minimum (2.00%) from G2 (Table 4). According to the study G10 tomatillo genotypes have the highest brix (%).

4.1.4 Lycopene

The genotype G10 recorded maximum lycopene content of the fruit (0.84 mg), while the minimum was observed by the genotype G17 (0.25 mg) in case of 472 nm. Colour of fruit is an important quality parameter both for table purpose and processing varieties. Potaczek and Michalik (1998) have observed that environmental factors especially temperature and light intensity exerted a great influence on lycopene level than on carotene contents in tomato fruits. Red-fruiting cultivars also have higher lycopene content than yellow, orange and black- fruiting cultivars (Cox *et al.*, 2003).

4.1.5 Shelf Life

From the result of the experiment it was observed that shelf life of tomatillo fruit was varied significantly among the eighteen tomatillo genotypes. The ranges of shelf life varies from 10.33 days to 36.67 days with the mean value of 25.11 (Table 3). Maximum shelf life 36.67 days was found in G7 whereas minimum (10.33 days) from G2 (Table 4). According to the study G7 tomatillo genotypes had the highest shelf life (days).

4.1.6 Non-Reducing Sugar

From the result of the experiment it was observed that amount of Non-reducing sugar of tomatillo fruit was varied significantly among the eighteen tomatillo genotypes. The ranges of Non-reducing sugar varies from 1.10g to 1.79 g with the mean value of 1.46g (Table 3). Maximum non-reducing sugar 1.79g was found in G8 whereas minimum 1.10 g from G6 (Table 4). According to the study G8 tomatillo genotypes had the highest non-reducing sugar (gram).

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4.1.7 Reducing Sugar

From the result of the experiment it was observed that amount of reducing sugar of tomatillo fruit was varied significantly among the eighteen tomatillo genotypes. The ranges of reducing sugar varies from 1.16 g to 1.58 g with the mean value of 1.37 g (Table 3). Maximum reducing sugar 1.58 g was found in G1 whereas minimum 1.16 g from G10 (Table 4). According to the study G1 tomatillo genotypes had the highest reducing sugar (gram).

4.1.8 Total Sugar

From the result of the experiment it was observed that amount of total sugar of tomatillo fruit was varied significantly among the eighteen tomatillo genotypes. The ranges of total sugar varies from 2.56 g to 3.27 g with the mean value of 2.81 g (Table 3). Maximum reducing sugar 3.27 g was found in G8 whereas minimum 2.56 g from G6 (Table 4). According to the study G8 tomatillo genotypes had the highest total sugar (gram).

4.2 Estimates of Genetic Parameters

The development of suitable plant type is of great importance for all the crops through planned design programme. Attempts have, therefore, been made by several scientists to analyse different chemical characters to provide meaningful information about the significance of characters in relation to tomatillo fruit. An ideal plant ideotype would only be defined if the different components of fruit of tomatillo are analysed and their relative importance can be assessed. In the present study, genetically diverse tomatillo genotypes collected from different sources were examined and quality component analyses were carried out to identify important fruit quality components.

Genetic variability Estimates including genotypic variance (σ^2_g) phenotypic variance (σ^2_p) environmental variance (σ^2_e) phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H %), genetic advance (GA) and genetic advance as percent mean (GAM) were summarized in Table 6.

4.2.1. Estimates of Variance Components

The variance components include genotypic variance, environmental variance and phenotypic variance which are presented in (Table 6) and discussed here. The highest environmental variance was observed 17.683 was for Shelf Life (Days) which indicated that environmental component in total variation was high. The highest genotypic variance and phenotypic variance were 1421.900 and 1422.360, respectively; indicates the presence of high variation for this trait. The lowest environmental, genotypic and phenotypic variances were also 0.001, 0.010 and 0.019. All of the above results showed the potential of variation that exist in different traits. According to Engida (2007) traits that showed the different genotypic, phenotypic and environmental values indicates the presence of variation among the traits used.

Table 5. Analysis of variance for different characters

Character	Mean sum of square		
	Replication (r-1)=2	**Genotype (g-1)=17**	**Error (r-1)(g-1)=34**
Reducing sugar	0.010	0.039**	0.009
Total sugar	0.002	0.078**	0.0010
Non-reducing sugar	0.0002	0.062**	0.0012
pH	0.503	0.537**	0.040
Lycopene	0.007	0.106**	0.002
Vit-C	1.590	4266.160**	0.460
Shelf Life(days)	51.389	131.020**	17.683
Brix Percentage	0.396	5.383**	0.092

** Denote Significant at 1% level of probability

Table 6. Estimation of genetic, phenotypic and environmental variance in eight traits

Parameters	σ^2_p	σ^2_g	σ^2_e
Reducing sugar	0.019	0.010	0.009
Total sugar	0.027	0.026	0.001
Non-reducing sugar	0.022	0.020	0.001
pH	0.206	0.166	0.040
Lycopene	0.036	0.035	0.002
Vit-C	1422.360	1421.900	0.460
Shelf Life(days)	55.462	37.779	17.683
Brix Percentage	1.856	1.764	0.092

σ 2p = Phenotypic variance, σ 2g = Genotypic variance and σ 2 e = Environmental variance, PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, ECV = Environmental coefficient of variation.

4.2.2. Estimates of Genotypic and Phenotypic Coefficient of Variation

The highest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were 167.701 and 167.673, respectively both of which were recorded for Vit-C content (Table 7). In this case, the PCV values of Vit-C were a little more than GCV values. This indicated that the environmental effect was small for the expression of these characters (Kassaye, 2006). The magnitudes of phenotypic and genotypic coefficient of variation were lowest for total sugar (%) 5.796 & 5.689, respectively. In these cases, the PCV values were higher than GCV values across the environment. This indicated the presence of environmental influence on these characters (Sharma *et al* 2009). PCV and GCV were classified as suggested by Shivasubramanian and Menon (1973) as follows, 0–10% -Low, 10-20 – Moderate, 20% and above – High.

High to moderate GCV and PCV values were shown by all the characters except total sugar (%) that showed low GCV and PCV values. The present result revealed that, higher genotypic coefficients of variations were recorded for Vitamin C (167.673%), brix (29.894%), lycopene at 472 nm (34.107%) (Table 7). p^H (12.291%) had the moderate values. Total sugar (5.689%) had the lowest GCV values (Table 7). The highest phenotypic coefficient of variation value recorded for Vitamin C (167.701%), brix (30.664%), lycopene at 472 nm (34.85 (Table 7). p^H (13.69%) had the moderate PCV values. Total Sugar (5.80%) had the lowest PCV values. Phenotypic co-efficient of variation (PCV) agreed closely with the genotypic co-efficient of variation (GCV) (Table 7) which was well supported by Proshy *et al.*, 1984 ; Saper *et al.*,1984; Joshi *et al.*, 2004; Kumar *et al.*, 2006; Golani *et al.*,

2007. In all cases, the difference between PCV and GCV values was low indicating the low effects of environment in all of these characters. The difference in genotypic coefficient of variation and phenotypic coefficient of variation values were closer indicates that there was a minimum influence of environment on these characters. In general, phenotypic coefficient of variation values were higher than their corresponding genotypic coefficient variation values in all of the characters. This result is also in line with the results reported by Sharma *et al.,* (2009) who revealed that the magnitude of PCV was higher than GCV for all the characters.

High proportion of GCV to PCV is desirable in selection process because it depicts that the traits are much under the genetic control rather than the environment (Kaushik *et al.,* 2007). The proportion of GCV in PCV observed in this study ranged from 71.70% in reducing sugar to 99.98% in Vitamin C (mg/100g). The traits with high proportion of GCV in PCV are reliable for selection in quality genetic improvement of tomatillo genotypes. Trait (Reducing Sugar) whose expression was environmentally dependent may not be reliable descriptor for quality characterization.

Table 7. Estimation of phenotypic and genotypic coefficient variation

Parameters	**PCV (%)**	**GCV (%)**	**PCV:GCV**
Reducing sugar	10.077	7.225	71.702
Total sugar	5.796	5.689	98.142
Non-reducing sugar	10.047	9.756	97.103
pH	13.691	12.291	89.779
Lycopene	34.849	34.107	97.871
Vit-C	167.701	167.673	99.984
Shelf Life(days)	29.657	24.477	82.533
Brix Percentage	30.664	29.894	97.486

σ 2p = Phenotypic variance, σ 2g = Genotypic variance and σ 2 e = Environmental variance, PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, ECV = Environmental coefficient of variation.

4.2.3. Estimates of Heritability and Genetic Advance

Broad sense heritability values were higher (more than 51.41%) for all the characters. Broad sense heritability ranged from 51.41% (Reducing Sugar) to 99.99 % (Vit-C). These broad sense heritability values were likely to be over- estimated as in this calculation it was not possible to exclude variation due to different genetic components and their interactions.

The present estimation of heritability was 80.60% for p^H value, 99.99% for Vitamin C, 96.32% for Total sugar, 95.04% for brix, 95.79% for lycopene at 472 nm were higher. The heritability of the highest magnitude was noticed for Vit-C (99.99%) (Table 8). Thus, it indicated that larger proportion of phenotypic variance had been attributed to genotypic variance and reliable selection could be made for almost all the traits on the basis of phenotypic expression.

High estimates of heritability in broad sense indicated that substantial improvement can be made using standard selection procedures. In general, characters which exhibited high heritability suggest that the selection would be more effective whereas characters showing low heritability indicate that the selection would be affected by environmental factors.

Based on the observation, in the present study, it can be surly concluded that selection of genotype based on p^H value, vitamin C, brix, lycopene at 472,total sugar & non-reducing sugar would be more satisfactory.

The heritability estimates were, therefore, to be considered with these limitations in view. However, genetic advance (GA) expressed as percentage of mean was high (>20%) for the characters like p^H, vitamin C, shelf life, brix, lycopene at 472 nm The estimate of genetic advance as percent of mean was highest (345.35%) for vit-C at 472 nm and lowest (11.50%) for Total Sugar. Most of the traits studied had high genetic advance as percent of mean though it was moderate for

Total Sugar (10.67%). These results were in agreement with Singh *et al.,* (2005). According to Johnson *et al.*, (1955) high heritability estimates along with the high genetic advance is usually more helpful in predicting gain under selection than heritability estimates alone. The estimates of heritability accompanied by estimates of genetic advance as percent of means are more meaningful from the point of expected genetic gain. Genetic advance is the measure of improvement that can be achieved by practicing selection in a population.

Table 8 .Estimation of heritability and genetic advance

Parameters	**Heritability**	**Genetic advance (5%)**	**Genetic advance (% of mean)**
Reducing sugar	51.412	0.146	10.672
Total sugar	96.318	0.324	11.501
Non-reducing sugar	94.291	0.285	19.515
pH	80.603	0.753	22.732
Lycopene	95.788	0.376	68.764
Vit-C	99.968	77.666	345.352
Shelf Life(days)	68.117	10.450	41.616
Brix Percentage	95.035	2.667	60.033

4.3 Character Association

Association analysis of different quality characters with brix of tomatillo fruit and their interrelationships were investigated through the study of both phenotypic and genotypic correlation coefficients. In the present study, eight quality characters were recorded and their genotypic and phenotypic correlation coefficients were analyzed (Table 9).

Phenotypic and genotypic correlation co-efficient, in general, agreed very closely. However, the genotypic correlations were higher than phenotypic correlations in most of the cases. These could occur when the genes governing two traits were similar and environmental factors played a small part in the expression of these traits.

4.3.1 Phenotypic Correlation

Phenotypic correlation among eight characters were computed and presented in the Table 9 and described below:A high positive significant correlation of Reducing Sugar ($r=0.411^{**}$) with Total Sugar, p^H($r = 0.299^{*}$) and Shelf Life ($r=0.293^{*}$); and Total Sugar ($r = 0.766^{**}$) with Non-reducing Sugar and Total sugar ($r=0.449^{**}$) with p^H at 1% level of significant, suggested that more Reducing Sugar along with high Shelf Life and High p^H would be an appropriate selection criterion to get high quality fruit of tomatillo. Reducing Sugar was positively and significantly correlated with Total Sugar (r =0.411**), p^H (r = 0.299*) and Shelf Life ($r = 0.496^{*}$).Total sugar had positively significant correlated with non-reducing sugar ($r = 0.766^{**+}$), and p^H ($r = 0.449^{**}$). Vitamin C had positively significant association with shelf life ($r = 0.429^{*}$) and brix ($r = 0.328^{*}$) (Table 9). This implies that lycopene and moisture increased with increasing of vitamin C indicating that simultaneous selection of such traits were possible. Non-reducing sugar had positively significant correlation with p^H ($r = 0.368^{**}$).p^H had significant and positive correlation with Lycopene at 472 nm.The significant positive association of any character with the other character suggests that increase in any one of these traits may results in increase in other trait.

Table 9. Phenotypic correlation coefficients among different pairs of yield and yield contributing characters for different genotype

	Reducing sugar	Total sugar	Non-reducing sugar	pH	Lycopene	Vit-C	Shelf Life(days)	Brix Percentage
Reducing sugar	1							
Total sugar	0.411**	1						
Non-reducing sugar	-0.061^{NS}	0.766**	1					
pH	0.299*	0.449**	0.368**	1				
Lycopene	-0.080^{NS}	-0.119^{NS}	0.036^{NS}	-0.453**	1			
Vit-C	-0.046^{NS}	0.035^{NS}	0.072^{NS}	-0.207^{NS}	0.086^{NS}	1		
Shelf Life(days)	0.293*	0.210^{NS}	0.037^{NS}	0.229^{NS}	-0.191^{NS}	-0.429**	1	
Brix Percentage	-0.051^{NS}	0.111^{NS}	0.175^{NS}	-0.075^{NS}	-0.040^{NS}	0.328*	0.182^{NS}	1

4.3.2 Genotypic Correlation

Genotypic correlations among eight characters were computed and presented in (Table 10) and described below. The genotypic correlation coefficient revealed positive and significant association of reducing sugar with total sugar (r = 0.666^{**}), pH (r = 0.270^{*}) and shelf life (r = 0.473^{**}).In the present study Total sugar had positive significant correlation with Non-reducing sugar (r = 0.788^{**}) and pH (r = 0.521^{**}). From this result positive and significant correlation of non-reducing sugar with pH (r = 0.389^{**}). pH showed positive and significant association with lycopene at 472 nm (r =0.521^{**}) and shelf life (r=0.346^{*}). This indicated that if pH was increased the traits Shelf life and lycopene were increased as same degree because they were positively associated with pH. Vit-C had significant and positive correlation with shelf life (r=0.523**) and Brix (r= 0.335*).

Table 10. Genotypic correlation coefficients among different pairs of yield and yield contributing characters for different genotype

	Reducing sugar	**Total sugar**	**Non-reducing sugar**	**pH**	**Lycopene**	**Vit-C**	**Shelf Life(days)**	**Brix Percentage**
Reducing sugar	1							
Total sugar	0.666**	1						
Non-reducing sugar	-0.052^{NS}	0.788**	1					
pH	0.270*	0.521**	0.389**	1				
Lycopene	-0.118^{NS}	-0.134^{NS}	0.031^{NS}	-0.521**	1			
Vit-C	-0.068^{NS}	0.035^{NS}	0.074^{NS}	-0.232^{NS}	0.088^{NS}	1		
Shelf Life(days)	0.473**	0.239^{NS}	-0.015^{NS}	0.346*	-0.255^{NS}	-0.523**	1	
Brix Percentage	-0.081^{NS}	0.116^{NS}	0.182^{NS}	-0.093^{NS}	-0.047^{NS}	0.335*	0.219^{NS}	1

** = Significant at 1%. * = Significant at 5%.

4.4 Path Co-efficient Analysis

The correlation values decide only the nature and degree of association existing between pair of characters. Yield is dependent on several mutually associated component characters and hence change in any one of the components is likely to affect the whole network of cause and effect. This in turn might affect the true association of component characters both in magnitude, direction and tend to vitiate the association of yield and yield attributes. Hence, it is necessary to partition the correlation of component characters into direct and indirect effects through components (Table 11).

Path analysis of direct and indirect effects revealed that reducing sugar (0.044), non-reducing sugar (0.648), vitamin C (0.628), shelf life (0.712) exerted direct positive effect on brix percentage whereas the direct effect of total sugar (-0.491), pH (-0.278) and lycopene at 472 nm (-0.146) were negative direct effect on brix percentage (Table 11). Shelf life exerted the highest positive direct effect (0.712) on brix percentage and exerted positive indirect effect through reducing sugar (0.021), and lycopene at 472 nm (0.037). The residual (0.54) indicated that characters which included in these genotypic path analysis explained (99.46%) of the total variation in brix or all other characters which are not included in this study have negative direct effect on brix. The higher positive direct effect was observed in shelf life can be used as either direct or indirect selection criteria to improve brix percentage and other traits those are positively associated with it. Selecting plants with higher shelf life of fruits will increase other associated qualitative traits specially fruit brix.

Table 11. Partitioning of genotypic correlations into direct (bold) and indirect effects of eight important characters by path analysis

Trait	Reducing sugar	Total sugar	Non-reducing sugar	pH	Lycopene	Vit-C	Shelf Life(days)	Genotypic correlation with Brix Percentage
Reducing sugar	**0.044**	-0.327	-0.034	-0.075	0.017	-0.043	0.337	-0.081 NS
Total sugar	0.029	**-0.491**	0.510	-0.145	0.020	0.022	0.170	0.116 NS
Non-reducing sugar	-0.002	-0.386	**0.648**	-0.108	-0.005	0.047	-0.011	0.182 NS
pH	0.012	-0.256	0.252	**-0.278**	0.076	-0.146	0.246	-0.093 NS
Lycopene	-0.005	0.066	0.020	0.145	**-0.146**	0.056	-0.182	-0.047 NS
Vit-C	-0.003	-0.017	0.048	0.064	-0.013	**0.628**	-0.372	0.335*
Shelf Life(days)	0.021	-0.117	-0.010	-0.096	0.037	-0.328	**0.712**	0.219 NS

Residual effect: 0.54 * = Significant at 5%.

CHAPTER V

SUMMARY AND CONCLUSION

The seed sowing was carried out on October 28, 2018 in the seedbed. Harvesting was started from March 4, 2019 and completed by April 28, 2019.

An investigation was undertaken at the Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka. The experiment was established in seedbed on October 28, 2018 and harvesting was done on March 4, 2019. Quality traits were observed on April 30, 2019 to study genetic variability in tomatillo. Eighteen tomatillo genotypes were used for the variability and correlation and path analysis. The experimental data was subjected to statistical analysis for elucidating the information on genetic variation existing for different component characters of quality trait. The genetic variability was assessed using the parameters like genotypic (GV) and phenotypic (PV) variance. Genotypic coefficient variation (GCV) and phenotypic coefficient of variation (PCV), heritability and genetic advance over mean (GAM) were studied. The inter character correlation and path coefficient analysis were also carried out to know the relationship among various quality. Analysis of variance indicated highly significant difference among all the accessions for all characters under study.

The maximum p^H was observed in genotype G17 (4.33) and the lowest p^H found in the genotype G11 (2.74). Maximum vitamin C (170.94 mg/100g) was found in G5 whereas minimum (5.94 mg/100g) from $G_{16.}$ The maximum dry shelf life was observed by the genotype G7 (36.67 days) and minimum in G2 (10.33 days). Maximum brix (6.87%) was found in G10 whereas minimum (2.00%) from G2. The genotype G10 recorded maximum lycopene content of the fruit (0.84 mg), while the minimum was observed by the genotype G17 (0.25 mg) in case of 472 nm. Maximum total sugar was found by the genotype G8 (3.27g) and the minimum was observed from the genotype G6 (2.56g). Maximum reducing sugar sugar was found by the genotype G1 (1.58g) and the minimum was observed from the genotype G10 (1.16g). Maximum non-reducing sugar was found by the genotype G8 (1.29g) and the minimum was observed from the genotype G6 (2.56g).

Phenotypic coefficient of variation values was higher than their corresponding genotypic coefficient variation values in all of the characters. The highest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were 147.68 and 147.59 respectively both of which were recorded for lycopene (mg/100g) at 472 nm. Higher genotypic coefficients of variations were recorded for Vitamin C (167.673%), brix (29.894%), lycopene at 472 nm (34.107%) (Table 7). The highest phenotypic coefficient of variation value recorded for Vitamin C (167.701%), brix (30.664%), lycopene at 472 nm (34.85 (Table 7). The proportion of GCV in PCV observed in this study ranged from 71.70% in Reducing Sugar to 99.98% in Vitamin C (mg/100g). The traits with high proportion of GCV in PCV are reliable for selection in quality genetic improvement of tomatillo genotypes. Trait (Reducing Sugar) whose expression was environmentally dependent may not be reliable descriptor for quality characterization. Broad sense heritability values were higher (more than 51.41%) for all the characters. Broad sense heritability ranged from 51.41% (Reducing Sugar) to 99.99 % (Vit-C). These broad sense heritability values were likely to be over- estimated as in this calculation it was not possible to exclude variation due to different genetic components and their interactions.

The present estimation of heritability was 80.60% for p^H value, 99.99% for Vitamin C, 96.32% for Total sugar, 95.04% for brix, 95.79% for lycopene at 472 nm were higher. The heritability of the highest magnitude was noticed for Vit-C (99.99%) (Table 8).

Genotypic correlations among eight characters were computed and presented in (Table 10) and described below. The genotypic correlation coefficient revealed positive and significant association of reducing sugar with total sugar ($r = 0.666^{**}$), pH ($r = 0.270^{*}$) and shelf life ($r = 0.473^{**}$).In the present study Total sugar had positive significant correlation with Non-reducing sugar ($r = 0.788^{**}$) and pH ($r = 0.521^{**}$). From this result positive and significant correlation of non-reducing sugar with pH ($r = 0.389^{**}$).pH showed positive and significant association with lycopene at 472 nm ($r =0.521^{**}$) and shelf life ($r=0.346^{*}$). This indicates that if pH will increase the traits Shelf life and lycopene will increase as same degree because they were positively associated with pH.Vit-C had significant and positive correlation with shelf life (r=0.523**) and Brix (r= 0.335*).

Path analysis of direct and indirect effects revealed that reducing sugar (0.044), non-reducing sugar (0.648), vitamin C (0.628), shelf life (0.712) exerted direct positive effect on brix percentage whereas the direct effect of total sugar (-0.491), pH (-0.278) and lycopene at 472 nm (-0.146) were negative direct effect on brix percentage (Table 11).

The result of the analysis of variance indicated that mean square due to accession of all traits were highly significant ($p<0.01$). The major qualitative traits of tomatillo were observed vitamin C, shelf life and non-reducing sugar.

Considering the quality performance of tomatillo genotypes, G5 could be selected for high vit-C. G7 could be selected for higher shelf life. And G8 could be selected for higher non-reducing sugar. Higher Brix percentage as well as Lycopene contents belong to G10 genotype.

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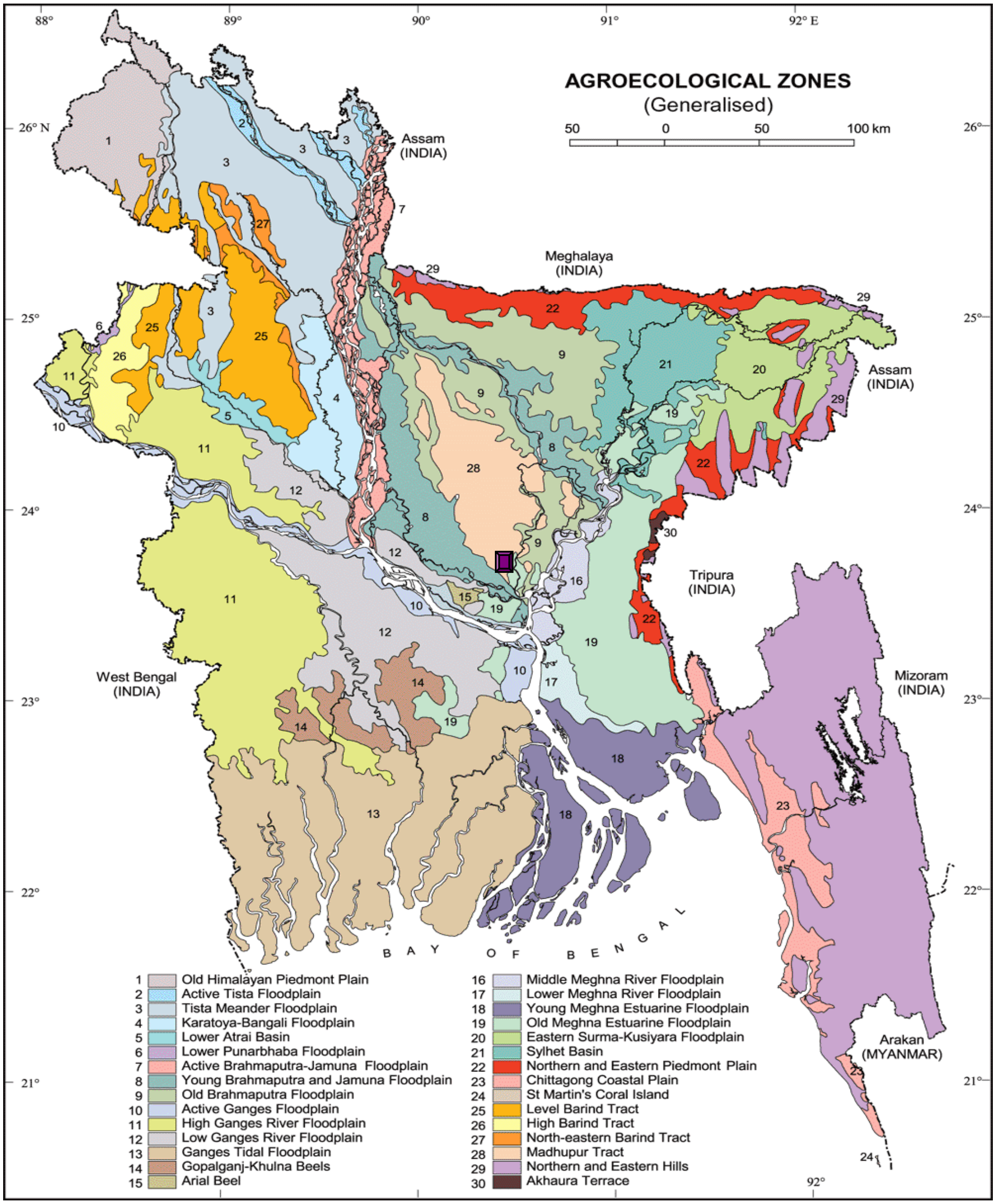
APPENDICES

Appendix I. Map showing the experimental site under the study



The experimental site under study

Appendix II. Monthly average Temperature, relative humidity and total rainfall and sunshine of the experimental site during the period from 10 October, 2018 to 12 March, 2019.

Month	Air temperature (ºC)		Relative humidity (%)	Rainfall (mm) (total)	Sunshine (h)
	Maximum	Minimum			
November, 2018	34.8	18.0	77	227	5.8
December, 2018	32.3	16.3	69	0	7.9
January, 2019	29.0	13.0	79	0	3.9
February, 2019	28.1	11.1	72	1	5.7

Source: Bangladesh Meteorological Department (Climate and Weather Division), Agargoan, Dhaka – 1212

Appendix III: Mechanical, physical and chemical characteristics of initial soil (0-15 cm depth) of the experimental site

A. Physical composition of the soil

Soil separates	%
Sand	36.90
Silt	26.40
Clay	36.66
Texture class	Clay loam

B. Chemical composition of the soil

Sl. No.	Soil characteristics	Analytical data
1	Organic carbon (%)	0.82
2	Total N (kg/ha)	1790.00
3	Total S (ppm)	225.00
4	Total P (ppm)	840.00
5	Available N (kg/ha)	54.00
6	Available P (kg/ha)	69.00
7	Exchangeable K (kg/ha)	89.50
8	Available S (ppm)	16.00
9	pH (1:2.5 soil to water)	5.55
10	CEC	11.23

Source: Central library, Sher-e-Bangla Agricultural University, Dhaka.

Appendix IV. Showing a part of experimental field of the present study with research supervisor