MITIGATION OF LATE PLANTING-INDUCED COLD STRESS IN TOMATO WITH GIBBERELLIC ACID AND CALCIUM

By

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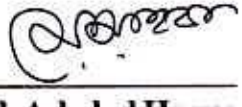

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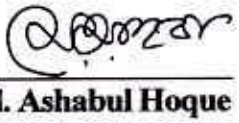

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CERTIFICATE

This is to certify that thesis entitled, "**MITIGATION OF LATE PLANTING-INDUCED COLD STRESS IN TOMATO WITH GIBBERELLIC ACID AND CALCIUM**" submitted to the Department of Agricultural Botany, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **AGRICULTURAL BOTANY**, embodies the result of a piece of *bona fide* research work carried out by **SHAHIDUL ISLAM**, Registration No. **08-02891** under my supervision and guidance. No part of the thesis has been submitted for any other degree.

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

Dated: December, 2014
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DEDICATED

TO

MY

BELOVED PARENTS

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MITIGATION OF LATE PLANTING-INDUCED COLD STRESS IN TOMATO WITH GIBBERELLIC ACID AND CALCIUM

ABSTRACT

The experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka, during the period from 15 November 2013 to 15 April 2014 to find out the role of exogenous application of gibberellic acid (GA_3) and calcium (Ca^{2+}) on the improvement of morpho-physiology and fruit yield of tomato placed to late transplanting-induced cold injury. In this study, variety BARI Tomato 15 was used as a planting material and the treatments consisted of three different times of transplanting, T_1 = First transplanting time (10 December2013), T_2 = Second transplanting time (20 December2013), T_3 = Third transplanting time (30 December2013); and six different combination of gibberellic acid and calcium *viz.* M_0 = 0 ppm GA_3 and 0 mM Ca^{2+}, M_1 = 20 ppm GA_3 and 0 mM Ca^{2+}, M_2 = 0 ppm GA_3 and 5 mM Ca^{2+}, M_3 = 20 ppm GA_3 and 5 mM Ca^{2+} , M_4= 0 ppm GA_3 and 10 mM Ca^{2+} and M_5 = 20 ppm GA_3 and 10 mM Ca^{2+}. The experiment was laid out in two factors Randomized Complete Block Design (RCBD) with three replications. The total treatment combinations were 18 (3x6). Most of the results of this experiment showed differences to the treatments. The first transplanting time significantly influenced to increased morpho-physiological characters: plant height, number of leaves plant^{-1}, number of branches plant^{-1} and SPAD value of leaf; yield contributing characters: number of flowers plant^{-1}, number of fruits plant^{-1}, fruit length, fruit diameter and yield plot^{-1} and ha^{-1} compared to late or third transplanting time-induced cold stress . The maximum yield (80.46 t ha^{-1}) was obtained from T_1 whereas the lowest yield was found (58.53 t ha^{-1}) from T_3 transplanting and suggesting that early transplanting time improves fruit yield through promoting the morpho-physiological features of tomato. In this study it was found that different combination of GA_3 and Ca^{2+} mitigated the adverse effects of late transplanting-induced cold stress in tomato. Exogenous foliar application of 20 ppm gibberellic acid (GA_3) along with 5 mM calcium (Ca^{2+}) improved the morphological characters of tomato except number of branch plant^{-1}. The yield contributing characters and fruit yield showed statistically significant increased with 20 ppm gibberellic acid (GA_3) along with 5 mM calcium (Ca^{2+}). The highest yield 83.99 t ha^{-1} yield were found with the 20 ppm gibberellic acid (GA_3) along with 5 mM calcium (Ca^{2+}) but the lowest 59.92 t ha^{-1} yield were recorded from control. These results indicated that combined application of GA_3 along with Ca^{2+} has positive impact on fruit yield. The interaction between date of transplanting and sole or combined application of GA_3 and Ca^{2+} influenced all the morpho-physiological and yield contributing characters and yield of tomato. The highest yield (93.54 t ha^{-1}) of tomato was obtained with the first transplanting time along with 20 ppm GA_3 with 5 mM Ca^{2+} (T_1M_3) treatment combination, whereas the lowest yield (49.79 t ha^{-1}) was recorded from T_3M_0, third transplanting date and 0 ppm GA_3 and 0 mM Ca^{2+} treatment combination. In addition the treatments combination T_3M_3 influenced to produce the highest yield 69.09 t ha^{-1} out of all the treatment under late transplanting and suggested that 20 ppm GA_3 along with 5 mM Ca^{2+} successfully mitigate the detrimental effect of cold stress in variety BARI tomato 15.

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LIST OF ABBREVIATION AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BARI	=	Bangladesh Agricultural Research Institute
BBS	=	Bangladesh Bureau of Statistics
Ca^{2+}	=	Calcium
cm	=	Centi-meter
CV%	=	Percentage of Coefficient of Variance
DAT	=	Days after transplanting
df	=	Degree of freedom
et. al.	=	And others
FAO	=	Food and Agriculture Organization
Fig.	=	Figure
GA_3	=	Gibberellic Acid
gm	=	Gram (s)
IAA	=	Indole Acetic Acid
kg	=	Kilogram
LSD	=	Least Significant Difference
m	=	Meter
m^2	=	Meter square
mM	=	Millimolar
No.	=	Number
NS	=	Non significant
PGRs	=	Plant growth regulators
ppm	=	Parts per million
RCBD	=	Randomized Complete Block Design
SAU	=	Sher-e-Bangla Agricultural University
$t\ ha^{-1}$	=	Ton per hectare
%	=	Percent
^0C	=	Degree Celsius

Chapter 1
Introduction

CHAPTER 1

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is a fruit producing annually cultivated vegetable fruit crop under the family of Solanaceae (Haque *et al.*, 1999). It is native to Western South America and used as a nutritious edible fruit throughout the planet that is grown in both greenhouse and field conditions (Smith, 1994). Tomato is generally grown in the winter season in Bangladesh. Usually, it is typically herbaceous, 1 to 3 m in height that often sprawl over the ground. The type of fruit of tomato is berry with variable in size and weight found in different varieties that can contribute to yield.

Many previous authors stated that tomato is an excellent source of food nutrients for human health due to be full of antioxidants. Lycopene is an antioxidant which is rich in tomato and shows protective function to prevent prostate cancer, improve the skin's ability to protect against harmful UV rays (Redenbaugh *et al.*, 1992 and Wilcox *et al.*, 2003). In addition, Rao *et al.* (2002) reported that tomato helps to relieve the oxidative stress and managing human neurodegenerative diseases. It is also an exceptional source of vitamins and minerals such as thiamin (Vit-B_1), niacin (Vit-B_3), pantothenic acid (Vit-B_5), pyridoxine (Vit-B_6), biotin (Vit-B_7), folate (Vit-B9), ascorbic acid (Vit-C), alpha Tocopherol (Vit-E), vitamin K, copper, potassium, manganese, molybdenum, phosphorus, zinc, and iron, dietary fiber, zeaxanthin, protein, choline, along with low sodium (Yilmaz, 2000, Olaniyi *et al.*, 2010). BARI (2010) showed that ripen tomato contains 94 g water, 0.5 g minerals, 0.8 g fibre, 0.9 g protein, 356 mg carotene, 0.12 mg vitamin B-1, 0.06 mg vitamin B-2 and 27 mg vitamin C in each 100 g. At present, it is consumed as a raw salad, cooked or as processed food item such as Sauce, Ketchup, Jam, Jelly etc. Altogether, it is suggested that tomato has huge health benefits in view of its nutritional value.

It has been reported that the total production of tomato is 2.51 lac tons in the area of 26 thousands hectare and the average yield is being 95.437 ton ha^{-1} in Bangladesh (FAO, 2013 and BBS, 2013). This production rate is very much low in contrast with other tomato producing countries like China (506 lac tons), India (182 lac tons) and USA (125 lac tons) etc. The yield of tomato in our country is not sufficient in comparison to requirement (Aditya *et al.*, 1999). The low yield of tomato in Bangladesh is not the

indication of low yield potentiality of this crop but the fact that this may be attributed due to several reasons like; availability of improved variety, conventional management practices, inappropriate time of transplanting, different abiotic and biotic stress including temperature, salinity, insects, pathogens and residual effect of pesticides, proper application of plant nutrients and plant growth regulators (PGRs) etc. Among them actual time of sowing and foliar use of PGRs along with nutrients may contribute to improve the present yield status of tomato.

Climate change is a major threat for crop production not only in Bangladesh but also all over the world. In Bangladesh, early November planting time seems to be the best for tomato production (Hossain *et al.*, 1986) and late planting results lower yield and enhanced disease infestation (BARI, 1989). Went (1984) reported that fruit set was abundant only when night temperature was between 15°C and 20°C. It has been also reported that fruit set varies with temperature as low (7.2°C) and with temperature as high (26.6°C) (Curme, 1992). Tremendous reduction in fruit set due to high as well as low temperature which disturb mechanisms involved in the male and female parts of the flower (Lawhori *et al.*, 1963). In some areas of our country particularly in the northwestern part, the night temperature falls even sometimes go below 5-6°C which results remarkable yield loss in tomato. These information suggest that late time of sowing or transplanting-induced cold stress exhibit a significant reduction on both growth and yield of tomato.

It has been reported that plant growth regulators (PGRs) played essential functions on growth, flowering, fruit setting, ripening and quality of tomato (Kumar *et al.* 2014; Naeem *et. al.*, 2001 and Davies, 1995). Presently, growers in some countries are also commercially producing tomatoes at higher and lower temperature with exogenous application of synthetic PGRs. The PGRs are used extensively in tomato to enhance yield by improving fruit set, size and number and could have practical application for tomato growers (Batlang, 2008). The application of synthetic gibberellins or gibberellic acid (GA_3) is an effective tool in increasing both yield and quality of tomato (Gemici *et al.* 2006.). Fruit set in tomato can be increased by applying plant growth regulators to compensate the deficiency of natural growth substances required for its development (Singh and Choudhury, 1966). Application of certain PGRs like auxin and GA_3 carry the possibility of tomato production under adverse environmental conditions. Tomato

fruit setting was promoted by GA_3 at low concentration. In fact the use of GA_3 had improved the production of tomato including other vegetables like cucumber in respect of better growth and quality, which ultimately led to generate interest among the scientists and farmers for commercial application of plant growth regulators (Rafeekher *et al.*, 2002). In addition, Wang *et al.* (2014) stated that GA_3 help to improve cold tolerance in wheat. The most widely available plant growth regulator is GA_3 which induces stem and internode elongation, seed germination, enzyme production during germination and fruit setting and growth in tomato (Davies, 1995). Gibberellic acid is an important growth regulator that may have many uses to modify the growth, yield and yield contributing characters of tomato (Rafeekher *et al.*, 2002). However, to my knowledge information is not enough whether exogenous application of GA_3 improves the morpho-physiological processes and yield to tomato in relation to late planting-induced clod stress in our country.

Generally, calcium (Ca^{2+}) is taken up from the soil and translocated to the leaves for vigorous leaf growth (Kadir, 2004). Hirschi (2004) stated that Ca^{2+} is an essential macro nutrient which fulfills a fundamental role in plant membrane stability and cell wall-stabilization. The Ca^{2+} is not only essential structural elements that strengthen plant cell walls and membranes but also is a well-known secondary messenger to mitigate the abiotic stress in plants (White and Broadley, 2003). It has been also reported that (Ca^{2+}) nutrition showed an encouraging effect on growth, fruit yield and quality tomato (Hao and Papadopoulos, 2004). In addition, leaf Ca^{2+} deficiency in tomato reduces leaf size, and causes necrosis of young leaves and yield loss (Holder and Cockshull, 1990). Low supply of Ca^{2+} to tomato fruit leads to more fruit with blossom-end rot (Saure, 2001). In contrast, excessive supply of Ca^{2+} to fruit causes gold spot, cells containing a granular mass of tiny calcium oxalate crystals which not only affects the appearance of the fruit, but also reduces its shelf life (Ho *et al.*, 1999). Usten *et al.* (2006) reported that Ca^{2+} enhances resistance to bacterial and viral diseases. Therefore, limited study has elucidated that whether foliar application of Ca^{2+} can modify the morpho-physiological attributes and yield of tomato under cold stress at SAU campus during late transplanting.

Tomato production in Bangladesh is largely affected due to adverse environmental conditions. Therefore, this experiment was conducted to examine the role of exogenous

combine or sole application of GA_3 and Ca^{2+} on morpho-physiology, yield and quality of tomato at different time of transplanting as well as late transplanting-induced cold stress with the following objectives:

- To examine the effects of late transplanting-induced cold stress on the morpho-physiological characters and yield of tomato variety BARI Tomato 15.
- To examine the independent effects of GA_3 and Ca^{2+} on the morpho-physiological characters and yield of tomato variety BARI Tomato 15.
- To analyze the effectiveness of different combinations of GA_3 and Ca^{2+} on mitigation of late transplanting-induced cold stress in tomato in relation to morpho-physiology and yield.

Chapter 2
Review of literature

CHAPTER 2

REVIEW OF LITERATURE

Tomato is an important crop plant which supply Vitamin C as well as used as a vegetables by the people of Bangladesh. The proper management practices, time schedule and foliar application of plant growth regulators (PGRs) and macronutrient essentially influence its morphological characters and yield performance. Experimental evidences showed that there is a profound influence of time of transplanting and foliar application of calcium (Ca^{2+}) and Gibberellic acid (GA_3) on this tomato. A brief of the relevant works performed in the past are presented in this Chapter.

2.1 Effect of different transplanting time on morphological parameters and yield of tomato:

Sing *et al.*, (2005) carried out an experiment was conducted to study the effect of transplanting time and mulching on growth and yield of tomato. Among different dates of planting, early planting (10th December) recorded the highest vegetative growth, yield attributes, early and total fruit yield; whereas it was *vice-versa* in case of 20th January planting. Among different mulch materials, black polyethylene retained higher soil moisture and temperature as compared to other materials and control. Further, fruit yield was significantly higher with black polyethylene mulch compared to other mulch materials. Highest net returns (Rs. 52,700/ha) was recorded under early (10th December) planting date and mulching with black polyethylene treatment combination, which was significantly superior to all other treatment combinations.

Madhumathi and Sadarunnisa (2013) done an experiment on transplanting of tomato during 15th of October recorded significantly higher number of fruits plant^{-1} (33.31), yield plant^{-1} (1.25kg), fruit size (length, diameter and volume), fruit weight (42.63 g), pulp content (54.01%), ascorbic acid (20.81 mg/100 g pulp) and number of seeds fruit^{-1} (192.21) over other dates of planting. Among the varieties, maximum number of fruits plant^{-1}, yield plant^{-1}, titrable acidity, ascorbic acid content, number of seeds fruit^{-1} and seed weight fruit^{-1} were recorded in Pusa Ruby, whereas Pusa Early Dwarf recorded maximum fruit size, fruit weight, pulp content, TSS and 1000 – seed weight.

Among the treatment combinations Pusa Ruby planted on October 15th emerged as the best combination with regard to fruit quality and seed characters.

An investigation was carried out by Mira *et al.* (2011) to determine the effect of four sowing times viz. October 25, November 09, November 25, and December 09, on growth and yield of two tomato varieties (Roma VF and Ratan). Ratan showed better performance, in respect quality when sown on November 09. Sugar, organic acid, ascorbic acid and β-carotene contents in fruits of both varieties were recorded maximum when sown on November 09. No significant change of lycopene content in fruit was recorded during the sowing times from October 25 to December 09. Labile change of nitrogen, phosphorus and potassium contents was recorded with the change of sowing times, whereas late sowing showed significant decline of calcium contents in fruit.

Ahammad *et al.* (2009) was conducted an experiment at Jessore to observe the effect of planting date and variety on the yield of late planting tomato. The potentiality of fruiting in the late season were evaluated for BARI tomato 4, 5, 6 and 12 by planting December 01, December 16, January 01, January 16 and February 01. A combination of December 01 planting with BARI Tomato 5 variety performed better in respect of yield (57.07 t/ha). The variety BARI Tomato 5 also showed potential fruiting capability during late winter season and February 01 planting produced 11 ton/ha of potential yield. All the four varieties showed potential fruiting capability during late winter season and February 01 planting produced 4-6 tons of potential yield during late season.

The experiment was conducted by Hossain *et al.* (2013) at Agricultural Research Station, Thakurgaon, Bangladesh during October 2009 to March 2010 to observe the effect of sowing dates on yield of tomato genotypes. Three sowing dates viz. October 1, October 15 and October 30 were considered as factor A and tomato variety viz., BARI Tomato-2, BARI Tomato-3, BARI Tomato-4, BARI Tomato-9 and BARI Hybrid Tomato-4 considered as factor B. The experiment was laid out in RCBD (Factorial) with three replications. Early flowering (52.40 days) as well as early fruit harvesting (119.13 days) was occurred in October 1 sowing, whereas sowing on October 30 resulted in delayed flowering (71.73 days) and fruit harvesting (140.67 days), respectively. Number of fruits plant^{-1} was also the highest (27.40) in October 1 sowing and the lowest (13.73) was in October 30 sowing. Seed sowing of October 1

was found better in respect of yield (74.75 t ha^{-1}) compared to October 15 (58.55 t ha^{-1}) and October 30 (24.60 t ha^{-1}) sowing. Among the variety, BARI Tomat-2 produced the highest (68.12 t ha^{-1}) marketable yield followed by BARI Tomato-9 (56.16 t ha^{-1}) and BARI Tomato-3 while BARI Tomato-4 gave the lowest (36.91 t ha^{-1}) marketable yield.

Tongova and Zhelev (1975) reported that both early sowing and early planting of tomato gave increased yield. The highest early and total yield were produced by plants sown on 20 September and transplanted at the 4-5 leaf stage.

Adelana (1976) reported that the earliest planting of tomato seedlings resulted in greater leaf area, higher yield and number of fruits $plant^{-1}$ and greater average fruit weight than later planting.

Sanjoy (1999) studied the impact of seedling age (15 or 30 days old) and planting time (early: 16 November or late: 16 December) on the fruit yield performance of tomato (*Lycopersicon lycopersicum*) cultivars BT 18, BT 12, BT 10, BT 2 and MIX ENT in upland rice (cv. Annada)-based cropping system. All cultivars performed well when planted early (with 15-day-old seedlings) and showed a declining trend in fruit yield and other yield-attributing characters when planted late with 30 days old seedlings. Among the tomato cultivars, remarkably good fruit yields of 60.7 and 47.0 t/ha were recorded from BT 18 during 1994-95 and 1995-96, respectively, when planted early with 15 days old seedlings. BT 12 gave fruit yields of 59.7 and 41.9 t/ha during 1994-95 and 1995-96, respectively. The economics of different tomato cultivars also showed the same trend. The gross return, net return and net return per rupee were highest in BT 18, followed by BT 12, irrespective of seedling age and planting time.

Haque *et al.* (1999) reported that cluster $plant^{-1}$ of tomato were significantly influenced by sowing dates. The highest number of clusters $plant^{-1}$ was obtained from early sowing.

Hossain *et al.* (1986) reported that early sowing enhanced total number of flowers $plant^{-1}$.

Taha *et al.* (1984) said that fruit size and fruit weight of early sowing was bigger than others sowing and the late sowing scored the lowest number of fruits $plant^{-1}$.

Peyvast (2001) reported that the earliest sowing date resulted in a significantly higher total fruit yield compared to the later sowing date.

Singh and Tripanthy (1995) showed variation in yield of tomato when sown in different dates from June to August at Orissa of India.

Went (1984) assured that tomato fruit set was abundant only when night temperature was between 150C and 200C, which might over simplify the issue.

Curme (1992) reported that fruit set in certain varieties with temperature as low (7.2 ^{0}C) and with temperature as high (26.6 ^{0}C).

Omara (1995) said that maximum fruit size, average fruit weight and number of fruits plant^{-1} of tomato during early sowing dates.

Abdul and Harris (1978) reported that temperature affected the level of endogenous hormones.

2.2 Effect of macronutrient (Ca^{2+}) and plant growth regulators (GA_3) on morphological parameters and yield of tomato:

2.2.1 Effect of Gibberellic acid (GA_3) on morphological parameters and yield of tomato:

This study was conducted by Kumar *et al.* (2014) with the objective to determine the effects of Gibberellic acid (GA_3) on growth, fruit yield and quality of tomato. The experiment consisted of one tomato variety- Golden, and six treatments with five levels of gibberellic acid (GA_3 - 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm), arranged in randomized block design with three replications. The highest plant height, number of leaves, number of fruits, fresh fruit weight has been observed and ascorbic acid, total soluble solid (TSS) was estimated for GA_3 50 ppm.

The effect of applied gibberellin (GA) and auxin on fruit-set and growth has been investigated by Serrani *et al.* (2007) in tomato (*Solanum lycopersicum* L.) cv Micro-Tom. It was found that to prevent competition between developing fruits only one fruit per truss should be left on the plant. Unpollinated ovaries responded to GA_3 and to different auxins [indol-3-acetic acid, naphthaleneacetic acid, and 2,4-dichlorophenoxyacetic acid (2,4-D)], 2,4-D being the most efficient. Simultaneous

application of GA_3 and 2,4-D produced parthenocarpic fruits similar to pollinated fruits, but for the absence of seeds, suggesting that both kinds of hormones are involved in the induction of fruit development upon pollination. It is concluded that Micro-Tom constitutes a convenient model system, compared to tall cultivars, to investigate the hormonal regulation of fruit development in tomato.

An experiment was conducted by Rai *et al.* (2006) during the 2003 winter season in Meghalaya, India, on tomato cv. Manileima to study the effect of plant growth regulators on yield. The treatments comprised 25 and 50 mg GA_3/litre, water spray. Data were recorded for growth, flowering and fruiting characteristics GA_3 significantly reduced the number of seeds fruit^{-1} but increased plant height and number of branches plant^{-1}.

Khan *et al.* (2006) conducted an experiment to study the effect of 4 levels of gibberellic acid spray on the growth, leaf-NPK content, yield and quality parameters of 2 tomato cultivars *(Lycopersicon esculentum* Mill.*)*, namely Hyb-SC-3 and Hyb-Himalata. They reported that irrespective of its concentration, spray of gibberellic acid proved beneficial for most parameters, especially in the case of Hyb-SC-3.

Nibhavanti *et al.* (2006) carried out an experiment on the effects of gibberellic acid, NAA, 4-CPA and boron at 25 or 50 ppm on the growth and yield of tomato (cv. Dhanshree) during the summer season of 2003. Plant height was greatest with gibberellic acid at 25 and 50 ppm (74.21 cm and 75.33 cm, respectively) and 4-CPA at 50 ppm (72.22 cm). The number of primary branches plant^{-1} did not significantly vary among the treatments. Gibberellic acid at 50 ppm resulted in the lowest number of primary branches plant^{-1}.

Sasaki *et al.* (2005) studied the effect of plant growth regulators on fruit set of tomato *(Lycopersicon esculentum* cv. Momotaro) under high temperature and in a field (Japan) under rain shelter. Tomato plants exposed to high temperature (34/20 degrees C) had reduced fruit set. Treatments of plant growth regulators reduced the fruit set inhibition by high temperature to some extent.

Bhosle *et al.* (2002) reported the effects of NAA (25, 50 and 75 ppm), gibberellic acid (15, 30 and 45 ppm) and 4-CPA (25, 50 and 75 ppm) on the growth and

yield of tomato cultivars 'Dhanashree' and 'Rajashree' during the summer of 1997. They reported that the number of flowers cluster^{-1}, fruit weight and marketable yield increased with increasing rates of the plant growth regulators. Treatment with 30 ppm gibberellic acid resulted in the tallest plants, whereas treatment with 25 ppm 4-CPA and 45 ppm gibberellic acid resulted in the highest number of primary branches of 'Dhanashree' (4.16) and Rajashree' (5.38), respectively. The highest marketable yield of 'Dhanashree and Rajashree' was also found from treatment with 75 ppm 4-CPA.

Sun *et al.* (2000) reported the role of growth regulators on cold water for irrigation reduces stem elongation of plug-grown tomato seedlings. The effect of growth regulators (abscisic acid, gibberellic acid (GA), paclobutrazol, ethephon, IAA and silver thiosulfate) and cold water irrigation at different temperatures (5, 15, 25, 35, 45 and 55 °C) on the reduction of stein elongation of plug-grown tomato seedlings was investigated. Paclobutrazol, ethephon and GA reduced the stem length of the tomatoes at several water temperatures. Cold water irrigation with the addition of 1.8 ppm GA or irrigation at room temperature could promote stem elongation. Irrigation at room temperature with the addition of 10 ppm paclobutrazol (GAs biosynthesis inhibitor) or cold water irrigation could inhibit stem elongation. The reduction in stem elongation in plug-grown tomato seedlings was due to the relationship of GAs metabolism and sensitivity.

El-Habbasha *et al.* (1999) studied the response of tomato plants to foliar spray with some growth regulators under late summer conditions. Field experiments were carried out with tomato (cv. Castelrock) over two growing seasons (1993-94) at Shalakan, Egypt. The effects of GA_3, IAA, TPA (tolylphthalamic acid) and 4-CPA (each at 2 different concentrations) on fruit yield and quality were investigated. Many of the treatments significantly increased fruit set percentage and total fruit yield, but also the percentages of puffy and parthenocarpic fruits, compared with controls.

Tomar and Ramgiry (1997) found that plants treated with GA_3 showed significantly greater plant height, number of branches/plant, number of fruits/plant and yield than untreated controls. GA_3 treatment at the seedling stage offered valuable scope for obtaining higher commercial tomato yields.

El-Abd *et al.* (1995) studied the effect of plant growth regulators for improving fruit set of tomato. Two tomato cv. Alicante crops were produced in pots in the greenhouse. When the third flower of the second cluster reached anthesis, the second cluster was sprayed with IAA, GA_3 or ABA at 10^{-4}, 10^{-6} or 10^{-8} M each and ACC at 10^{-9}, 10^{-10} or 10^{-11} M. All concentrations of IAA, GA_3, ACC and ABA induced early fruit set compared with controls sprayed with distilled water. GA_3 led to the formation of leafy clusters, with the number of leaves formed increasing with GA_3 concentration.

Groot *et al.* (1987) reported that GA was indispensable for the development of fertile flowers and for seed germination, but only stimulated in later stages of fruit and seed development.

Sumiati (1987) reported that tomato cultivars, 'Gondol', 'Meneymaker', 'Intan' and Ratan sprayed with 1000 ppm chlorflurenol, 100 ppm IAA, 50 ppm NAA or 10 ppm GA_3 or left untreated, compared with controls, fruit setting was hastened by 4-5 days in all cultivars following treatment with 100 ppm IAA or 10 ppm GA_3 .

Leonard *et al.* (1983) observed that inflorescence development in tomato plants (cv. King plus) grown under a low light regime was promoted by GA applied directly on the inflorescence.

In China, Wu *et al.* (1983) sprayed one month old transplanted tomato plants with GA at 1, 10 or 100 ppm. They reported that GA at 100 ppm increased plant height and leaf area.

Onofeghara (1981) conducted an experiment on tomato sprayed with GA at 1000 ppm and NAA at 25- 50 ppm. He observed that GA promoted flower primodia production and the number of primordia and NAA promoted flowering and fruiting.

Saleh and Abdul (1980) conducted an experiment with GA_3 (25 or 50 ppm) which was applied 3 times in June or early July. They reported that GA_3 stimulated plant growth. It reduced the total number of flowers plant^{-1}, but increased the total yield compared to the control. GA_3 also improved fruit quality.

Mehta and Mathi (1975) reported that treatments with NAA at 0.1 or 0.2 ppm improved the yield of tomato irrespective of planting date. Maximum fruit set, early and total yield, fruit number and weight were obtained in response to 4-D at 5 ppm followed by NAA at 0.2 ppm. He also reported that GA treatments at 10 or 25 ppm improved the yield of tomato cv. Pusa Ruby irrespective of planting date. GA gave earlier fruit setting and maturity.

Kaushik *et al.* (1974) carried out an experiment with the application of GA_3 at 1, 10 or 100 mg/L on tomato plants at 2 leaf stage and then at weekly interval until 5 leaf stage. They reported that GA_3 increased the number and weight of fruits plant^{-1} at higher concentration.

Hossain (1974) investigated the effect of gibberellic acid along with parachlorophenoxy acetic acid on the production of tomato. He found that GA_3 applied at 50, 100 and 200 ppm produced an increased fruit set. However, GA_3 treatment induced a small size fruit production. A gradual increase in the yield plant^{-1} was obtained with higher concentration of GA_3.

Choudhury and Faruque (1972) reported that the percentage of seedless fruit increased with an increase in GA_3 concentration from 50 ppm to 100 ppm and 120 ppm. However, the fruit weight was found to decrease by GA_3 effects.

Jansen (1970) reported that tomato plants treated with GA neither increased the yield nor accelerated fruit ripening. He also mentioned that increasing concentration of GA reduced both the numbers and size of the fruits.

Adlakha and Verma (1965) observed that when the first four clusters of tomato plants were sprayed three times at unspecified intervals with GA at 50 and 100 ppm, the fruit setting, fruit weight and total yield increased by 5, 35 and 23%, respectively with the higher concentration than the lower.

Adlakha and Verma (1964) sprayed GA in concentration of 50 and 100 ppm on flower cluster at anthesis and noted that the application of GA at 100 ppm could appreciably increase fruit size, weight, protein, sugar and ascorbic acid contents.

Rappaport (1960) noted that GA had no significant effect on fruit weight or size either at cool (11 °C) or warm (23°C) night temperatures; but it strikingly reduced fruit size at an optimal temperature (17 °C).

Bima *et al.* (1995) worked with gibberellic acid and found that GA_3 (5-10 ppm) enhanced germination of seeds and induced flowering.

Gustafson (1960) worked with different concentration of GA and observed that when 35 and 70 ppm GA were sprayed to the flowers and flower buds of the first three clusters, percentage of fruits set increased but there was a decrease in the total weight. When only the first cluster was sprayed, the number of fruit set and the total weight cluster^{-1} was increased, but this response did not occur in subsequent clusters.

A field experiment was carried out by Choudhury *et al.* (2013) at Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh, to assess the effect of different plant growth regulators on tomato during summer season 2011. Different plant growth regulators (PGR) viz. PGR = Control, 0 PGR1 = 4-CPA (4-chloro phenoxy acetic acid) @ 20 ppm, PGR2 = GA3 (Gibberellic Acid) @ 20 ppm and PGR3 = 4-CPA + GA3 @ 20 ppm of each were used in the study. The growth and yield contributing characters were significantly differed due to different plant growth regulators. The maximum plant height at 60 DAT (86.01cm), number of flowers cluster plant^{-1} (10.60), number of flowers plant^{-1} (39.69), number of fruits plant^{-1} (36.54), single fruit weight (74.01 g) and yield (28.40 t ha 1) were found in PGR and the minimum for all the parameters were found in control (PGR) 30 treatment.

A pot experiment was perform by Khan *et al.* (2006) according to a factorial randomized design at Aligarh to study the effect of 4 levels of gibberellic acid spray (0, 10^{-8}, 10^{-6} and 10^{-4} M GA_3) on the growth, leaf-NPK content, yield and quality parameters of 2 tomato cultivars (*Lycopersicon esculentum* Mill.), namely Hyb-SC-3 and Hyb-Himalata. Irrespective of its concentration, spray of gibberellic acid proved beneficial for most parameters, especially in the case of Hyb-SC-3.

Jong *et al.* (2009) had reported that the initiation of tomato fruit growth, fruit set, is very sensitive to environmental conditions. Therefore, an understanding of the mechanisms that regulate this process can facilitate the production of this agriculturally valuable fruit crop. Over the years, it has been well established that tomato fruit set

depends on successful pollination and fertilization, which trigger the fruit developmental programme through the activation of the auxin and gibberellin signaling pathways. However, the exact role of each of these two hormones is still poorly understood, probably because only few of the signaling components involved have been identified so far. Recent research on fruit set induced by hormone applications has led to new insights into hormone biosynthesis and signaling. The aim of this review is to consolidate the current knowledge on the role of auxin and gibberellin in tomato fruit set.

Srivastava and Srivastava (2007) observed that GA_3 significantly reduce time to flowering and increased flower number and size.

Davies and Zalman (2006) reported that GA_3 significantly increased the total number of fruits, the fruit weight plant^{-1} by reducing pre-harvest fruit drop in orange.

Graham and Ballesteros (2006) reported that GA_3 promote vegetative growth and reproductive organ formation with extended flowering, maturity period and less fruit size formation.

Ilias *et al.* (2007) said that most of the vegetative and reproductive growth parameters especially plant height increases with GA_3.

Abdel-Mouty and El-Greadly (2008) reported that GA_3 increases most of the vegetative and reproductive growth parameters especially plant height tomato.

2.2.2 Effect of calcium (Ca^{2+}) on morphological parameters and yield of tomato:

Hao and Papadopoulos (2003) conducted an experiment on Tomato (*Lycopersicon esculentum* Mill.) with two concentrations of calcium (150 and 300 mg L^{-1}) in combination with four concentrations of magnesium (20, 50, 80 and 110 mg L^{-1}) in fall, 1999, to investigate their effects on plant growth, leaf photosynthesis, and fruit yield and quality (fruit firmness, dry matter, soluble solids and russeting). High Ca (300 mg L^{-1}) concentration increased fruit yield and reduced the incidence of blossom-end rot (BER) and fruit russeting, compared with the low Ca concentration (150 mg L^{-1}). High Ca concentration reduced fruit firmness but did not affect fruit size and leaf photosynthesis. Plants grown at 20 mg L^{-1} Mg started to show leaf chlorosis on both the middle and bottom leaves 8 week after planting. Leaves with moderate chlorosis

lost about 50% of their photosynthetic capacity. Fruit yield in the late growth stage decreased at 20 mg L^{-1} Mg. Blossom-end rot incidence increased linearly with increasing Mg concentration in the early growth stage at low Ca, but BER incidence at high Ca was not affected by Mg concentration. Fruit firmness increased with increasing Mg concentration at low Ca. At high Ca, Mg concentration affected fruit firmness only late in the season; fruit firmness at 80 mg L^{-1} Mg was higher than at 50 mg L^{-1} Mg concentration. Fruit resetting in mid-season was affected by nutrient treatments, being the least at 300/50 mg L^{-1} Ca/Mg. Therefore, for a fall greenhouse tomato crop, the optimum Ca/Mg concentration for tomato production is estimated to be 300/50-80 mg L^{-1}. The Mg concentration may be started at 50 mg L^{-1} and gradually increased to 80 mg L^{-1} towards the end of the season, to improve plant growth and fruit firmness.

The effects of calcium chloride on growth and leaf ions concentration of tomato (*Lycopersicon esculentum* L.) were investigated by Lolaei (2012) in Gorgan, Iran. A factorial experiment was conducted based on RCBD with four NaCl levels (0, 50, 100, and 150 mM) and four $CaCl_2$ levels (0, 100, 200 and 300 mg L^{-1}). Increasing Ca^{2+} concentration in the nutrient solution increased the fruit yield. Tomato in its response to nutrient solution, salinized with sodium chloride and calcium chloride.

The study was carried out by Kazemi (2012) to evaluate the effects of foliar application of humic acid and calcium chloride on vegetative and reproductive growth, yield, and quality of tomato plants as a completely randomized block design with 4 replications, each consisting of 3 pots with each pot containing one plant. Humic acid (15 and 30 ppm) and calcium chloride (10 and 15 mM) solutions were applied as foliar sprays either alone or in combination. Data were recorded for plant height, branches $plant^{-1}$, flowers $cluster^{-1}$, fruits $plant^{-1}$, yield, fruit weight, fruit firmness and total soluble solid content of the fruit. Results showed that humic acid (30ppm) and calcium chloride (15 mM) spray either alone or in combination (30 ppm HA+15 mM Ca) affected on vegetative and reproductive growth and chlorophyll content, significantly. Mean comparisons indicated yield, and quality of tomato plants was improved by increasing humic acid and calcium chloride concentration up to 30 ppm and 15 mM. Foliar application of Ca (15 mM) + HA (30 ppm) resulted in the maximum TSS (5.14 °Brix), vitamin C (25.14), nitrate reductase activity (6.4), yield (25.36 t ha^{-1}), fruit firmness (3.91 kg cm^{-2}), fruit lycopene content (2.14) and the lowest blossom end rot incidence

(5%). In finally, humic acid and calcium chloride application can be helpful for yield improvement and prevent of decreasing yield.

The influence of $CaCl_2$ and borax on growth, yield, and quality of tomato was investigated by Rab and Haq (2012) during the years 2009 and 2010. The experiment was laid out with a randomized complete block design. Calcium chloride (0.3% and 0.6%) and borax (0.2% and 0.4%) solutions were applied as foliar sprays either alone or in combination and data were recorded for plant height, branches plant^{-1}, flowers cluster^{-1}, fruits plant^{-1}, yield, fruit weight, fruit firmness, and total soluble solid content of the fruit. The application of $CaCl_2$ alone significantly increased the plant height and fruits plant^{-1} and decreased the incidence of blossom end rot. Borax alone significantly enhanced the number of branches plant^{-1}, number of flowers cluster^{-1}, fruits cluster^{-1}, fruits plant^{-1}, fruit weight, fruit firmness, and total soluble solid content of the fruits. Foliar application of $CaCl_2$ (0.6%) + borax (0.2%) resulted in the maximum plant height (86.60 cm), branches plant^{-1} (7.21), flowers cluster^{-1} (32.36), fruits plant^{-1} (96.37), fruit weight (96.33 g), yield (21.33 t ha^{-1}), fruit firmness (3.46 kg cm^{-2}), and total soluble solids (6.10%) and the lowest blossom end rot incidence (6.25%). However, the difference among 0.6% $CaCl_2$ + 0.2% borax, 0.3% $CaCl_2$ + 0.2% borax, and 0.6% $CaCl_2$ + 0.4% borax was nonsignificant.

An experiment was carried out Ilyas *et al.* (2014) at Agriculture Extension and Model Farm Service Center Timergara, Khyber Pakhtunkhwa, Pakistan during summer 2010 to investigate the response of tomato (*Lycopersicon esculentum* L.) cv 'Rio Grand' to different levels of calcium (Ca) and magnesium (Mg). The experiment was laid out in a randomized complete block design (RCBD) with two factors i.e. Ca and Mg levels; treatments were replicated three times. Three levels of Ca (0, 3 and 6%) and three levels of Mg (0, 2 and 4%) were applied as foliar spray and the data were recorded on plant height, number of branches plant^{-1}, number of flower cluster $^{-1}$, number of fruits clusrer^{-1}, number of fruits plant^{-1}, weight of fruit (gm), yield ha^{-1} (ton) and Blossom End Rot fruits %. Both Ca and Mg and their interaction significantly increased the growth and yield parameters. Among the different levels of Ca, 6% level showed significant increase in plant height (84.10 cm), number of branches plant^{-1} (6.35), number of flowers cluster^{-1} (24.42), number of fruits cluster^{-1} (5.68), number of fruits plant^{-1} (6.92), fruit weight (78.01 gm), yield ha^{-1} (21.14 tons) and low percentage of blossom end rot fruits (8.22). Magnesium also significantly affected growth and yield

components. Among the different levels of Mg, 4% showed significant increase in plant height (85.68 cm), number of flowers cluster^{-1} (27.62), number of fruits cluster^{-1} (5.95), number of fruits plant 1 (6.22), yield ha^{-1} (20.26 tons) and less percentage of blossom end rot fruits (6.76). Based on the above results, it is recommended that 6% Ca concentration and 4% Mg concentration should be collectively applied to tomato for better growth and yield under the agro climatic conditions of Timergara Dir Pakistan.

A pot experiment was carried out by Tuna *et al.* (2005) with tomato (*Lycopersicon esculentum* Mill.) cv. "Target F1" in a mixture of peat, perlite, and sand (1:1:1) to investigate the effects of supplementary calcium sulphate on plants grown at high NaCl concentration (75 mM). The treatments were: (i) control (C), nutrient solution alone; (ii) salt treatment (C + S), 75 mM NaCl; (iii) salt plus calcium treatment 1 (C+S+Ca1), 75 mM NaCl plus additional mixture of 2.5 mM $CaSO_4$ in nutrient solution; (iv) salt plus calcium treatment 2 (C+S+Ca2), 75 mM NaCl plus additional mixture of 5 mM $CaSO_4$ in nutrient solution. The plants grown under salt stress produced low dry matter, fruit weight, and relative water content than those grown in standard nutrient solution. Supplemental calcium sulphate added to nutrient solution containing salt significantly improved growth and physiological variables affected by salt stress (e.g. plant growth, fruit yield, and membrane permeability) and also increased leaf K^+,Ca^{2+}, and N in tomato plants. The effects of supplemental $CaSO_4$ in maintaining membrane permeability, increasing concentrations of Ca^{2+}, N, and K^+ and reducing concentration of Na^+ (because of cation competition in root zone) in leaves could offer an economical and simple solution to tomato crop production problems caused by high salinity.

An experiment was done by Abbasi *et al.* (2013) Where tomato plants were foliar sprayed with naphthalene acetic acid (0.02%) and calcium chloride (0.5%, 1%) individually as well as in combination to determine its effect on growth, nutrient uptake, incidence of blossom end rot, fruit yield, and enhancement of shelf life. The results showed increased absorption of calcium in tomato plants and fruits, which were treated with NAA in combination with $CaCl_2$. Higher level of $CACl_2$ (1 %) with NAA (0.02%) increased plant growth and yield by improving mineral uptake of tomato plants. The improved calcium absorption also resulted in lowering occurrence of blossom end rot in tomato fruits. In addition, it was also observed that during storage at ambient conditions (20-25°C) for sixteen days, tomato fruits maintained best quality for longer period of time when treated with calcium chloride (1%) along with naphthalene acetic

acid (0.02%) as compared to other treatments. Although, fruit quality was lowered with passage of storage time but tomato fruits from treated plants maintained their quality for longer duration as compared to control.

Islam *et al.* (1987) reported that solution calcium concentrations required for the growth of a range of plant species, including both monocotyledons and dicotyledons, were determined in two experiments in which plants were grown in flowing solution culture at constantly maintained calcium concentrations ranging from 0.5 to 3000 μM. Calcium chloride was used as the calcium source in the first experiment, calcium sulphate was used in the second. At calcium concentrations of 10 μM and below, all species developed calcium deficiency symptoms. The severity of the deficiency was more pronounced in the dicotyledons than in the monocotyledons. However, cassava was much more tolerant than all other dicotyledons and equally as tolerant as rice, the most tolerant monocotyledon. Solution calcium concentrations required for 90% of maximum yield were generally lower for monocotyledons (3 to 20 μM) than for dicotyledons (7 to 720 μM) when calcium chloride was used as the calcium source. When calcium sulphate was used, 7 out of 11 species, including 3 monocotyledons, required external calcium concentrations of 1200 μM and above. The results are discussed in relation to effects of solution composition and the choice of counter-ions on plant response to calcium and other macronutrient cations. It is concluded that yield depressions due to toxicity of excesses of chloride, and possibly other counter-ions, can lead to serious underestimation of limiting external cation concentrations for plant growth.

White and Broadley (2003) stated that Calcium is an essential plant nutrient. It is required for various structural roles in the cell wall and membranes, it is a counter-cation for inorganic and organic anions in the vacuole, and the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is an obligate intracellular messenger coordinating responses to numerous developmental cues and environmental challenges. This article provides an overview of the nutritional requirements of different plants for Ca, and how this impacts on natural flora and the Ca content of crops. It also reviews recent work on (a) the mechanisms of Ca^{2+} transport across cellular membranes, (b) understanding the origins and specicity of $[Ca^{2+}]_{cyt}$ signals and (c) characterizing the cellular $[Ca^{2+}]_{cyt}$ - sensors (such as calmodulin, calcineurin B-like proteins and calcium-dependent protein kinases) that allow plant cells to respond appropriately to $[Ca^{2+}]_{cyt}$ signals.

Chaum *et al.* (2012) reported that Calcium (Ca) is a signaling molecule that plays an active role in regulating various mechanisms involved in recognition and response to abiotic stresses in plants.

Calcium is an essential plant nutrient and has a role in metabolic activities, like stabilization of membranes, signal transduction through second messenger, and control of enzyme activity in Cassia angustifolia (Arshi *et al.* 2006).

Yaseen *et al.* (2006) had reported that Calcium carbide (CaC_2) is a rich source of the nitrification inhibitor acetylene (C_2H_2) and plant hormone ethylene (C_2H_4). C_2H_4 formed from biotic reduction of C_2H_2 released from CaC_2 may accumulate in soil at physiologically active concentrations. Laboratory studies were conducted to evaluate the potential of encapsulated CaC_2 for gradually releasing C_2H_2 and its product C_2H_4 in soil. The GC-FID analysis revealed that encapsulated CaC_2 released a copious amount of C_2H_2 (up to 23700 nmol kg^{-1} soil), which was gradually reduced to C_2H_4 over a period of time via a strictly biotic reaction as no C_2H_4 was detected in CaC_2-amended sterilized soil. Ammonium oxidation was suppressed by the encapsulated CaC_2 indicating that C_2H_2 acted as a nitrification inhibitor. Results of pot trials conducted in the net house indicated that encapsulated CaC_2 applied at 30 mg kg^{-1} soil significantly increased the number of tillers (up to 45.5%), root weight (up to 14.9%), straw (up to 32.8%) and grain yield (up to 37.3%) of wheat over the fertilizer application alone. In the case of cotton, the number of bolls, root, shoot and seed weight were also significantly increased in response to the application of encapsulated CaC_2. Moreover, application of encapsulated CaC_2 resulted in greater N-use efficiency (NUE) (up to 61.1%) by both wheat and cotton crops than that observed at the same rates of N fertilizer alone. These findings imply that CaC_2 affects plant growth through hormonal action of C_2H_4 as well as improved NUE; however, the latter factor might be a relatively more contributing. It is desirable that CaC_2 is formulated for gradually slow release of C_2H_2 and C_2H_4 in soil air.

Effects of calcium (Ca) nutrition on growth, fruit yield and quality of greenhouse tomato have been investigated extensively in northern Europe (Ho *et al.*, 1999).

Adams *et al.* (1988 and 1992) reported that leaf Ca deficiency in tomato reduces leaf size, and causes necrosis of young leaves and yield loss in extreme cases.

Chapter 3
Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted during the period from 15 November 2013 to 15 April 2014. The materials and methods those were used and followed for conducting the experiment have been described under the following headings.

3.1 Experimental site

This study was conducted in the Agricultural farm of Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. The location of the experimental site is 23°74′N latitude and 90°35′E longitude at an altitude of 8.6 meter above the sea level (Anonymous, 2004), which have been shown in the Appendix II.

3.2 Characteristics of soil

The soil of the experimental area belongs to the Modhupur Tract (Anonymous, 1988) under AEZ No. 28. The characteristics of the soil under the experiment were analyzed in the Laboratory of Soil science Department, SAU, Dhaka and details of soil characteristics have been presented in Appendix I.

3.3 Climatic condition of the experimental site

The experimental site is situated in the subtropical monsoon climatic zone, which is characterized by heavy rainfall during the months from April to September (Kharif season) and scanty of rainfall during rest of the year (Rabi season). Plenty of sunshine and moderately low temperature prevail during October to March (Rabi season), which are suitable for growing of tomato in Bangladesh. The weather information regarding temperature prevailed at the experimental site during the cropping season November 2013 to April 2014 have been presented in Appendix VII.

3.4 Planting materials

Seedlings of 25 days of BARI Tomato-15 were used. The seedlings of tomato were grown at the seedbed of Sher-e-Bangla Agricultural University. BARI Tomato-15, a high yielding variety of Tomato was developed by the Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh.

3.5 Treatments of the experiment

The experiment consisted of two factors:

Factor A: Different days of transplanting

10 December 2013
First transplanting time (T_1)

20 December 2013
Second transplanting time (T_2)

30 December 2013
Third transplanting time (T_3)

Factor (B): Different combination of GA_3 and Ca^{2+} as mitigating agent of cold stress

GA= 0 ppm and Ca^{2+}= 0 mM marked as M_0

GA= 20 ppm and Ca^{2+}= 0 mM marked as M_1

GA= 0 ppm and Ca^{2+}= 5 mM marked as M_2

GA= 20 ppm and Ca^{2+}= 5 mM marked as M_3

GA= 0 ppm and Ca^{2+}= 10 mM marked as M_4

GA= 20 ppm and Ca^{2+}= 10 mM marked as M_5

Total 18 treatment combinations were as follows:

T_1M_0	T_2M_0	T_3M_0
T_1M_1	T_2M_1	T_3M_1
T_1M_2	T_2M_2	T_3M_2
T_1M_3	T_2M_3	T_3M_3
T_1M_4	T_2M_4	T_3M_4
T_1M_5	T_2M_5	T_3M_5

3.6 Design and layout of the experiment

The two factors experiment was laid out in Randomized Complete Block Design (RCBD) with three transplanting time and six different combination of GA_3 and Ca^{2+}. Three replications were maintained in this experiment. The total number of unit plots was 54 (3×18). Each plot was 1.8 m × 1.5 m = 2.7 m^2. The distance between blocks was 1 m and distance between plots was 0.5 m and plant spacing was 50 cm × 60 cm. The layout of the experiment is presented in Appendix III.

3.7 Seedling raising

A common procedure was followed in raising of seedlings in the seedbed. Tomato Seedlings were raised in three different seedbed on a relatively high land in the farm of Sher-e-Bangla Agricultural University, Dhaka. The size of each seedbed was 3m× 1 m. The soil was well prepared with spade and made into loose friable and dried mass to obtain fine tilth. All weeds and stubbles were removed and 5 kg well rotten cow dung was applied during seedbed preparation. The seeds were sown in the seedbed at 15 November, 25 November and 05 December, 2013 respectively to get 25 days old seedlings. Germination was visible 3 days after sowing of seeds. After sowing, seeds were covered with light soil to a depth of about 0.6 cm. Heptachlor 40 WP was applied @ 4 kg ha^{-1} around each seedbed as precautionary measure against ants and worm. The emergence of the seedlings took place within 5 to 7 days after sowing. Weeding, mulching and irrigation were done from time to time as and when required and no chemical fertilizer was used in this seedbed.

3.8 Land preparation

The land was ploughed with a rotary plough and power tiller for four times. Ploughed soil was then brought into desirable fine tilth and leveled by laddering. The weeds were cleaned properly. The final ploughing and land preparation were done on 1 December, 2013. According to the layout of the experiment the entire experimental area was divided into blocks and prepared the experimental plot for the transplanting of tomato seedlings. In addition, irrigation and drainage channels were made around the plot.

3.9 Uprooting and transplanting of seedlings

Healthy and uniform 25 days old seedlings were uprooted separately from the seedbed and were transplanted in the experimental plots in the afternoon of 10 December, 20 December and 30 December, 2013 maintaining nine seedlings in each plot. The seedbed was watered before uprooting the seedlings from the seedbed so as to minimize damage to roots with ensuring maximum retention of roots. The seedlings were watered after transplanting.

3.10 Transplanting dates

Tomato seedlings were transplanted to the main field from seed bed for three times at 10 days interval at 10 Dec 2013, 20 Dec 2013 and 30 Dec 2013.

3.11 Application of the treatments

Gibberellic acid (GA_3) and calcium (Ca^{2+}) were applied to the tomato plant according to the treatments. As treatment combinations, different concentration of GA_3 and Ca^{2+} were sprayed exogenously with 0.01% of Tween 20 by a hand sprayer in the early morning at 25 and 50 DAT. The Ca^{2+} was used in the form of $CaSO_4.0.5H_2O$ of Merck India and GA_3 of Sigma Chemical Co., St. Louis, U.S.A.

3.12 Intercultural operations

3.12.1 Irrigation

Light watering was provided with water cane immediately after transplanting the seedlings and this technique of irrigation was used as every day at early morning and sometimes also in evening throughout the growing period. But the frequency of irrigation became less in harvesting stage.

3.12.2 Staking

When the plants were well established, staking was given to each plant by bamboo sticks for support to keep them erect.

3.12.3 Weeding

Weeding was done whenever it was necessary, mostly in vegetative stage.

3.12.4 Plant protection measures

Melathion 57 EC was applied @ 2 ml L^{-1} of water against the insect pests like cutworm, leaf hopper, fruit borer and others. The insecticide application was made fortnightly after transplanting and was stopped before second week of first harvest. Furadan 10G was also applied during plot preparation as soil insecticide. During foggy weather precautionary measure against disease attack of tomato was taken by spraying Diathane M-45 fortnightly @2 gm L^{-1} of water at the early vegetative stage. Aktara was used against Aphid @ 0.2 g L^{-1}. Ridomil gold was also applied @ 2 gm L^{-1} of water against blight disease of tomato.

3.13 Harvesting

Fruits were harvested at 3 days interval during early ripe stage when they developed slightly red color. Harvesting was started from 12 March, 2014 and was continued up to 2nd week of April 2014.

3.14 Recording of data

Experimental data were recorded from 60 days after transplanting and continued until last harvest. The following data were recorded during the experimental period.

A. Morphological characters

1. Plant height (cm)
2. Number of leaves $plant^{-1}$
3. Number of branches $plant^{-1}$

B. Physiological characters

4. SPAD value

C. Yield contributing and yield characters

5. Number of flowers $plant^{-1}$
6. Number of fruits $plant^{-1}$
7. Fruit Diameter (cm)
8. Fruit Length (cm)

9. Fruits weight plot^{-1} (kg)

10. Yield (t ha^{-1})

3.15 Detailed procedures of recording data

A brief outline of the data recording procedure followed during the study is given below:

A. Morphological characters

3.15.1. Plant height (cm)

Plant height was measured at 60 DAT. The height of the plant was determined in centimeter by measuring the distance from the soil surface to the tip of the highest leaf.

3.15.2. Number of leaves plant^{-1}

Leaf number was counted at 60 DAT. The number of leaves plant^{-1} was counted from each plant.

3.15.3. Number of branches plant^{-1}

The total number of branches plant^{-1} was counted from each plant at 60 DAT.

B. Physiological characters

3.15.4. SPAD value

SPAD value was measured using a hand-held SPAD meter (CCM-200, Opti-Science, USA). At each evaluation the value was measured 5 times from five leaves at different positions plant^{-1} and the average was used for analysis.

C. Yield contributing and yield characters

3.15.5. Number of flowers plant^{-1}

The number of flowers produced plant^{-1} was counted and recorded.

3.15.6. Number of fruits plant^{-1}

The number of fruits plant^{-1} was counted and recorded.

3.15.7. Fruit diameter (cm)

The length of fruit was measured with a slide calipers from the neck of the fruit to the bottom of 10 fruits from each plant and their average was taken and expressed in cm.

3.15.8. Fruit length (cm)

Diameter of fruit was measured at middle portion of 10 fruits from each plant with a slide calipers. Their average was taken and expressed in cm.

3.15.9. Fruits weight (kg plot^{-1})

Fruits weight of tomato plot^{-1} was calculated and expressed in kilogram (kg).

3.15.10. Yield (t ha^{-1})

Yield hectare^{-1} of tomato fruits was calculated by converting the weight of plot yield into hectare on the basis of total plant population of tomato hectare^{-1} and expressed in ton.

3.16 Statistical analysis

All the data collected on different parameters were statistically analyzed following the analysis of variance (ANOVA) technique using MSTAT-C computer package program and the mean differences were adjudged by least significant difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

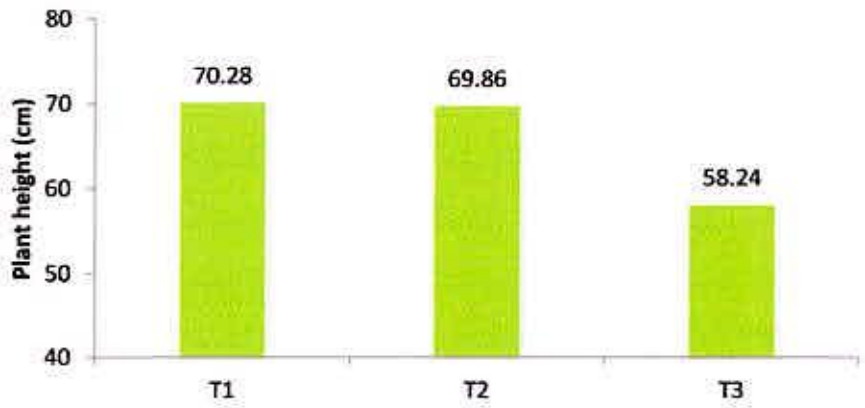
Chapter 4
Results and Discussion

CHAPTER 4

RESULTS AND DISCUSSION

Climate change is major threat of crop production not only in Bangladesh but also all over the world. The north western part of Bangladesh are highly sensitive to tomato production due to presence of severe cold along with drought during the winter season. In this study I used GA_3 and Ca^{2+} alone or in combination as a cold stress mitigation agent for improvement of mrpho-physiology and yield of tomato. The results obtained from date of transplanting and foliar application of gibberellic acid (GA_3) and calcium (Ca^{2+}) alone or in combination are presented and discussed in this chapter. Data about morpho-physiological parameters, yield contributing characters and yield of tomato have been presented in both Tables and Figures and analyzes of variance and corresponding degrees of freedom have been shown in Appendix.

4.1 Plant height (cm):

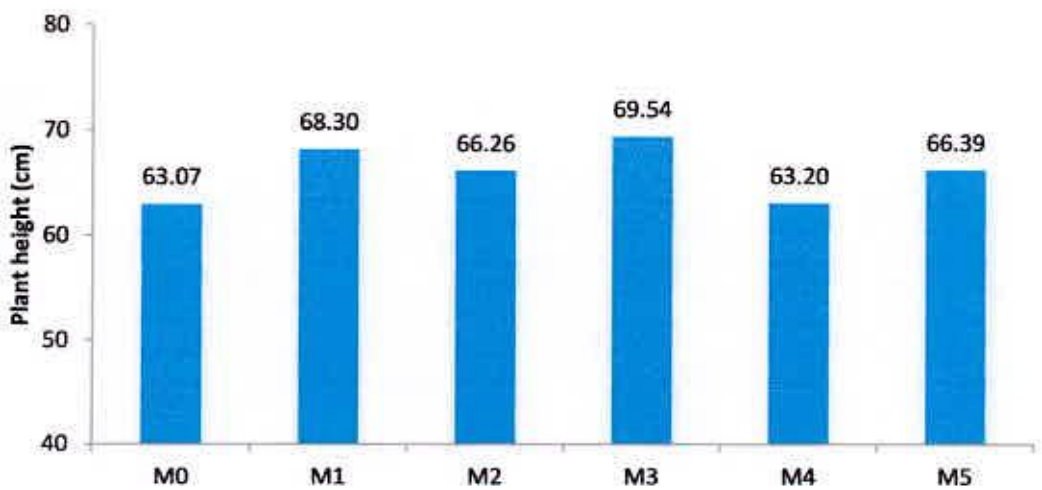


T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Fig. : 1. Effect of transplanting time on the height of tomato plant at 60 days after transplanting (DAT) ($LSD_{0.05} = 3.429$).

It is usual that the effect of transplanting time in relation to change of temperature of the environment is reflected primarily in plant height. Many previous authors stated that different days of transplanting-induced cold condition showed the reduction of length ofplant axis or height (Chen *et al.*, 1999). In this experiment, the temperature

was gradually declining trend during November 2013 to January 2014 which were graphically presented in Appendix VII. The height of 25 day age seedling was also gradually decreasing with different days of sowing or late sowing (data not shown). In this study, late transplanting of tomato showed a significant reduction in plant height (cm) of tomato (Fig. 1 and appendix IV). The highest plant height (70.28 cm) was observed from the T_1 which was statistically similar to T_2 (69.86 cm) and the lowest (58.24 cm) was observed from T_3 time of planting at 60 days after transplanting (DAT). The variation of height of tomato plant at different DAT is identical to seedling height of tomato plant. These results indicate that late sowing of tomato have to withstand cold environment. These results are consistent with the findings of Lawahori *et. al.*, (1963) who stated that plant height decreased with decreasing trend of temperature. Recently, Srivastava and Srivastava (2007) reported that transplanting time had great effect on the regulation of plant architecture as well as plant height of tomato. Altogether, the present results suggest that plant height of tomato decreased with the late planting from optimum time of our environmental conditions.



M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}, M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}, M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}, M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

Fig.: 2. Effect of different combination of GA_3 and Ca^{2+} on the height of tomato plant at 60 days after transplanting (DAT) ($LSD_{0.05}$ = 4.849).

In this study, different combination of GA_3 and Ca^{2+} such as 0 ppm GA_3, 20 ppm GA3 and 0 mM Ca^{2+}, 5 mM Ca^{2+}, 10 mM Ca^{2+} were used as a mitigating agent of cold stress to investigate the adverse effect of late planting-induced cold condition on regulation

of plant height of tomato at 60 days after transplanting (DAT). The GA_3 and Ca^{2+} showed a significant impact to promote the plant height of tomato under late planting-induced cold stress (Fig. 2 and Appendix IV). The highest plant height (69.54 cm) was observed from the M_3 which was statistically similar to M_1 (68.30 cm), M_2 (66.26 cm) and M_5 (66.39 cm) levels of GA_3 and Ca^{2+}. The lowest plant height (63.07 cm) was observed from M_0 which was statistically similar to M_2 (66.26 cm), M_4 (63.20 cm) levels of GA_3 and Ca^{2+}. These experimental results of plant height showed that combined application of GA_3 and Ca^{2+} increased the plant height of tomato compared to sole application of either GA_3 or Ca^{2+} at early induction of cold stress (Appendix VII). In contrast, 20 ppm GA_3 along with higher amount of Ca^{2+} 10 mM i.e. M_5 significantly produced lower plant height as compared to M_3 levels of GA_3 and Ca^{2+}. These results suggest that higher amounts of foliar application of Ca^{2+} inhibit the plant height whereas lower concentration of Ca^{2+} promotes the plant height with GA_3 because higher cellular concentration of Ca^{2+} may reduce the rate of photosynthesis by regulating stomatal movement. These findings are in agreement with those of Kazemi (2012), Rab and Haq (2012) and Ilyas *et al.* (2014) who reported that both GA_3 and Ca^{2+} independently increased the plant height of tomato significantly.

The results of the present study showed that, the interaction effect between transplanting date and different combinations of GA_3 and Ca^{2+} showed significant effect on increment of plant height of tomato (Table 1 and Appendix IV). The highest plant height (75.50 cm) was observed from the T_1M_3 treatment which was statistically similar to T_1M_1 (73.34 cm), T_1M_5 (72.55 cm), T_2M_1 (69.83 cm), T_2M_3 (74.61 cm), and T_3M_3 (72.11 cm). The lowest (55.11 cm) was observed from T_3M_0 which was statistically similar to T_2M_0 (57.11 cm), T_2M_2 (63.33 cm), T_2M_4 (57.67cm), T_3M_2 (61.00 cm), T_3M_4 (56.00 cm) and T_3M_5 (62.56 cm) treatment combinations. The first transplanting (T_1) along with 20 ppm GA_3 5 mM Ca^{2+} (M_3) i.e. (T_1M_3) produced the highest value of plant height and the minimum value were found in third transplanting date (T_3) along with without GA_3 and Ca^{2+} (T_3M_0) treatment combinations and suggest that the plant height failed to increase under cold stress because the lowest temperature were recorded during T_3 planting date (Appendix VII) and the simultaneous application of GA_3 and Ca^{2+} successfully improved the plant height under cold stress. These findings are partially similar to the findings of Chen *et al.* (1999) who reported that plant height decreased with cold stress and Kumar *et al.* (2014), Bhosle *et al.* (2002), Tomar and

Ramgiry (1997), Wu *et al.* (1983), Abdel-Mouty and El-Greadly (2008) reported that GA_3 and Ca^{2+} individually increased the plant height of tomato. Taken together, these results indicate that cold condition decreases the plant height and foliar use of plant growth promoter, GA_3 and macronutrient Ca^{2+} enhance plant shoot structure including plant height of tomato.

Table 1. Combined effect of transplanting time and different combination of GA_3 and Ca^{2+} on the plant height of tomato at 60 DAT

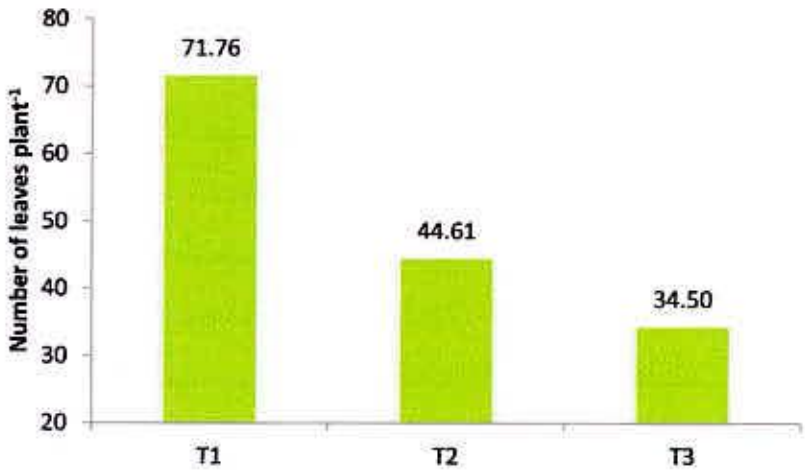
Treatment combination	Plant height
T_1M_0	65.22 bcde
T_1M_1	73.34 ab
T_1M_2	68.22 abcd
T_1M_3	75.50 a
T_1M_4	67.72 abcd
T_1M_5	72.55 ab
T_2M_0	57.11 ef
T_2M_1	69.83 abc
T_2M_2	63.33 cdef
T_2M_3	74.61 a
T_2M_4	57.67 ef
T_2M_5	69.11 abcd
T_3M_0	55.11 f
T_3M_1	69.28 abcd
T_3M_2	61.00 def
T_3M_3	72.11 ab
T_3M_4	56.00 f
T_3M_5	62.56 cdef
LSD $_{(0.05)}$	8.399
Significant level	*
CV (%)	7.65

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

CV = Co-efficient of variance
LSD = Least significant Difference
* = Significant at 5% level

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}
M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}
M_3 - 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}
M_5 - 20 ppm GA_3 + 10 mM Ca^{2+}

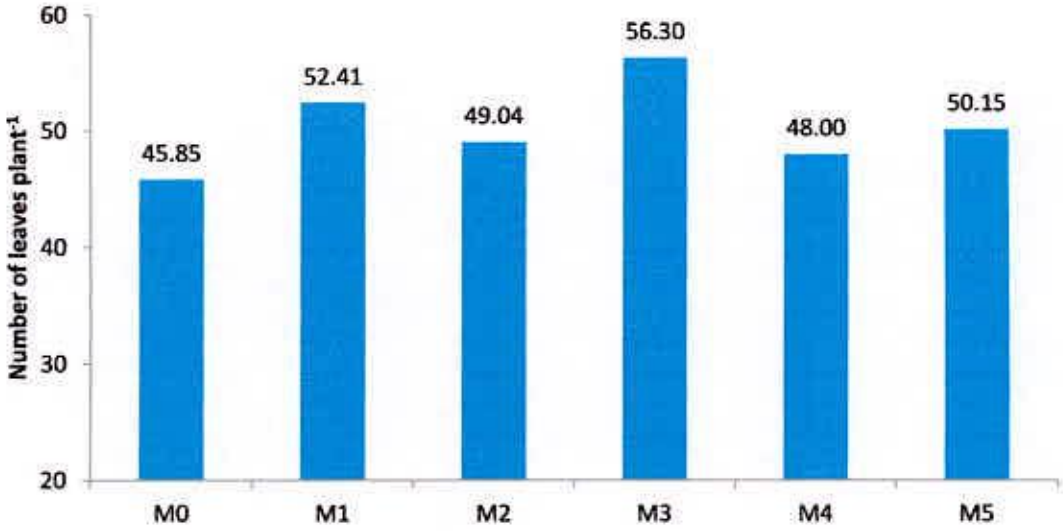
4.2 Number of leaves plant^{-1}



T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Fig. : 3. Effect of transplanting time on the number of leaves plant^{-1} of tomato at 60 DAT ($LSD_{0.05}$ = 6.205).

The leaf number is a fundamental morphological character for plant growth and development as leaf is the main photosynthetic organ. To investigate the effect of different days of transplanting of tomato on changes in the number of leaves plant^{-1} up to 60 DAT, the number of leaves were counted. Different days of transplanting showed a significant influenced on the formation of leaves plant^{-1} in tomato (Fig. 3 and Appendix IV). The highest number of leaves plant^{-1} (71.76) was observed from the T_1 and the lowest (34.50) was observed from late transplanting, T_3 time. These results showed that the highest number of leaves plant^{-1} found from early transplanting time and minimum number of leaves was observed at late transplanting when the temperature was gradually declining that creates cold stress to the late planting plants (Appendix VII). Hossain *et al.* (1986) reported that early sowing enhanced total number of leaves plant^{-1}. Therefore, altogether this experimental result indicates that the number of leaves plant^{-1} of tomato will be decreased at late planting under our environmental conditions.



M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}, M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}, M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}, M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

Fig.: 4. Effect of different combination of GA_3 and Ca^{2+} on the number of leaves plant^{-1} of tomato at 60 DAT ($LSD_{0.05}$ = 8.775).

It is reported by numerous authors that the plant growth regulators enhanced cell division with considerable stem elongation, bud and leaf formation. As an alleviator of cold stress, GA_3 and Ca^{2+} were used which played a significant effect on the number of leaves plant^{-1} of tomato (Fig. 4 and Appendix IV).The highest leaves number plant^{-1} (56.30) was observed from the M_3 which was statistically similar to all except M_0 (45.85) and the lowest (45.85) was observed from M_0 which was statistically similar to all except M_3 (56.30). Thus these results suggested that together application of GA_3 and Ca^{2+} produced higher number of tomato leaves with cold acclimation (Appendix VII). This fact was supported by authors like El-Abd *et al* (1995), Kumar *et al.* (2014), Tuna *et al.* (2005) and Adams *et al.* (1988 and 1992).

The results of the present study showed that, the interaction effect between planting time and different combinations of GA_3 and Ca^{2+} showed significant variation on leaves number plant^{-1} of tomato (Table 2 and Appendix IV). The highest leaves number plant^{-1} (83.56) was observed from the T_1M_3 treatment which was statistically similar to T_1M_1 (75.78), T_1M_2 (68.66), T_1M_5 (72.00), whereas, the lowest (30.22) was observed from T_3M_0 treatment which was statistically similar to T_2M_0 (41.67), T_2M_2 (44.44), T_3M_2 (32.22), T_3M_3 (39.33), and T_3M_5 (35.56). The first transplanting (T_1) along with 20 ppm

GA_3 5 mM Ca^{2+} (M_3) i.e. (T_1M_3) produced the highest value of leaves number and the minimum value were found in third transplanting date (T_3) without GA_3 and Ca^{2+} (T_3M_0) treatment combination and suggests that the late transplanting or T_3 planting date failed to produce leaves as T_1 planting date because the induction of cold might be responsible to influence the formation of leaf in tomato plants (Appendix VII) and the concurrent application of GA_3 and Ca^{2+} successfully improve the leaf number under cold stress.

Table 2. Combined effect of transplanting time and different combination of GA_3 and Ca^{2+} on the number of leaves plant^{-1} of tomato at 60 DAT

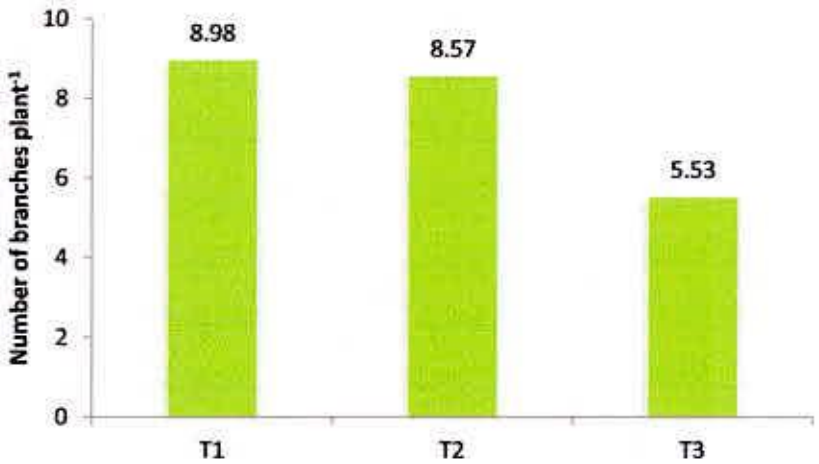
Treatment combination	Number of leaves plant^{-1}
T_1M_0	63.67 b
T_1M_1	75.78 ab
T_1M_2	68.66 ab
T_1M_3	83.56 a
T_1M_4	66.89 b
T_1M_5	72.00 ab
T_2M_0	41.67 cde
T_2M_1	46.00 cd
T_2M_2	44.44 cde
T_2M_3	47.33 c
T_2M_4	42.89 cde
T_2M_5	45.33 cde
T_3M_0	30.22 e
T_3M_1	37.89 cde
T_3M_2	32.22 cde
T_3M_3	39.33 cde
T_3M_4	31.78 de
T_3M_5	35.56 cde
LSD (0.05)	15.20
Significant level	*
CV (%)	18.21

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3 – Third transplanting time, 30 December 2013

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}
M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}
M_3 - 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}
M_5 - 20 ppm GA_3 + 10 mM Ca^{2+}

CV = Co-efficient of variance
LSD = Least significant Difference
* = Significant at 5% level

4.3 Number of branches plant^{-1}



T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Fig. : 5. Effect of transplanting time on the number of branches plant^{-1} of tomato at 60 DAT ($LSD_{0.05}$ = 1.121).

Branches plant^{-1} of tomato were significantly influenced by transplanting times (Fig. 5 and Appendix IV). The highest number of branches plant^{-1} (8.981) was observed from the T_1 which was statistically similar to T_2 (8.574) and the lowest (5.537) was observed from T_3. Among dates of planting, early planting recorded the highest vegetative growth Singh *et al.* (2005). Number of branches plant^{-1} was found to be gradually decreased with the late transplanting dates (Mira *et al.*, 2011). Therefore, the presented results are consistent with many other previous findings published.

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}, M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}, M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}, M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

Fig.: 6. Effect of different combination of GA_3 and Ca^{2+} on the number of branches plant^{-1} of tomato at 60 DAT ($LSD_{0.05}$ = 1.586).

Application of different combination of GA_3 and Ca^{2+} showed statistically insignificant effect on number of branches plant^{-1} of tomato (Fig. 6 and Appendix IV). The highest number of branches plant^{-1} (7.99) was observed from the M_3 and the lowest (7.52) was observed from M_0. In contrast, Tomar and Ramgiry (1997), Ilyas *et al.* (2014), Rab and Haq (2012), Kazemi (2012) observed significant effect of GA_3 and Ca^{2+} in increasing the number of branches in plant. Therefore, variety and environmental factors may have some influence to produce considerable branches which did not response to the late planting of present research.

There were significant interaction effects between transplanting time and different combination of GA_3 and Ca^{2+} on number of branches plant^{-1} of tomato (Table 3 and Appendix IV). The highest number of branches plant^{-1} (9.557) was observed from the T_1M_3 treatment which was statistically similar to T_1M_0 (8.333), T_1M_1 (9.553), T_1M_2 (8.890), T_1M_4 (8.887), T_1M_5 (9.110), T_2M_0 (7.443), T_2M_1 (8.667), T_2M_2 (8.557), T_2M_3 (9.113), T_2M_4 (8.553), and T_2M_5 (8.667) whereas, the lowest (4.887) was observed from T_3M_0 treatment which was statistically similar to T_3M_1 (5.777), T_3M_2 (5.443), T_3M_3 (6.223), T_3M_4 (5.337) and T_3M_5 (5.557). The first transplanting (T_1) along with 20 ppm GA_3 5 mM Ca^{2+} (M_3) i.e. (T_1M_3) produced the highest value of leaves number

and the minimum value were found in third transplanting date (T_3) without GA_3 and Ca^{2+} (T_3M_0) treatment combinations. These findings have partial agreement with the findings of Nibhavanti *et al.* (2006), Bhosle *et al.* (2002). From this result, it suggests that cold stress reduce the plant growth as well as branching. The application of GA_3 and Ca^{2+} at the rate of 20 ppm and 5mM, respectively minimize the adverse effects of cold stress by forming more branches in response to different days of transplanting (Appendix VII).

Table 3. Combined effect of transplanting time and different combination of GA_3 and Ca^{2+} on the number of branches plant^{-1} of tomato at 60 DAT

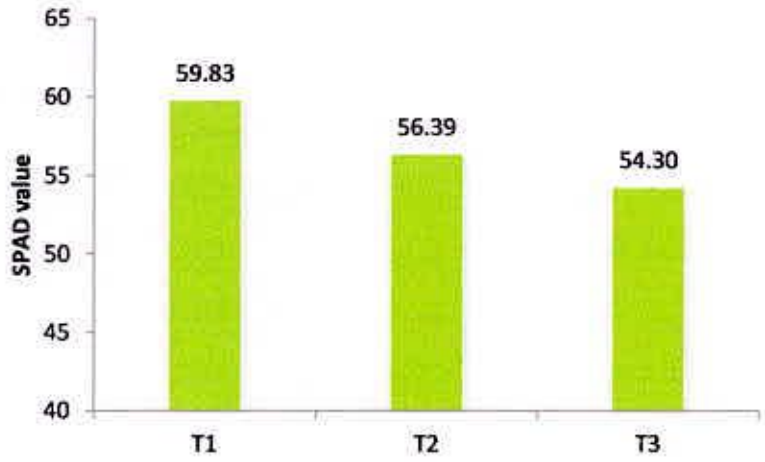
Treatment combination	Number of branches plant^{-1}
T_1M_0	8.333 abc
T_1M_1	9.553 a
T_1M_2	8.890 ab
T_1M_3	9.557 a
T_1M_4	8.887 ab
T_1M_5	9.110 a
T_2M_0	7.443 abcd
T_2M_1	8.667 ab
T_2M_2	8.557 ab
T_2M_3	9.113 a
T_2M_4	8.553 ab
T_2M_5	8.667 ab
T_3M_0	4.887 d
T_3M_1	5.777 cd
T_3M_2	5.443 d
T_3M_3	6.223 bcd
T_3M_4	5.337 d
T_3M_5	5.557 d
LSD $_{(0.05)}$	2.747
Significant level	**
CV (%)	19.16

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3 – Third transplanting time, 30 December 2013

CV = Co-efficient of variance
LSD = Least significant Difference
** = Significant at 1% level

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}
M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}
M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}
M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

4.4 SPAD value



T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Fig. : 7. Effect of transplanting time on the SPAD value of tomato plant leaf ($LSD_{0.05}$ = 1.383).

There was a clear effect of transplanting time on the SPAD value of tomato plant leaf for T_1, T_2 and T_3 (Fig. 7 and Appendix V). The SPAD value of tomato leaves higher in T_1 other than T_2 and T_3 decreased at late transplanting. The highest SPAD value was observed from the T_1 (59.83) and the lowest (54.30) was observed from T_3. From this experiment it was observed that the SPAD value decreased gradually when transplanting of tomato done at early December to late December.

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}, M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}, M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}, M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

Fig.: 8. Effect of different combination of GA_3 and Ca^{2+} on the SPAD value of tomato plant leaf ($LSD_{0.05}$ = 1.956).

Different combination of GA_3 and Ca^{2+} had insignificant effect on SPAD value of tomato plant leaf (Fig. 8 and Appendix V). The highest SPAD value (57.69) was observed from the M_3 and the lowest (56.09) was observed from M_0.

Interaction of different planting time and combination of GA_3 and Ca^{2+} showed significant variation on SPAD value of tomato leaves (Table 4 and Appendix V). The highest SPAD value (61.43) was observed from the T_1M_3 treatment which was statistically similar to T_1M_0 (58.70), T_1M_1 (60.17), T_1M_2 (59.37), T_1M_4 (59.27) and T_1M_5 (60.03) whereas, the lowest (52.57) was observed from T_3M_0 treatment which was statistically similar to T_2M_0 (54.93), T_2M_4 (55.10), T_3M_1 (55.77), T_3M_2 (53.80), T_3M_4 (53.77) and T_3M_5 (53.80). From my experiment it has been observed that cold stress reduce the SPAD value and GA_3 and Ca^{2+} 20ppm and 5mM respectively alleviate the effect of cold stress partially when the lowest temperature were recorded during T_3 planting date (Appendix VII)

Table 4. Combined effect of transplanting time and different combination of GA_3 and Ca^{2+} on the SPAD value of tomato plant leaf

Treatment Combination	SPAD value
T_1M_0	58.70 abcde
T_1M_1	60.17 ab
T_1M_2	59.37 abcd
T_1M_3	61.43 a
T_1M_4	59.27 abcd
T_1M_5	60.03 abc
T_2M_0	54.93 fgh
T_2M_1	57.13 bcdefg
T_2M_2	56.73 cdefg
T_2M_3	57.63 bcdef
T_2M_4	55.10 fgh
T_2M_5	56.80 bcdefg
T_3M_0	52.57 h
T_3M_1	55.77 efgh
T_3M_2	53.80 gh
T_3M_3	56.10 defg
T_3M_4	53.77 gh
T_3M_5	53.80 gh
LSD (0.05)	3.387
Significant level	*
CV (%)	3.59

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3 – Third transplanting time, 30 December 2013

CV = Co-efficient of variance
LSD = Least significant Difference
* = Significant at 5% level

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}
M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}
M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}
M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

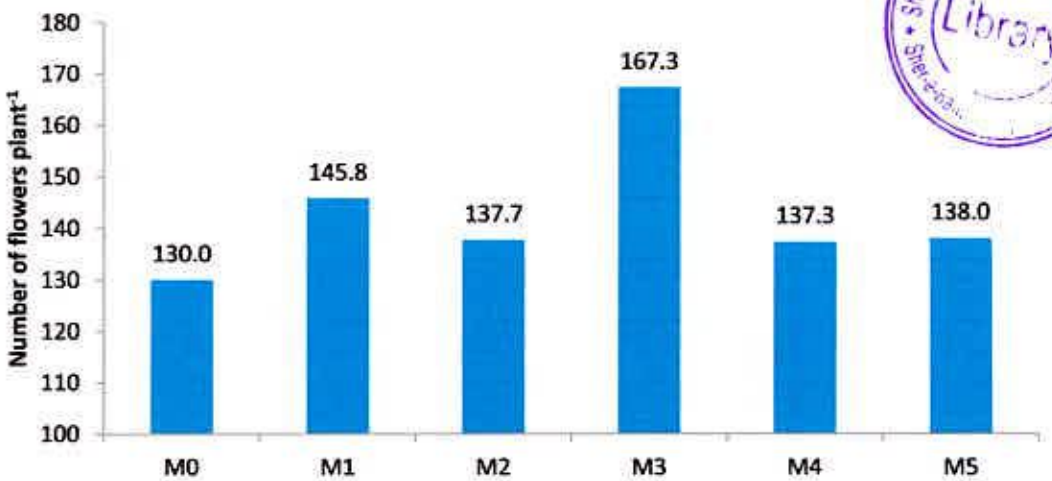
4.5 Number of flowers plant^{-1}

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Fig. : 9. Effect of transplanting time on the number of flowers plant^{-1} of tomato ($LSD_{0.05}$ = 7.555).

Planting time had significant effect on number of flowers plant^{-1} of tomato (Fig. 9 and Appendix VI). The highest number of flowers plant^{-1} was observed from the T_1 (149.9) and the lowest (137.3) was observed from T_3 treatment which was statistically similar to T_2 (140.9). Hossain *et al.* (1986) reported that early sowing enhanced total number of flowers plant^{-1}. From these results, it was found that the early transplanted tomato seedlings produce more flower than lately transplanted tomato seedlings.

Statistically significant variation was recorded for the number of flowers plant^{-1} of tomato for different combination of GA_3 and Ca^{2+} (Fig. 10 and Appendix VI).The highest number of flowers plant^{-1} (167.3) was observed from the M_3 and the lowest (63.07 cm) was observed from M_0 which was statistically similar to M_2 (137.7), M_4 (137.3) and M_5 (138.0) respectively. These results are in conformity with the findings of Srivastava and Srivastava (2007), Choudhury *et al.* (2013), Ilyas *et al.* (2014) and Kazemi (2012), Rab and Haq (2012). Bima *et al.* (1995) found that GA_3 (5-10 ppm) induced flowering. The first transplanting (T_1) along with 20 ppm GA_3, 5 mM Ca^{2+} (M_3) i.e. (T_1M_3) produced the highest value the number of flowers plant^{-1} and the minimum value were found in third transplanting date (T_3) without GA_3 and Ca^{2+} (T_3M_0)



M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}, M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}, M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}, M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

Fig.: 10. Effect of different combination of GA_3 and Ca^{2+} on the number of flowers plant^{-1} of tomato ($LSD_{0.05}$ = 10.68).

treatment combinations and suggest that the flowers produced plant^{-1} highest when the temperature near to optimum and lowest flowers produced under lower temperature was recorded during T_3 planting date (Appendix VII) and the simultaneous application of GA_3 and Ca^{2+} successfully improved the number of flowers plant^{-1} under cold stress.

Interaction effect of planting time and different combination of GA_3 and Ca^{2+} showed significant variation in terms of number of flowers plant^{-1} (Table 5 and Appendix VI). The highest number of flowers plant^{-1} (181.9) was observed from the T_1M_3 treatment which was statistically similar to T_2M_3 whereas, the lowest (118.3) was observed from T_3M_0 treatment which was statistically similar to T_1M_0 (133.2), T_2M_0 (124.7), T_2M_4 (136.3), T_3M_2 (128.3), T_3M_4 (128.1) and T_3M_5 (134.7). Many authors like Choudhury *et al.* (2013), Ilyas *et al.* (2014) and Kazemi (2012), Rab and Haq (2012) mentioned that GA_3 and Ca^{2+} individually increase number of flowers plant^{-1}. So it can be suggest that early transplanting at T_1 increase the flower number and late transplanting at T_3 time due to temperature condition but along with GA_3 and Ca^{2+} late transplanting partially overcome the unfavorable temperature condition.

Table 5. Combined effect of transplanting time and different combination of GA_3 and Ca^{2+} on the number of flowers plant^{-1} of tomato

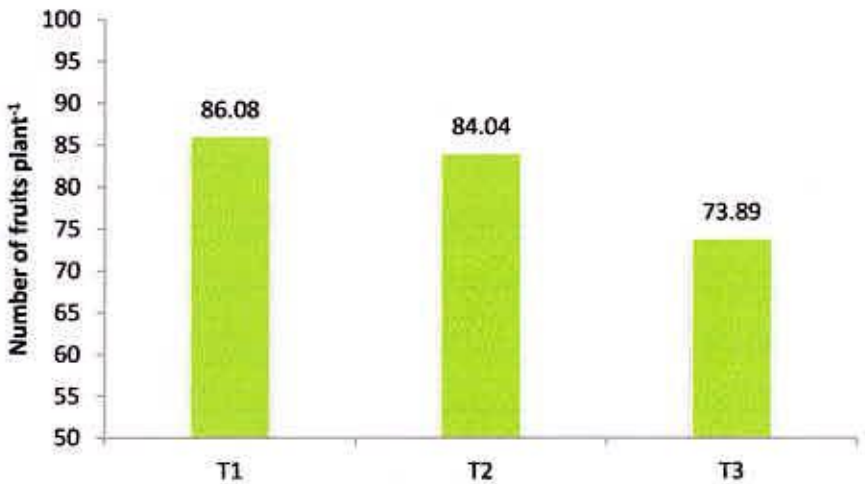
Treatment combination	Number of flowers plant^{-1}
T_1M_0	133.2 cdef
T_1M_1	152.0 b
T_1M_2	145.0 bcd
T_1M_3	181.9 a
T_1M_4	138.6 bcde
T_1M_5	148.6 bc
T_2M_0	124.7 ef
T_2M_1	147.8 bc
T_2M_2	138.6 bcde
T_2M_3	172.2 a
T_2M_4	136.3 bcdef
T_2M_5	147.4 bc
T_3M_0	118.3 f
T_3M_1	142.3 bcde
T_3M_2	128.3 def
T_3M_3	150.8 bc
T_3M_4	128.1 def
T_3M_5	134.7 bcdef
LSD $_{(0.05)}$	18.51
Significant level	*
CV (%)	7.82

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

CV = Co-efficient of variance
LSD = Least significant Difference
* = Significant at 5% level

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}
M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}
M_3 - 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}
M_5 - 20 ppm GA_3 + 10 mM Ca^{2+}

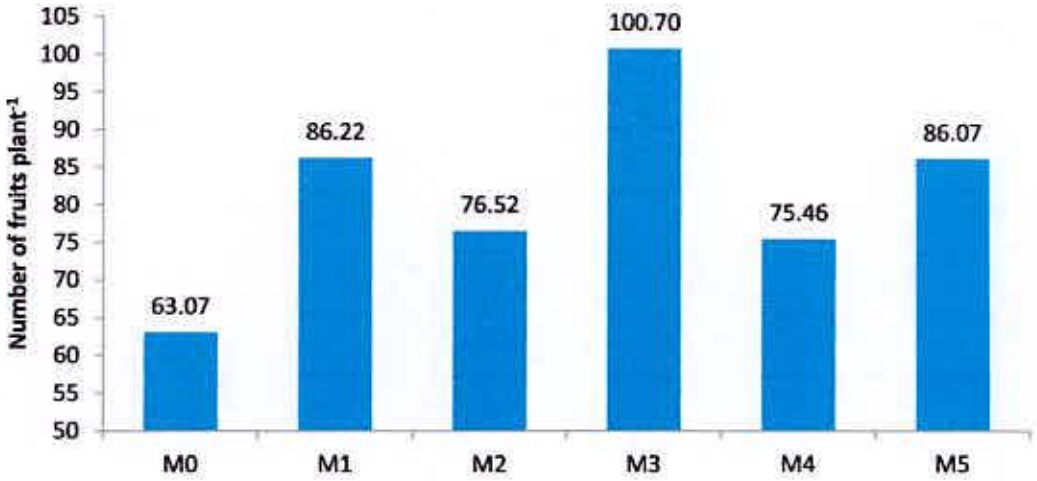
4.6 Number of fruits plant^{-1}



T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Fig. : 11. Effect of transplanting time on the number of fruits plant^{-1} of tomato ($LSD_{0.05}$ = 4.912).

Number of fruits plant^{-1} of tomato showed significant differences in response to transplanting time (Fig. 11 and Appendix). The highest Fruits number plant^{-1} (86.08) was observed from the T_1 which was statistically similar to T_2 (84.04) and the lowest (73.89) was observed from T_3. Maximum number of fruits plant^{-1} from early and the minimum from late transplanting due to high temperature (BARI, 1989). Adelana (1976) and Drost and Price (1991) had also reported that late transplanting reduce fruits number and early showed increasing trend. Jong *et al.* (2009) had reported that the initiation of tomato fruit growth, fruit set, is very sensitive to environmental conditions. So it can easily understand that environmental condition regulate the number fruits plant^{-1} as when near optimum temperature was present produced the highest number of fruits and in unfavorable temperature condition decreased the number of fruits plant^{-1}.



M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}, M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}, M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}, M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

Fig.: 12. Effect of different combination of GA_3 and Ca^{2+} on the number of fruits plant^{-1} of tomato ($LSD_{0.05}$ = 6.946).

Statistically significant variation was recorded for number of fruits plant^{-1} of tomato after the application of different combination of GA_3 and Ca^{2+} (Fig. 12 and Appendix VI). The highest Fruits number plant^{-1} (100.7) was observed from the M_3 and the lowest (63.07) was observed from M_0. The spraying of concentrations of GA_3 and Ca^{2+} had a great regulatory effect on number of fruits plant^{-1} and increased the fruits yield as suggested by Rai *et al.* (2006), Tomar and Ramgiry (1997), El-Habbasha *et al.* (1999), Mehta and Mathi (1975), Kaushik *et al.* (1974), Choudhury *et al.* (2013), Davies and Zalman (2006), Ho *et al.* (1999), Ilyas *et al.* (2014) and Kazemi (2012).

Number of fruits plant^{-1} varied significantly with the interaction effect of different transplanting time and combination of GA_3 and Ca^{2+} (Table 6 and Appendix VI). The highest fruits number plant^{-1} (110.4) was observed from the T_1M_3 treatment whereas, the lowest (56.00) was observed from T_3M_0 treatment which was statistically similar to T_2M_0 (63.33) and T_3M_4 (67.56).

Table 6. Combined effect of transplanting time and different combination of GA_3 and Ca^{2+} on the number of fruits plant^{-1} of tomato

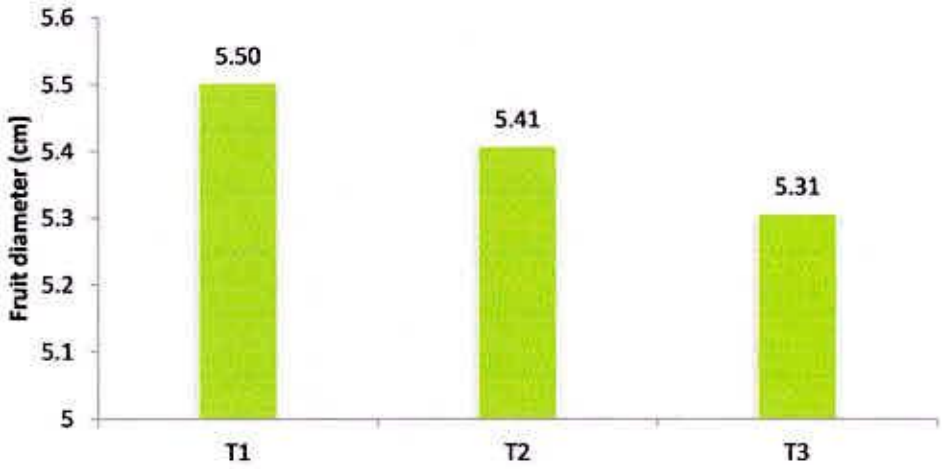
Treatment combination	Number of fruits plant^{-1}
T_1M_0	69.89 ghi
T_1M_1	91.44 bc
T_1M_2	83.22 cdef
T_1M_3	110.4 a
T_1M_4	78.78 defgh
T_1M_5	90.78 bcd
T_2M_0	63.33 ij
T_2M_1	89.44 bcde
T_2M_2	81.67 cdefg
T_2M_3	98.21 b
T_2M_4	75.28 fghi
T_2M_5	88.22 bcde
T_3M_0	56.00 j
T_3M_1	79.00 defgh
T_3M_2	69.44 hi
T_3M_3	93.33 bc
T_3M_4	67.56 hij
T_3M_5	78.00 efgh
LSD $_{(0.05)}$	12.03
Significant level	*
CV (%)	8.91

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

CV = Co-efficient of variance
LSD = Least significant Difference
* = Significant at 5% level

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}
M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}
M_3 - 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}
M_5 - 20 ppm GA_3 + 10 mM Ca^{2+}

4.7 Fruit diameter (cm)



T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Fig. : 13. Effect of transplanting time on the fruit diameter of tomato ($LSD_{0.05} = 0.1645$).

In this study planting time showed significant variation in the fruit diameter (cm) of tomato (Fig. 13 and Appendix VI). The highest fruit diameter (5.50) was observed from the T_1 which is statistically similar with T_2 (5.41) and the lowest (5.31) was observed from T_3 treatment. Madhumathi and Sadarunnisa (2013) reported that date of transplanting affected the fruit diameter of tomato. From the study of results it was found that early transplanting provide higher fruit diameter than the late transplanted tomato plant.

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}, M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}, M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}, M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

Fig.: 14. Effect of different combination of GA_3 and Ca^{2+} on the fruit diameter of tomato ($LSD_{0.05}$ = 0.2327).

A significant variation was recorded due to the different combination of GA_3 and Ca^{2+} on fruit diameter of tomato (Fig. 14 and Appendix VI). The highest fruit diameter (5.76 cm) was observed from the M_3 and the lowest (5.10 cm) was observed from M_0. Previous many authors reported that GA_3 and Ca^{2+} plays an important role on the fruit development and setting in many crops. All together the presented data suggest that GA_3 and Ca^{2+} had positive functions on fruit diameter (cm) as well as fruit yield of tomato as supported by Adlakha and Verma (1964), Serrani *et al.* (2007).

Fruit diameter (cm) was observed significant variation due to the interaction between the different transplanting time and application of different combination of GA_3 and Ca^{2+} on tomato. The highest fruit diameter (5.913) was observed from the T_1M_3 treatment which was statistically similar to T_1M_1 (5.647), T_2M_1 (5.560), T_2M_3 (5.700) and T_3M_3 (5.657) whereas, the lowest (5.050) was observed from T_3M_0 treatment which was statistically similar to all except T_1M_1 (5.647), T_1M_3 (5.913), T_1M_5 (5.510), T_2M_1 (5.560), T_2M_3 (5.700), T_2M_5 (5.463) and T_3M_3 (5.657). Madhumathi and Sadarunnisa (2013) reported significant increase of fruit diameter of tomato.

Table 7. Combined effect of transplanting time and different combination of GA_3 and Ca^{2+} on the fruit diameter of tomato

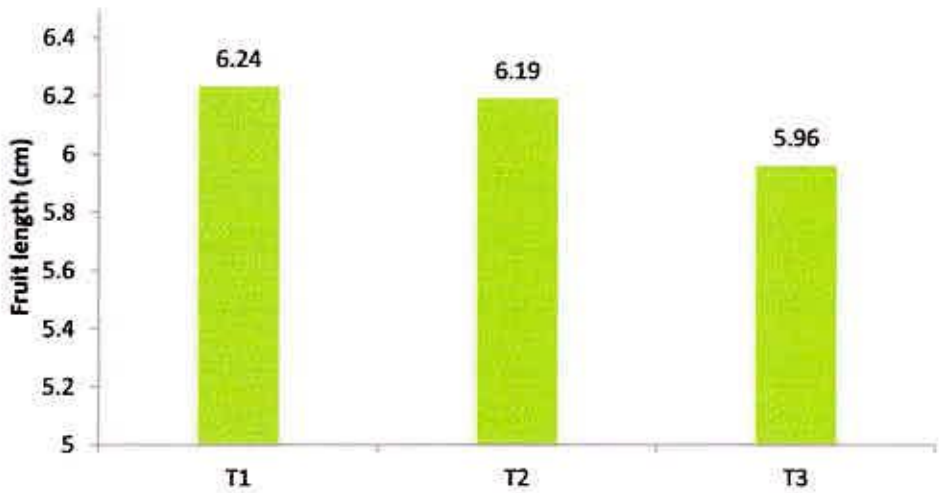
Treatment combination	Fruit diameter(cm)
T_1M_0	5.143 ef
T_1M_1	5.647 abc
T_1M_2	5.443 bcdef
T_1M_3	5.913 a
T_1M_4	5.397 bcdef
T_1M_5	5.510 bcde
T_2M_0	5.110 ef
T_2M_1	5.560 abcd
T_2M_2	5.397 bcdef
T_2M_3	5.700 ab
T_2M_4	5.227 def
T_2M_5	5.463 bcde
T_3M_0	5.050 f
T_3M_1	5.397 bcdef
T_3M_2	5.267 cdef
T_3M_3	5.657 abc
T_3M_4	5.147 ef
T_3M_5	5.277 cdef
LSD (0.05)	0.4030
Significant level	**
CV (%)	4.49

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3 – Third transplanting time, 30 December 2013

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}
M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}
M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}
M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

CV = Co-efficient of variance
LSD = Least significant Difference
**= Significant at 1% level

4.8 Fruit length (cm)



T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Fig. : 15. Effect of transplanting time on the fruit length of tomato ($LSD_{0.05} = 0.156$).

As consistent to fruit diameter planting time had significant influenced on fruit length (cm) of tomato (Fig. 15 and Appendix VI). The highest fruit length (6.24) was observed from the T_1 which was statistically similar to T_2 (6.19) and the lowest (5.96) was observed from T_3. These data showed that early transplanting time increased fruit length (cm) in contrast to late transplanting. Madhumathi and Sadarunnisa (2013) had reported that early transplanting showed the maximum fruit length of tomato fruit among different varieties.

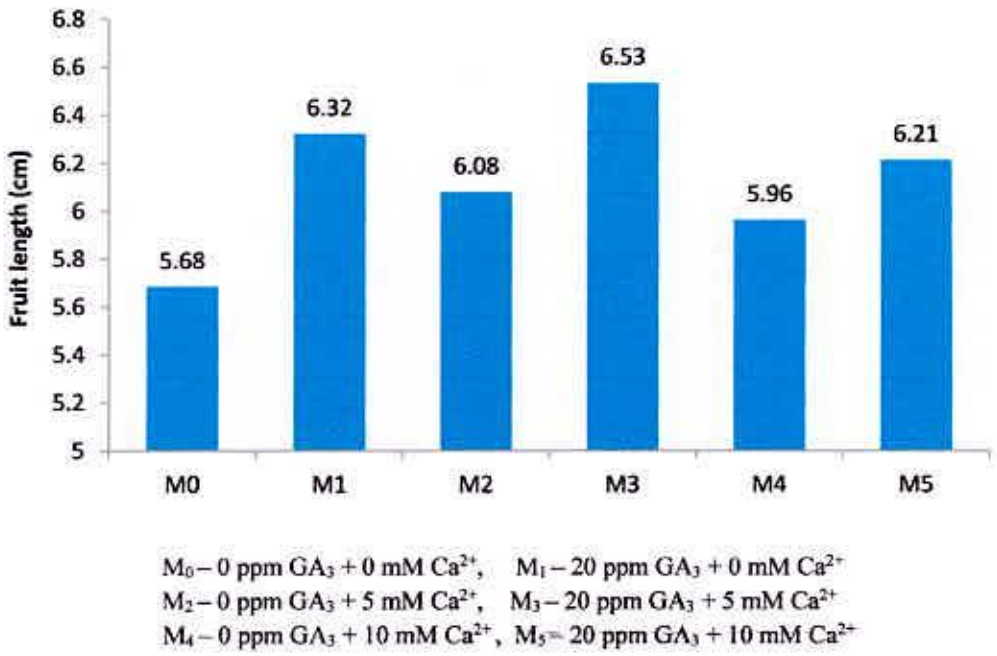


Fig.: 16. Effect of different combination of GA_3 and Ca^{2+} on the fruit length of tomato ($LSD_{0.05} = 0.2206$).

Different combination of GA_3 and Ca^{2+} had significant effect on fruit length of tomato (Fig. 16, Appendix VI). The highest Fruit Length (6.53) was observed from the M_3 which was statistically similar to M_1 (6.32) and the lowest (5.68) was observed from M_0. Here results showed that GA_3 and Ca^{2+} increased fruit length as reported by Madhumathi and Sadarunnisa (2013), Adlakha and Verma (1964) that application of GA_3 increased the fruit length of tomato. Previous many authors reported that GA_3 and Ca^{2+} plays an important role on the fruit development and setting in many crops. All together the presented data suggest that GA_3 and Ca^{2+} had positive functions on fruit length of tomato.

Interaction of planting time and different combination of GA_3 and Ca^{2+} showed significant variation on fruit length of tomato. The highest fruit length (6.713 cm) was observed from the T_1M_3 treatment which was statistically similar to T_1M_1 (6.417), T_1M_5 (6.407), T_2M_1 (6.400) and T_2M_3 (6.603) whereas, the lowest (5.653 cm) was observed from T_3M_0 treatment which was statistically similar to T_1M_0 (5.747 cm), T_2M_0 (5.653 cm), T_2M_4 (5.810 cm), T_3M_2 (5.853 cm), and T_3M_4 (5.790cm). Results showed that best combination (T_1M_3) increased fruit length at 20 ppm GA_3 and 5 mM Ca^{2+} as consistent with fruit diameter of tomato.

Table 8. Combined effect of transplanting time and different combination of GA_3 and Ca^{2+} on the fruit length of tomato

Treatment combination	Fruit length (cm)
T_1M_0	5.747 gh
T_1M_1	6.417 abc
T_1M_2	6.330 bc
T_1M_3	6.713 a
T_1M_4	6.113 cdefg
T_1M_5	6.407 abc
T_2M_0	5.653 h
T_2M_1	6.400 abc
T_2M_2	6.177 cde
T_2M_3	6.603 ab
T_2M_4	5.810 efgh
T_2M_5	6.223 bcd
T_3M_0	5.653 h
T_3M_1	6.157 cdef
T_3M_2	5.853 defgh
T_3M_3	6.280 bc
T_3M_4	5.790 fgh
T_3M_5	6.043 cdefg
LSD $_{(0.05)}$	0.3820
Significant level	**
CV (%)	3.74

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

CV = Co-efficient of variance
LSD = Least significant Difference
** = Significant at 1% level

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}
M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}
M_3 - 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}
M_5 - 20 ppm GA_3 + 10 mM Ca^{2+}

4.9 Yield (kg plot^{-1}) and (t ha^{-1})

Fig. : 17.A

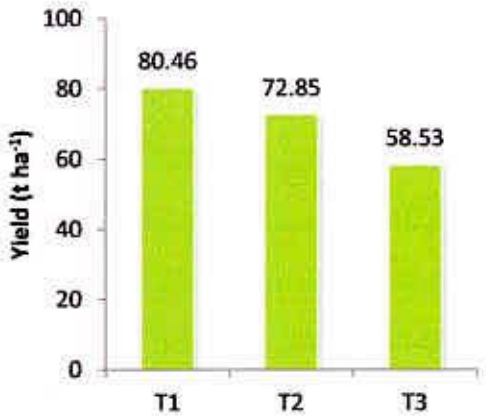


Fig. : 17.B

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Fig. : 17. Effect of transplanting time on the yield plot^{-1} in kg (A) and yield in t ha^{-1} (B) of tomato ($LSD_{0.05}$ = 1.150 and 4.259 for yield kg plot^{-1} and t ha^{-1} respectively).

As morphological characters the yield of tomato also significantly reduced by late transplanting-induced cold stress (Fig. 17 A and B, Appendix VI). The highest yield plot^{-1} (21.73 kg) and yield ha^{-1} (80.46 t) were observed from the T_1 and the lowest yield plot^{-1} (15.80 kg) and yield ha^{-1} (58.53 t) were observed from T_3 or late planting. The results of both yield (kg plot^{-1}) and yield (t ha^{-1}) of tomato is gradually decreasing with the late transplanting, T_3. These results are consistent with the yield contributing characters which are analyzed in this experiment such as number of flowers plant^{-1} (Fig. 9 and 10), fruit diameter (Fig. 13 and 14) and fruit length (Fig. 15 and 16). These information are also dependable on growth measuring parameters of this study ((Fig. 1 and 3). In addition, Sanjoy (1999) showed a declining trend in fruit yield and other yield attributing characters when planted lately. Tongova and Zhelev (1975) reported that early sowing or early planting of tomato gave increased yield. Previous authors reported as well that early transplanting of tomato gave increased fruit weight and yield of tomato (Adelana 1976). These results suggest that suitable transplanting time is more favorable to produce highest plant height, leaf number, branch number as a result

higher flower produced which enhance the higher fruit set and development i.e. fruit diameter and length of tomato which contribute to maximum yield than late transplanting-induced cold injury.

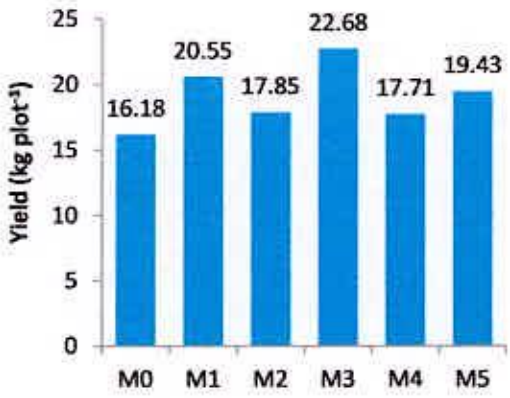


Fig. : 18.A

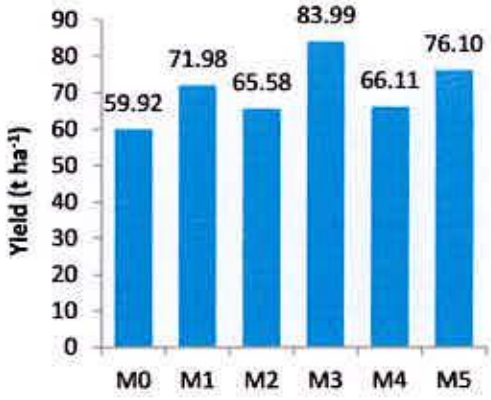


Fig. : 18.B

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}, M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}, M_3 – 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}, M_5 – 20 ppm GA_3 + 10 mM Ca^{2+}

Fig.: 18. Effect of different combination of GA_3 and Ca^{2+} on the yield plot^{-1} in kg (A) and yield in t ha^{-1} (B) of tomato ($LSD_{0.05}$ = 1.626 and 6.023 for yield kg plot^{-1} and t ha^{-1} respectively).

In this study, different combination of GA_3 and Ca^{2+} such as 0 ppm GA_3, 20 ppm GA_3 and 0 mM Ca^{2+}, 5 mM Ca^{2+}, 10 mM Ca^{2+} were used as a mitigating agent of cold injury to alleviate the late planting-induced cold injury on improve the yield of tomato. The GA_3 and Ca^{2+} showed a significant effect to promote the fruit yield tomato under late planting-induced cold stress (Fig.18 A and B; Appendix VI). The highest yield plot^{-1} 22.68 kg and yield 83.99 t ha^{-1} were observed from the M_3 and the lowest 16.18 kg plot^{-1} and 59.92 t ha^{-1} were observed from M_0 which was statistically similar to M_4 (17.71 kg). Lolaei (2012), Saleh and Abdul (1980), Adlakha and Verma (1965) reported that separately GA_3 and Ca^{2+} increase the yield of tomato. Hossain (1974) testified that GA_3 increased fruit set. Ca^{2+} increased the fruit yield of tomato (Lolaei, 2012). Therefore, altogether these results suggest that mutual application of GA_3 and Ca^{2+} increased the fruit yield of tomato.

The interaction effect between transplanting date and different combination of GA_3 and Ca^{2+} showed a significant positive effect on fruit yield of tomato (Table 9 and Appendix VI). The highest yield $plot^{-1}$ 25.26 kg and yield ha^{-1} 93.54 t were recorded from the T_1M_3 treatment which was statistically similar to T_1M_1 (22.77 kg $plot^{-1}$), (83.69 t ha^{-1}), and T_1M_5 (22.60 kg $plot^{-1}$), (84.34 t ha^{-1}) whereas, the lowest (13.45 kg $plot^{-1}$) and (49.79 t ha^{-1}) was observed from T_3M_0 treatment which was statistically similar to T_3M_2 (15.16 kg $plot^{-1}$, 53.90 t ha^{-1}) and T_3M_4 (14.55 kg $plot^{-1}$, 56.15 t ha^{-1}) treatment combinations. These results are consistent with the morpho-physiological data as well as fruit yield contributing characters of tomato (Table 4, 5, 6, 7 and 8). Singh *et al.* (2005), Ahammad *et al.* (2009), Peyvast (2001), Bhosle *et al.* (2002), Tomar and Ramgiry (1997), Hao and Papadopoulos (2003), Lolaei (2012), Kazemi (2012), Ilyas *et al.* (2014) reported that early transplanting, GA_3 and Ca^{2+} significantly increase the yield of tomato. Taken together, these results indicate that cold condition decreases the fruit yield and foliar application of plant growth promoter, GA_3 and macronutrient Ca^{2+} enhance the yield of tomato.

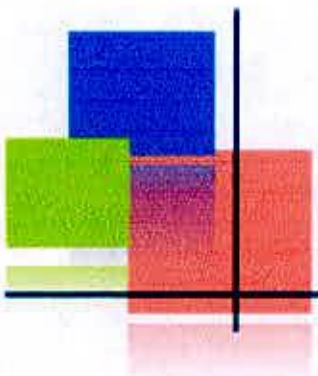
Table 9. Combined effect of transplanting time and different combination of GA_3 and Ca^{2+} on the yield (kg $plot^{-1}$) and yield (t ha^{-1}) of tomato

Treatment combination	Yield (kg $plot^{-1}$)	Yield (t ha^{-1})
T_1M_0	18.62 efgh	68.97 efgh
T_1M_1	22.77 abc	83.69 abc
T_1M_2	20.83 cde	75.07 cdef
T_1M_3	25.26 a	93.54 a
T_1M_4	20.27 cdef	77.14 cde
T_1M_5	22.60 abc	84.34 abc
T_2M_0	16.47 hij	61.01 hij
T_2M_1	21.80 bcd	73.19 defg
T_2M_2	18.30 efgh	67.77 efgh
T_2M_3	24.12 ab	89.34 ab
T_2M_4	17.56 fghi	65.05 fghi
T_2M_5	19.76 defg	80.76 bcd
T_3M_0	13.45 k	49.79 k
T_3M_1	17.06 ghij	59.05 hijk
T_3M_2	15.16 ijk	53.90 jk
T_3M_3	18.65 efgh	69.09 efgh
T_3M_4	14.55 jk	56.15 ijk
T_3M_5	15.94 hijk	63.20 ghij
LSD $_{(0.05)}$	2.817	10.43
Significant level	*	*
CV (%)	8.90	8.90

T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

CV = Co-efficient of variance
LSD = Least significant Difference
* = Significant at 5% level

M_0 – 0 ppm GA_3 + 0 mM Ca^{2+}
M_1 – 20 ppm GA_3 + 0 mM Ca^{2+}
M_2 – 0 ppm GA_3 + 5 mM Ca^{2+}
M_3 - 20 ppm GA_3 + 5 mM Ca^{2+}
M_4 – 0 ppm GA_3 + 10 mM Ca^{2+}
M_5 - 20 ppm GA_3 + 10 mM Ca^{2+}



Chapter 5
Summary and conclusion

CHAPTER 5

SUMMARY AND CONCLUSION

The experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka, during the period from 15 November 2013 to 15 April 2014 to find out a method to mitigate the late planting-induced cold stress with exogenous foliar application of gibberellic acid (GA_3) along with calcium (Ca^{2+}) on tomato. In this experiment, the treatments consisted of three different time of transplanting T_1= First transplanting time :10 December 2013, T_2= Second transplanting time :20 December 2013, T_3= Third transplanting time :30 December 2013 and six different combination of GA_3 and Ca^{2+} as mitigating agent of temperature stress *viz.* M_0 = 0 ppm GA_3 and 0 mM Ca^{2+}, M_1= 20 ppm GA_3 and 0 mM Ca^{2+}, M_2 = 0 ppm GA_3 and 5 mM Ca^{2+}, M_3= 20 ppm GA_3 and 5 mM Ca^{2+} , M_4= 0 ppm GA_3 and 10 mM Ca^{2+} and M_5= 20 ppm GA_3 and 10 mM Ca^{2+}. The experiment was laid out in two factors Randomized Complete Block Design (RCBD) with three replications. Data on different growth parameters, physiological parameters and yield with yield contributing characters of tomato were recorded. The collected data were statistically analyzed for evaluation of the treatment effect. A significant variation among the treatments was found while different transplanting time and gibberellic acid along with calcium were applied in different combinations.

There was significant difference among the different time of transplanting in respect of almost all parameters. The tallest plant height (70.28 cm) was recorded from T_1= First transplanting time. The maximum number of leaves plant^{-1} (71.76) was observed from the T_1= First transplanting time. The number of branches plant^{-1} showed significant variation during T_1= First transplanting time and the maximum number of branches plant^{-1} was (8.981). In case of lowest plant height, number of leaves and branches plant^{-1} was observed from T_3= Third transplanting time. Different planting time also had significant effect on SPAD value of tomato leaf, the highest (59.83) and lowest (54.30) observed from the T_1 and T_3. The maximum number of flowers and fruits plant^{-1} 149.9 and 86.08, respectively were obtained from the T_1. The highest fruit length and diameter 6.237 cm and 5.503 cm, respectively were obtained from T_1= First transplanting time. The highest yield plot^{-1} (21.73 kg) was observed from the T_1. The maximum yield (80.46 t ha^{-1}) was observed from the T_1, whereas the minimum yield (58.53 t ha^{-1}) was obtained from T_3= Third transplanting time.

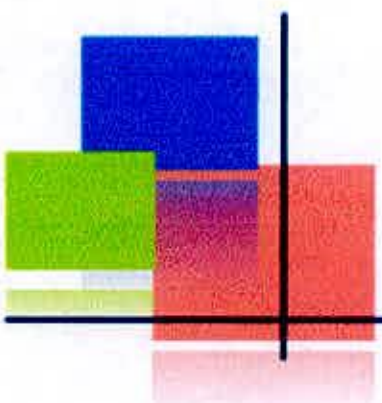
Plant height of tomato showed significant difference in response of exogenous foliar application of gibberellic acid (GA_3) with calcium (Ca^{2+}). The tallest plant height 69.54 cm was observed from the M_3 (20 ppm GA_3 and 5 mM Ca^{2+}). The results showed significant variation and the maximum number of leaves plant^{-1} and branch plant^{-1} 56.30 and 7.999, respectively were observed from M_3 (20 ppm GA_3 and 5 mM Ca^{2+}). In case of SPAD value there was no significant variation observed hence not affected by different levels of gibberellic acid (GA_3) with calcium (Ca^{2+}) on tomato plant. The maximum number of flower and fruit plant^{-1} 167.3 and 100.7, respectively were obtained in plants which received 20 ppm GA_3 and 5 mM Ca^{2+} (M_3). The highest fruit length and diameter 6.532 cm and 5.757 cm, respectively were obtained from M_3 (20 ppm GA_3 and 5 mM Ca^{2+}). The maximum yield plot^{-1} 22.68 kg was recorded from M_3 (20 ppm GA_3 and 5 mM Ca^{2+}). The maximum yield (83.99 t ha^{-1}) was obtained from M_3 (20 ppm GA_3 and 5 mM Ca^{2+}), whereas the minimum yield (59.92 t ha^{-1}) was obtained from M_0 (0 ppm GA_3 and 0 mM Ca^{2+}).

The combinations of time of transplanting and gibberellic acid with calcium had significant effect on almost all growth and yield contributing parameters. The tallest plant height 75.50 cm was found from T_1M_3 (First transplanting time with 20 ppm GA_3 and 5 mM Ca^{2+}) treatment combination. The results showed significant differences on number of leaves plant^{-1} and branches plant^{-1} of tomato. The maximum number of leaves plant^{-1} 83.56 and branches plant^{-1} 9.557 were found in T_1M_3 treatment combination (First transplanting time with 20 ppm GA_3 and 5 mM Ca^{2+}). Different combinations of time of transplanting and gibberellic acid with calcium also had significant effect on SPAD value of tomato plant leaf, the highest 61.43 was observed from the T_1M_3 treatment. All the yield contributing characters of tomato showed significant variations with all treatment combinations. The maximum number of flowers plant^{-1} (181.9), fruits plant^{-1} (110.4), fruit length (6.713 cm) and fruit diameter (5.913cm) were found in T_1M_3 (First transplanting time with 20 ppm GA_3 and 5 mM Ca^{2+}) treatment combination. The maximum yield plot^{-1} 25.26 kg was recorded from T_1M_3 treatment. The highest yield 93.54 t ha^{-1} was obtained from T_1M_3 (First transplanting time with 20 ppm GA_3 and 5 mM Ca^{2+}) treatment, whereas the lowest yield (49.79 t ha^{-1}) was obtained from T_3M_0 treatment combination. Third transplanting time and without gibberellic acid and calcium (0 ppm GA_3 and 0 mM Ca^{2+}).

Considering the above results, it may be summarized that morphological parameters, yield contributing characters and yield of tomato consistent with time of transplanting and exogenous foliar application of gibberellic acid (GA_3) along with calcium (Ca^{2+}). Therefore, the present experimental results suggest that the combined use of 20 ppm GA_3 with 5 mM Ca^{2+} can mitigate the detrimental effect of late transplanting-induced cold stress and increase the yield of tomato variety BARI Tomato 15 under the climatic and edaphic condition of Sher-e-Bangla Agricultural University, Dhaka.

Considering the situation of the present experiment, further studies in the following areas may be suggested:

1. Such study is needed in different agro-ecological zones (AEZ) of Bangladesh for analogy the accuracy of the experiment.
2. It needs to conduct more experiments with transplanting time and GA_3 with Ca^{2+} to find out whether these can regulate the morpho-physiology and yield of tomato var. BARI Tomato 15.
3. It needs to conduct related experiments with other varieties of tomato.
4. Scope to conduct advance experiments how, transplanting time and GA_3 with Ca^{2+} physiologically increase yield of tomato.



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Appendices

APPENDICES

Appendix I: Physical and chemical characteristics of initial soil (0-15 cm depth)

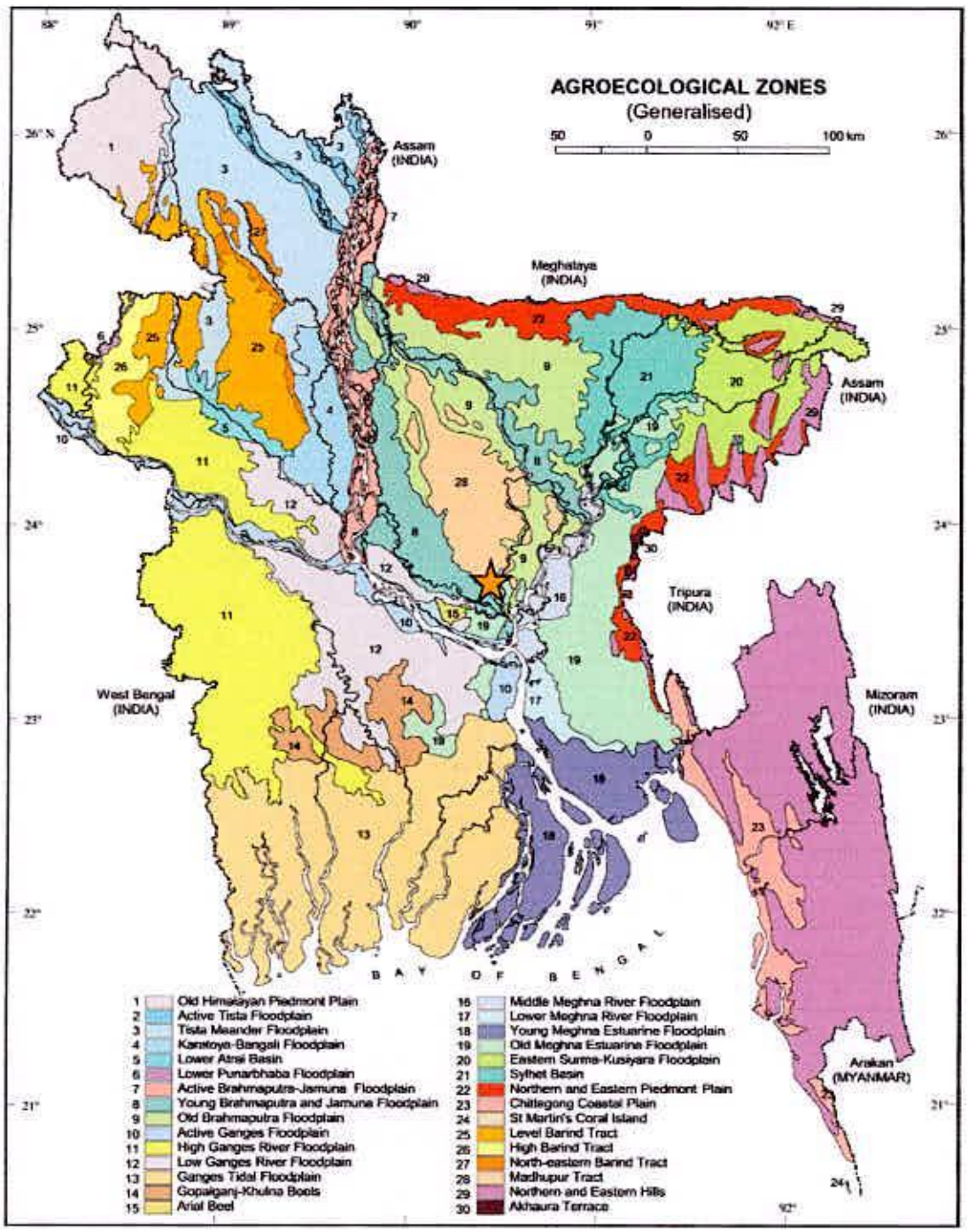
A. Physical composition of soil

Soil separates	(%)	Methods employed
Sand	36.90	Hydrometer method (Day, 1995)
Silt	26.40	-do-
Clay	36.66	-do-
Texure class	Clay loam	-do-

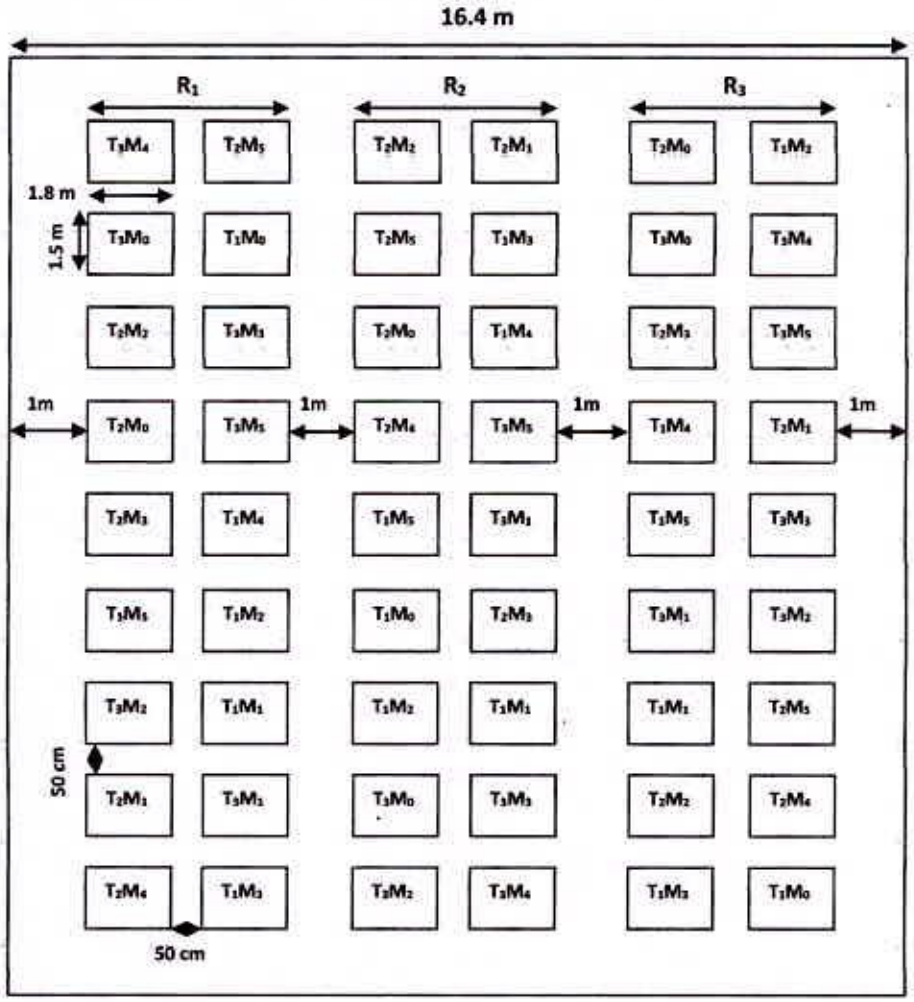
B. Chemical composition of soil

SI.	Soil characteristics	Analytical data	Methods employed
1	Organic carbon (%)	0.82	Walkley and Black, 1947
2	Total N (kg/ha)	1790.00	Bremner and Mulvaney, 1965
3	Total S (ppm)	225.00	Bardsley and Lancster, 1965
4	Total P (ppm)	840.00	Olsen and Sommers, 1982
5	Available N (kg/ha)	54.00	Bremner and Mulvaney, 1965
6	Available P (kg/ha)	69.00	Olsen and Dean, 1965
7	Exchangeable K (kg/ha)	89.50	Pratt, 1965
8	Available S (ppm)	16.00	Hunter, 1984
9	P^H (1:2.5 soil to water)	5.55	Jackson,1958
10	CEC	11.23	Chapman, 1965

Appendix II: Experimental location on the map of agro-ecological zones of Bangladesh



Appendix III. Layout of the experimental plot

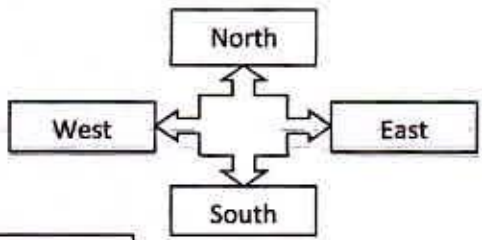


Date of transplanting:

T_1 – First transplanting time, 10 December 2013

T_2 – Second transplanting time, 20 December 2013

T_3– Third transplanting time, 30 December 2013



Different combination of GA_3 and Ca^{2+} as mitigating agent of cold stress

M_0 – 0 ppm GA + 0 mM Ca^{2+}, M_1 – 20 ppm GA + 0 mM Ca^{2+}

M_2 – 0 ppm GA + 5 mM Ca^{2+}, M_3 – 20 ppm GA + 5 mM Ca^{2+}

M_4 – 0 ppm GA + 10 mM Ca^{2+}, M_5 – 20 ppm GA + 10 mM Ca^{2+}

Appendix IV: Analysis of variance of the data on plant height, Number of leaves, Number of branches of tomato as influenced by different transplanting time and GA_3 along with Ca^{2+}

Sources of variation	Degree of freedom	Mean square		
		Plant height	Number of leaves	Number of branches
Replication	2	150.807	2381.702	23.694
Factor A (T)	2	840.076**	6682.384**	63.743**
Factor B (C)	5	61.706*	120.728**	0.292^{NS}
A X B	10	26.950*	42.398*	1.072**
Error	34	25.620	83.899	2.174

** significant at 1% level of probability,
* significant at 5% level of probability,
NS- Non significant

Appendix V: Analysis of variance of the data on SPAD value of tomato plant leaf as influenced by different transplanting time and GA_3 along with Ca^{2+}

Sources of variation	Degree of freedom	Mean square
		SPAD value
Replication	2	21.854
Factor A (T)	2	140.186**
Factor B (C)	5	3.725^{NS}
A X B	10	4.109**
Error	34	4.167

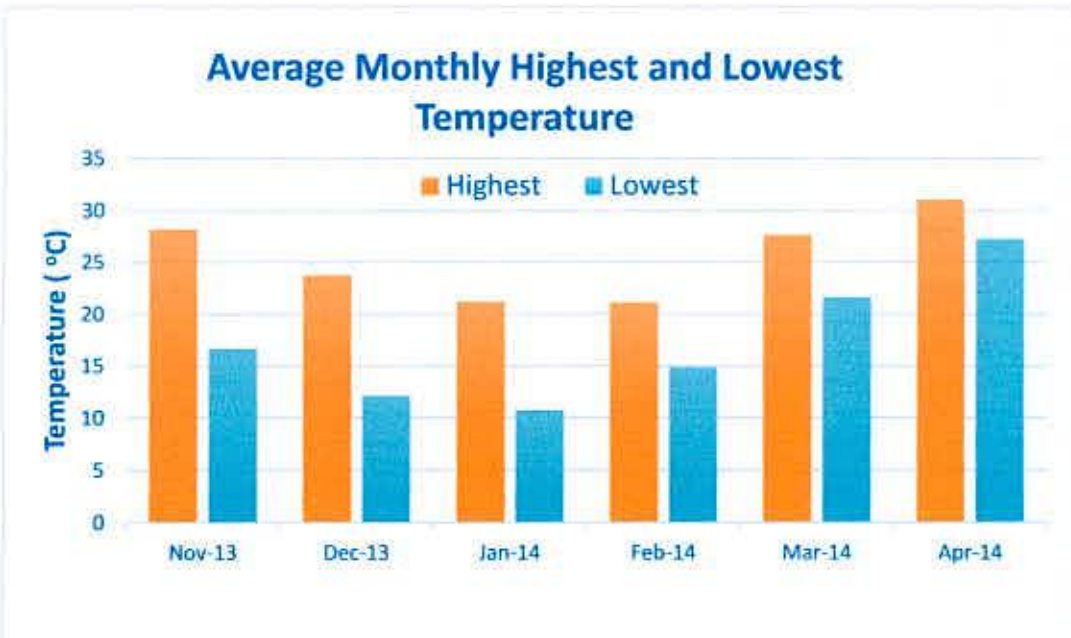
** significant at 1% level of probability,
* significant at 5% level of probability,
NS- Non significant

Appendix VI: Analysis of variance of the data on yield contributing characters and yield of tomato as influenced by different transplanting time and GA_3 along with Ca^{2+}

Sources of variation	Degree of freedom	Mean square					
		Number of flowers plant^{-1}	Number of fruits plant^{-1}	Fruit Diameter (cm)	Fruit length (cm)	Yield (Kg plot^{-1})	Yield (t ha^{-1})
Replication	2	1766.371	2151.691	0.294	0.303	85.785	1176.822
Factor A (T)	2	749.045**	767.707**	0.175*	0.393**	162.727**	2232.07*
Factor B (C)	5	1531.258**	1459.969**	0.419*	0.782**	48.624**	667.411*
A X B	10	349.298*	38.258*	0.032**	0.062**	1.204*	16.477*
Error	34	124.389	52.570	0.059	0.053	2.882	39.520

** significant at 1% level of probability,
* significant at 5% level of probability,
NS- Non significant

Appendix VII: Monthly averaged highest and lowest temperature of Dhaka City during November 2013 to April 2014



Source: http://climatevo.com