ALLEVIATION OF LOW TEMPERATURE INJURY IN TOMATO WITH SALICYLIC ACID AND CALCIUM

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CERTIFICATE

This is to certify that the thesis entitled **"ALLEVIATION OF LOW TEMPERATURE INJURY IN TOMATO WITH SALICYLIC 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 results of a piece of *bona fide* research work carried out by **NIGAR AFSANA,** Registration No. **08-02951** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

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

Dated:
Dhaka, Bangladesh

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DEDICATED TO

MY

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ABSTRACT

The experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka, during the period of 15 November 2013 to 15 April 2014 to find out the role of exogenous foliar application of salicylic acid (SA) and calcium (Ca^{2+}) on the changes of morpho-physiology and fruit yield of tomato under low temperature injury owing to late-transplanting. In this experiment, 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 December 2013), T_2 = Second transplanting time (20 December 2013), T_3 = Third transplanting time (30 December 2013); and six different combination of SA and Ca^{2+} *viz*. A_0 = 0 mM of SA and 0 mM Ca^{2+}, A_1= 0.25 mM SA and 0 mM Ca^{2+}, A_2 = 0 mM SA and 5 mM Ca^{2+}, A_3 = 0.25 mM SA and 5 mM Ca^{2+}, A_4 = 0 mM of SA and 10 mM Ca^{2+} and A_5 = 0.25 mM SA 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). SA and Ca^{2+} were applied to the plant according to the treatments. Different composition of SA and Ca^{2+} were sprayed exogenously by a hand sprayer in the morning at 15, 30, and 45 DAT**.** Most of the results of this experiment showed differences to the treatments. The first transplanting time (T_1) significantly 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 flower clusters plant^{-1}, number of flowers plant^{-1}, number of fruits plant^{-1}, fruit length, fruit diameter, yield (kg plot^{-1}) and (t ha^{-1}) and compared to third or late transplanting time (T_3) induced cold injury. The maximum yield (66.46 t ha^{-1}) was obtained from the first transplanting time (T_1) whereas the lowest yield (45.62 t ha^{-1}) was recorded from T_3 transplanting time 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 composition of SA and Ca^{2+} alleviated the adverse effects of late transplanting induced cold injury in tomato. Exogenous foliar application of 0.25 mM SA along with 5 mM Ca^{2+} improved the morphological characters of tomato. The yield contributing characters and fruit yield showed significant increased with 0.25 mM SA along with 5 mM Ca^{2+}. The highest tomato yield 72.57 t ha^{-1}were found with 0.25 mM SA along with 5 mM Ca^{2+} but the lowest 47.45 t ha^{-1} yield were recorded from control. These results indicated that combined application of SA along with Ca^{2+} shows positive impact on fruit yield by altering the morpho-physiological and yield contributing characters of tomato. The interaction between date of transplanting and sole or together application of SA and Ca^{2+} influenced all the morpho-physiological and yield contributing characters and yield of tomato. The highest yield (86.62 t ha^{-1}) of tomato obtained with the first transplanting time along with 0.25 mM SA and 5 mM Ca^{2+} (T_1A_3) treatment combination, whereas the lowest yield (36.59 t ha^{-1}) was recorded from T_3A_0, third transplanting time with 0 mM SA and 0 mM Ca^{2+} treatment combination. Therefore, the treatment combination T_1A_3 increased fruit yield 61.2% with T_1A_0 and T_3A_3 increased fruit yield 38.41% with T_3A_0 and suggest that combined application of SA and Ca^{2+} successfully alleviates low temperature injury in tomato under SAU environmental conditions.

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LIST OF ABBREVIATION AND ACRONYMS

AEZ	Agro- Ecological Zone
Anon.	Anonymous
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
Ca	Calcium
Cm	Centi-meter
CV %	Percentage of Coefficient of Variance
DAT	Day After Transplanting
Df	Degree of freedom
et al.	And others
FAO	Food and Agricultural Organization
G	Gram (s)
Kg	Kilogram (s)
LSD	Least Significant Difference
L	Liter
M	Meter
m^2	Square meter
mM	Millimolar
Na	Sodium
NaCl	Sodium Chloride
No.	Number
NS	Non significant
PGRs	Plant Growth Regulators
ppm	Parts per Million
PRP	Pathogenesis- related protein
RCBD	Randomized complete block design
ROS	Reactive Oxygen Species
SA	Salicylic acid
SAU	Sher-e- Bangla Agricultural University
t ha^{-1}	Ton per hectare
var.	Variety

wt.	Weight
%	Percentage
^0C	Degree Celsius

Chapter 1
Introduction

CHAPTER 1

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most important popular fruit vegetable which belong to Solanaceae family. It might be the world's largest vegetable crop after potato and a common canned vegetable. This is originated in Central and South America and grown throughout the world including Bangladesh. Generally, tomato is grown in the winter season, November-April in Bangladesh. It is usually red and some varieties showed yellow, orange and purple in colors after ripening.

In terms of human health, tomato is a major component in the daily diet and constitutes of important sources including antioxidants- like lycopene, which acts as an anti-carcinogen and improves skin's ability to protect against harmful ultra violet (UV) rays (Redenbaugh *et al.* 1992 and Wilcox *et al*., 2003). The lycopene from tomatoes has no effect on the risk of developing diabetes, whereas it relieves the oxidative stress in human who already have diabetes. It has been also reported that increasing evidence from clinical trials shows that lycopene supplementation is effective at lowering lower density lipoprotein (LDL) cholesterol. It decreases the risk of blood clotting. It is rich in 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-B_9), vitamin E (Alpha Tocopherol), vitamin K, potassium, copper, molybdenum, manganese, phosphorus, zinc, iron, dietary fiber, zeaxanthin, protein, choline, along with low sodium (Olaniyi *et al*., 2010). Usually, 100 g 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 (BARI, 2010). Now-a- days, it consumes as a raw salad, cooked and processed food item such as sauce, ketchup, jam, jelly etc. Therefore, it suggests that tomato has excellent health benefit to human in aspect of nutritional attributes.

The total arable land of our country is decreasing at alarming rate due to over population, road, construction, urbanization and changes of environment. Thus, need to take necessary steps to increase crop yield including tomato. In Bangladesh, the total production of tomato 2.51 lac tons in the area of 26 thousands hectare and the average yield being 9.96 ton ha^{-1} were reported by FAO, 2013 and BBS, 2013. This fruit yield is lower in contrast with other tomato producing countries like China (49.87 ton ha^{-1}), India (20.11 ton ha^{-1}) and USA (87.96 ton ha^{-1}). The yield of tomato of Bangladesh is not enough 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. Therefore, the proper time of transplanting and use of PGRs along with nutrients are believed to be effective and modern agricultural techniques to improve the fruit yield of tomato under the existing climatic conditions.

It is well known that climate change is a frightening issue on reduction of crop yield not only in Bangladesh but also all over the world. Presently, drought, changes of temperate, salinity, heavy metal contamination etc affect the growth, development and yield of agricultural crops. In Bangladesh, usually early November is the planting time seems to be the best (Hossain *et al*., 1986) and late planting results in lower yield and enhanced disease infection in tomato. It was reported that fruit set was abundant only when night temperature was between 15°C and 20°C (Went, 1984). Curme (1992) also showed that fruit set varies with temperature as low (7.2°C) and with temperature as high (26.6°C). Tremendous decline in fruit set due to high as well as low temperature which disturb mechanisms involved in the development of male and female parts of the flowers (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 findings suggest that late time of sowing or transplanting-induces cold injury which exhibits a significant reduction on both growth and yield of tomato.

Intercellular communication in higher plants is mediated by the action of chemical messengers called plant growth regulators (PGRs) or plant hormones which influence the growth and development of plant including plant cell division, enlargement and differentiation, photosynthesis, flowering, fruiting etc. Numerous studies illustrated that exogenous application of plant bio-regulators improve the morpho-physiology and yield of tomato as a smart agriculture. Presently, tomato cultivators are also commercially producing tomatoes both at higher and lower temperature with foliar application of PGRs. Batlang (2008) reported that the fruit yield of tomato is influenced by number of fruit in each clusters, size which are improved with PGRs and become popular to tomato growers. In addition, abiotic as well as biotic stresses are being alleviated with numerous

plant growth regulators together with abscisic acid (ABA), cytokinin (CK), auxin (IAA), gibberellin (GA), jasmonate (JA), salicylic acid (SA).

Salicylic acid (SA) is considered to be the potent plant hormone (Raskin, 1992a) because of its diverse regulatory roles in plant metabolism (Popova *et al.,* 1990). It is synthesized in cells; can move freely in and out of the cells, tissues and organs (Kawano *et al*., 2004) and this movement is finely regulated by reactive oxygen species (ROS) and Ca^{+2} (Chen and Kuc, 1999; Chen *et al*., 2001). The SA has been found to play key role in the regulation of plant growth, development, interaction with other organisms and in the responses to environmental stresses (Raskin, 1992a, b; Yalpani *et al*., 1994; Senaratna *et al*., 2000). Moreover, many previous authors reported that its role is evident in seed germination, fruit yield, glycolysis, flowering in plants ion uptake and transport, photosynthetic rate, stomatal conductance and transpiration (Harper and Balke, 1981; Klessig and Malamy, 1992; Khan *et al*., 2003). Arberg (1981) stated that both abiotic and biotic stress tolerance increases in plants to fungi, bacteria, viruses, chilling, heat, drought, salinity in presence of SA. Fariduddin *et al*. (2003) reported that lower concentrations of SA were found to be beneficial in enhancing the photosynthesis, growth and various other physiological and biochemical characteristics of plants. On the other hand, at higher concentrations, SA itself may cause a high level of stress in plants. The exogenous SA application enhances the activities of antioxidant enzyme activities as well as the enzymes of nitrate metabolism under stressful environments. Therefore, it suggests that SA alters various physiological functions and biochemical processes in plants for regulating their growth and productivity in relation to change of plant environment.

The exogenous application of SA improves yield and quality of tomato reported by Javaheri *et al.* (2014) who showed that the application of salicylic acid at the concentration of 10^{-6} M increased tomato fruit weight (3059.5 g per bush) whereas non-treated plants (2220 g per bush). In addition, Ding *et at*. (2002) reported that pretreatment of tomato with lower concentrations of exogenous salicylic acid increased their resistance to cold injury and there was also a decreased incidence of decay in low temperature storage and an increase in synthesis of Pathogenesis Related (PR) proteins. It was also reported that SA potentially alleviates the damaging effects of low temperatures in numerous crops including rice and wheat (Szalai *et al*., 2002; Tasgin *et al*., 2003), bean (Senaratna *et al*., 2000) and banana (Kang *et al*. 2003a). However, little is known whether exogenous SA regulates the morpho-physiology and yield of tomato at late transplanting-induced cold injury.

Many authors reported that calcium (Ca^{2+}) is an essential macro nutrient for vigorous plant growth which fulfills a fundamental role as a second messenger in plant membrane stability and cell wall-stabilization (Hirschi, 2004; Kadir, 2004). In 2003, White and Broadley elaborated that though it is an essential structural element in strengthening plant cell walls and membranes, it is also a well-known secondary messenger to mitigate the abiotic stress in plants. Hao and Papadopoulos (2004) reported that Ca^{2+} nutrition showed an encouraging effect on growth, fruit yield and quality of tomato. In *Arabidopsis*, cytoplasmic calcium levels increase rapidly in response to low temperature, largely due to an influx of calcium from extracellular stores (Polisensky, 1996). Holder and Cockshull (1990) clarified that Ca^{2+} deficiency in tomato reduces leaf size, causes necrosis of young leaves and yield loss. The low Ca^{2+} supply leads to blossom-end rot in the fruit of tomato (Saure, 2001). Separately, 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-physiology and fruit yield of tomato under cold injury at SAU campus during late transplanting.

As we know that tomato production in Bangladesh is largely affected due to adverse environmental conditions. To minimize the effect of these adverse conditions and to fulfill the current need of tomato for over population of our country, the yield of tomato needs to be increased through proper use of alleviating agents to alleviate the low temperature injury with late transplanting to increase the cropping intensity. However, to my knowledge little is known about the studies investigating the role of exogenous SA and Ca^{2+} in improving the morpho-physiology and yield of tomato under low temperature injury in Bangladesh.

OBJECTIVES

Therefore this study has been taken under consideration to achieve the following objectives:

- To examine the effects of low temperature injury on the morpho-physiology and yield of tomato variety BARI Tomato 15.
- To examine the independent effects of SA and Ca^{2+} on the morpho-physiology and yield of tomato variety BARI Tomato 15.
- To analyze the effectiveness of different combinations of SA and Ca^{2+} on alleviation of late transplanting-induced low temperature injury in tomato in relation to morpho-physiology and yield.

Chapter 2
Review of literature

CHAPTER 2

REVIEW OF LITERATURE

Tomato is globally cultivated for its fleshy fruits and known as protective food because of its special nutritive value and its wide spread production. Tomato is an important crop plant which is used as a vegetable all over Bangladesh. The proper management practices, time schedule and foliar application of plant growth regulators (PGRs) and micronutrient 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 Salicylic Acid (SA) and calcium (Ca^{2+}) on tomato. To facilitate the research works different literatures have been reviewed and presented in this chapter under the following headings.

2.1 Effect of different transplanting time on morpho-physiological attributes and yield of tomato:

Sing *et al.* (2005) conducted an experiment to study the effect of transplanting time and mulching on growth and yield of tomato. Among different dates of planting, early planting (l0th 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 (l0th December) planting date and mulching with black polyethylene treatment combination, which was significantly superior to all other treatment combinations.

More *et al*. (2013) conducted a field experiment to study the effect of transplanting dates and mulching on fruit characters, yield and quality of tomato. Among the different dates of transplanting, early planting (5th November) recorded highest fruit yield and it was vice-versa in case of late transplanting date (15th December).

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 l3-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.

According to Chen *et al.* (1999) seedlings of tomato (*Lycopersicon esculentum* Mill.) and cabbage (*Brassica oleracea* L. var. Capitata) were planted in 240-cell plug trays in the greenhouse and subjected to irrigation with water at different temperatures once a day. Irrigation with cold (5 to 15 °C) water reduced stem length of tomato by 28% to 32% in comparison with irrigation with water at room temperature (27.5 to 30.5°C). Use of water at 10°C did not affect total shoot dry weight but increased the shoot dry weight per centimeter of stem. Irrigation with water at 5°C reduced stem length of cabbage seedlings 40%, but use of water at 10 and 15°C did not. Both shoot and root dry weights were increased by irrigation with water at 10°C. These results demonstrate that irrigation with cold water provides an effective method for improving the quality of plug-grown seedlings.

Hossain *et al.* (2014) carried out an experiment 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, where as sowing on October 30 resulted in delayed flowering (71.73 days) and fruit harvesting (140.67 days), respectively. Number of fruits per plant 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 Tomato-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.

Madhumathi and Sadarunnisa (2013) set an experiment on transplanting of tomato during 15th of October recorded significantly higher number of fruits per plant (33.31), yield per plant (1.25 kg), fruit size (length, diameter and volume), fruit weight (42.63 g), pulp content (54.01 %), ascorbic acid (20.81 mg/l00 g pulp) and number of seeds per fruit (192.21) over other dates of planting. Among the varieties, maximum number of fruits per plant, yield per plant, titrable acidity, ascorbic acid content, number of seeds per fruit and seed weight per fruit 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.

Ahammad *et al.* (2009) carried out 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^{-1}). The variety BARI Tomato 5 also showed potential fruiting capability during late winter season and February 01 planting produced 11 t ha^{-1}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.

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

Adelana (1976) observed that the earliest planting of tomato seedlings resulted in greater leaf area, higher yield and number of fruits per plant and greater average fruit weight than later planting.

Sanjoy (1999) reported 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 Iycopersicum*) 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.

Robert *et al*. (1990) conducted a research where tomato seedlings (*Lycopersicon esculentum* Mill. 'Sunny') were exposed to cyclic cold stress at 2 ± 1^0C, then to 29 ± 6^0C in a greenhouse before being transplanted to the field. Cold-stressed seedlings were transplanted when the risk of ambient cold stress was negligible. In the first year of a 2-year study, transplants were exposed to 2^0C for 3, 6, or 12 hours for 1, 3, or 6 days before field planting. In the second year, transplants were exposed to 2^0C for 6, 12, or 18 hours for 4, 7, or 10 days before field planting. In the first year, cold stress generally stimulated increases in seedling height, leaf area, shoots and root dry weights but decreased chlorophyll content. In the second year, all seedling growth characteristics except leaf area and plant height were diminished in response to longer cold-stress treatment. In both years, earliness, total productivity, and quality were unaffected by any stress treatment. Therefore, cold stress occurring before transplanting has a negligible effect on earliness, yield, or quality.

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

Wurr (1986) reported that Lettuce crops of cv Saladin were grown from transplants raised in Techniculture plugs with a volume of only 4 ml. The transplants were raised at day/night temperatures of 20/10, 15/10 and 15/5°C, fed with nutrients either every two or every seven days and transplanted at three different ages in 1983 and 1984. In both years less than 1% of transplants failed to establish. In 1983 but not in 1984, 2-day feeding produced heavier heads than 7-day feeding. There was no effect of raising temperature on head weight at maturity in either year. In 1983 head weight decreased with the use of older transplants, whereas the reverse was true in 1984. The use of the youngest transplants gave the lowest CV of head weight, while transplants up to 19 days old

appeared to offer greater stability of mean head weight, between years, than using older transplants.

Hossain *et al.* (1986) observed that early sowing enhanced total number of flowers per plant.

Taha *et al.* (1984) briefed that fruit size and fruit weight of early sowing was bigger than others sowing and the late sowing scored the lowest number of fruits per plant.

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 15^0C and 20^0C, which might over simplify the issue.

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

Omara (1995) said that maximum fruit size, average fruit weight and number of fruits per plant of tomato during early sowing date.

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

2.2 Effect of plant growth regulator Salicylic Acid (SA) and macronutrient calcium (Ca^{2+}) on morpho-physiological attributes and yield of different crops including tomato:

Kazemi (2014) conducted experiment to study the effect of salicylic acid and methyl jasmonate as pre- harvest treatments on the tomato vegetative growth, yield and fruit quality. The experiment was completely randomized experimental design with four replications. These factors included salicylic acid in 2 levels (0.5 and 0.75 $mmolL^{-1}$) and methyl jasmonate in 3 levels (0.25, 0.5 and 0.75 $mmolL^{-1}$) applied on tomato. Results indicated that salicylic acid (0. 5 $mmolL^{-1}$) and methyl jasmonate (0.25 $mmolL^{-1}$) either alone or in combination (0.5 $mmolL^{-1}$ SA+ 0.25 $mmolL^{-1}$ MJ) increased vegetative and

reproductive growth, yield and chlorophyll content. The application of salicylic acid (0. 5 $mmolL^{-1}$) alone significantly increased the leaves-NK content and dry weight and decreased the incidence of blossom-end rot, but methyl jasmonate application alone or in combination had not significant effect on blossom-end rot and leaves-NK content. The TSS, TA and vitamin C content of tomato fruit had significantly affected by the application of salicylic acid and methyl jasmonate either alone or in combination (0.5 $mmolL^{-1}$ SA+ 0.25 $mmolL^{-1}$ MJ). Application of salicylic acid with methyl jasmonate improved the yield contributing factors that resulted in significant increase in tomato fruit yield.

Zhao-Min *et al*. (2013) observed the effects of SA on tolerance of tomato seedlings to cold stress were studied, with the tomato seedlings at three-leaf stage treated with a series of concentration of salicylic acid (SA) (0.5, 2.0, and 4.0 mmol/L). The results showed that SA could enhance the tolerance of tomato seedlings to cold stress, with the most effective for the concentration of SA at 2.0 mmol/L. Compared to non-treated tomato seedlings with cold stress, the rate of electrolyte leakage of tomato seedlings could be detected in the leaves of tomato seedlings treated with the concentration of SA at 2.0 mmol/L for 4 days after cold stress, which was significantly lower than other ones. While malondialdehyde (MDA) had a least increase of 73.01%. The content of soluble sugar had a highest increase of 87.35%. Chlorophyll content had a decrease of 16.47%. Therefore, the results suggested that tolerance of tomato seedlings to cold stress could be increased after a pre-treatment with SA at concentration of 2.0 mmol/L.

A field experiment was conducted by Mohammad (2013) to investigate the effect of seed presoaking of shikimic acid (30, 60 and 120 ppm) on growth parameters, fruit productivity and quality, transpiration rate, photosynthetic pigments and some mineral nutrition contents of tomato plants. Shikimic acid at all concentrations significantly increased fresh and dry weights, fruit number, average fresh and dry fruit yield, vitamin C, lycopene, carotenoid contents, total acidity and fruit total soluble sugars of tomato plants when compared to control plants. Seed pretreatment with shikimic acid at various doses induces a significant increase in total leaf conductivity, transpiration rate and photosynthetic pigments (Chl.a, chl.b and carotenoids) of tomato plants. Furthermore, shikimic acid at various doses applied significantly increased the concentration of nitrogen, phosphorus and potassium in tomato leaves as compared to control non-treated tomato plants. Among all doses of shikimic acid treatment, it was found that 60 ppm

treatment caused a marked increase in growth, fruit productivity and quality and most studied parameters of tomato plants when compared to other treatments. On the other hand, no significant differences were observed in total photosynthetic pigments, concentrations of nitrogen and potassium in leaves of tomato plants treated with 30 ppm of shikimic acid and control plants. According to these results, it could be suggested that shikimic acid used for seed soaking could be used for increasing growth, fruit productivity and quality of tomato plants growing under field conditions.

Javaheri *et al*. (2014) studied the effects of salicylic acid on some quality characters of tomato different concentration of salicylic acid (10^{-2}, 10^{-4}, 10^{-6}, 10^{-8} molar and control) in seedling stage as foliar replication. Measured characters was including (number of panicle in a bush, yield, fruit number in panicle, fruit number in bush, fruit weight and fruit diameter). Obtained results of this study show that salicylic acid significantly affected number of panicle in a bush, yield, fruit number in panicle, fruit number in bush, fruit weight and fruit diameter. Among foliar application, the highest rate of tomato yield with mean of 3059.5 g obtained in SA3 (SA at 10^{-6} M), highest numbers of panicle in tomato bushes with mean of 31.25 measured in SA1 (SA at 10^{-2} M). Highest fruit number in panicle and highest fruit number in bush obtained by mean of 3.5 and 66.75 in SA1 (SA at 10^{-2} M), respectively and minimum amount of all this characters was recorded in control treatment and the highest amount of fruit weight and also fruit diameter was measured in control treatment with mean of 61.50 g and 51.75 mm, respectively.

Salem *et al*. (2013) held three experiments (laboratory, field and pots) those were conducted at Giza Agric. Res. Station, ARC, Egypt, during the two successive summer seasons 2012 and 2013. Seed of teosinte variety (local) were primed in five concentrations of salicylic acid (0.4, 0.6, 0.8, 1.0 and 1.2 g/L) for 24 hours, as well as control with non-priming. The aims of this study was to determine the best level of salicylic acid of pre-sowing treatment for teosinte seeds to improve germination performance, germination speed, seedling characters, anti-oxidant enzyme activity and forage yield. A completely randomized design (CRD) at laboratory experiment, a randomized complete block design (RCBD) at field experiment and a split plot design at pot experiment with four replications were used. The results showed that seed priming with 0.6 g/L salicylic acid gave the highest germination speed, germination percentageage, shoot and radical length and increased plant leaf area at pot experiment. And it increased fresh and dry forage yield fed, plant height, number of tillers plant, number of leaves plant and stem diameter of teosinte plants at the field experiment.

Bondok (2013) completed an experiment aimed to investigate the effect of foliar application with salicylic acid (2 mM/L) alone or combined with chitosan (0.1%) with or without TMV inoculation on improving resistance, growth, productivity and quality of tomato Hybrid Super Jackal F. as tomato (*Solanum lycopersicum* L.) plants are considered sensitive to tomato mosaic virus especially during the reproductive growth phase. The 1 study was conducted in the Experimental Farm, Faculty of Agriculture, Ain Shams University, Shoubra ElKheima, Egypt, during the two growing summer seasons of 2012 and 2013. All inoculated plants with TMV after 14 and 30 days from foliar application with combination of salicylic acid and chitosan, exhibited symptoms later and less severe than the plants inoculated with TMV in the other treatments. The SA plus CH foliar application without TMV inoculation gave the highest significant values of vegetative growth in both seasons. Combination treatment of SA plus CH increased significantly N, P, K, Fe and Zn concentration. This treatment was also effective in increasing tomato yield compared with treatment of infection alone. Our results showed that SA or CH alone or combined significantly increased ascorbic acid concentration compared with control treatment.

Kazemi (2013) conducted an experiment in order to study effect of salicylic acid and calcium foliar application on growth, yield and yield components of strawberry plants as a factorial in completely randomized experimental design with four replications. These factors included of salicylic acid in 3 levels (0.25, 0.5 and 0.75 mM) and calcium in 2 levels (2.5 and 5 mM) spray on strawberry. Results showed that salicylic acid (0.25 mM) and calcium chloride (2.5 mM) spray either alone or in combination (0.25 mM SA+ 2.5 mM Ca^{2+}) affected on vegetative and reproductive growth, significantly. Mean comparisons indicated yield, and quality of strawberry plants was improved in low salicylic acid and calcium chloride concentration. In Finally, salicylic acid and calcium chloride application can be helpful for yield improvement and prevent of decreasing yield.

Sahu *et al*. (2007) investigated the effect of various concentrations of salicylic acid (SA) on the growth, pigment content and the activity of antioxidants in the laboratory grown wheat plants. The root and shoot growth was affected at higher concentration of SA in early days of growth. The activities of catalase (CAT), ascorbate peroxidase (APX) and guaicol-specific peroxidase (POX) declined with the application of SA (50, 500 and 1000 µM), the decrease being more pronounced with the increase in SA concentrations both in the root and leaf tissues. On the other hand superoxide dismutase (SOD) activity

increased with the application of SA. At low concentrations, SA has no effect on the activities of these enzymes *in vitro*. Salicylic acid at higher concentrations (5 and 10 mM) though inhibited CAT activity; the activities of APX and POX remain unchanged. High concentration of SA increased the level of H_2O_2 and malondialdehyde both in root and leaf tissues. Thus, SA though has been reported to be a signal molecule for inducing various physiological and morphological attributes in plants, this study indicated the negative effect of the compound on growth and the activity of major enzymatic antioxidants.

Shakirova (2007) recorded enhanced germination and seedling growth in wheat, when the grains were subjected to pre-sowing seed-soaking treatment in salicylic acid.

Hussein *et al*. (2007) conducted a pot experiment where they sprayed salicylic acid to the foliage of wheat plants, irrigated with Mediterranean sea water and reported an enhanced productivity due to an improvement in all growth characteristics including plant height, number and area of green leaves, stem diameter and dry weight of stem, leaves and of the plant as a whole. Moreover, the plants that received treatment with SA had more proline content.

Eraslan *et al*. (2007) carried out an experiment to elucidate the effect of exogenously applied salicylic acid on growth, physiology and antioxidant activity of carrot plant. The results of their experiment revealed that salicylic acid significantly enhanced the overall growth, root dry mass, sulphur concentration, carotenoids and anthocyanin contents with a concomitant enhancement of total antioxidant activity of shoot and that of storage root. The SA application also regulated the proline accumulation both in shoot and storage root.

Pancheva et al. (1996) reported that a delayed leaf emergence and a decrease in the growth of leaves and roots of barley plants in a dose-dependent manner, when salicylic acid was applied exogenously.

Hayat and Ahmad (2007) found that exogenous application of SA helped to shift the nutrient status leading to a decreased uptake of phosphate and potassium by roots and this decrease was found to be dependent on pH, suggesting a higher activity of protonated form of SA. The soil nutrient solution enters the plant body through its roots and besides some other factors a healthy root system plays a key role in enhancing the growth and

productivity of plants.

Shakirova (2007) reported that wheat seedlings increased the size and mass of plantlets significantly when salicylic acid applied exogenously to them, compared to the untreated control.

A trial was conducted by Sadra et al. (2013) to study the effects of SA application and irrigation intervals on yield and yield components of coriander using a split-plot layout in a randomized complete block design with three replications. Two levels of irrigation including irrigation every 4th day & irrigation every 8th day were compared in main plots. Four levels of salicylic acid (SA) including: 0, 0.01, 0.1 and 1 mM of SA were assigned in sub-plots. Results showed that the reduction of irrigation interval from 8 to 4 days statistically improved umbels number per plant, seeds number per plant and seed yield. Application of lower doses of SA increased the number of umbels and seeds per plant and seed yield. Evaluation of interaction effects of irrigation and SA revealed that in optimal conditions of water availability the crop was more responsive to the lowest dose of SA, on the other hand in water deficit conditions the median dose of SA (0.1 mM) was more efficient in improving seed yield, indicating the positive and enhancing role of SA under water deficiency stress conditions.

This study was conducted by Javaheri *et al*. (2011) with the objective to determine the effects of salicylic acid on yield quantity and quality of tomato. The experiment was arranged in randomized complete blocks design with four replications. Foliar application of five concentrations of salicylic acid (0, 10-^{2}, 10^{-4}, 10^{-6}, 10^{-8} M) were used. Results showed that application of salicylic acid affected tomato yield and quality characters of tomato fruits so that tomato plants treated with salicylic acid 10^{-6} M significantly had higher fruit yield (3059.5 g per bush) compared to non-treated plants (2220 g per bush) due to an increase in the number of bunch per bush. Fruit of tomato plants treated with salicylic acid 10^{-2} M significantly had higher vitamin C (32.5 mg per 100 g of fruit fresh weight) compared to non treated plants (24 mg per 100g fruit fresh weight). Salicylic acid concentration 10^{-2} M also increased the diameter of fruit skin (0.54 mm) more than two fold compared to control (0.26 mm). Fruit Brix index of tomato plants treated with salicylic acid 10^{-2} M significantly increased (9.3) compared to non-treated plants (5.9). These results suggest that foliar application of salicylic acid may improve quantity and quality of tomato fruits.

Ding *et al*. (2001) stated that changes in heat shock protein (HSP) gene expression induced by vapor application of methyl jasmonate (MeJA) and methyl salicylate (MeSA) in tomato fruit were investigated and compared to the well-described heat shock response. Northern hybridization experiments involving six cDNAs, encoding class I and II tomato small HSPs (sHSPs) and three members of HSP 70 family, showed that accumulation of class I and II sHSP mRNAs was increased significantly by MeJA and MeSA. When the treated fruits were transferred to low temperature, class I and II mRNA levels initially decreased, but then subsequently increased. Accumulation of HSP transcripts was also observed in non-treated fruit between 7 and 14 days at low-temperature storage, but all decreased to undetectable levels after 21 days. Following MeJA and MeSA treatments, the transcripts of HSP 70 family accumulated to higher levels than following the heat treatment. MeJA- and MeSA-treatments were clearly shown to alleviate chilling injury (CI), whereas tomato fruit stored at 5°C without pretreatment developed typical symptoms and severe decay. These results demonstrated that MeJA and MeSA induced the accumulation of sHSP transcripts in tomato. The increased transcript abundance of HSPs, especially class II sHSPs, was correlated with protection against CI.

Jazi *et al*. (2011) conducted a research; the objective of this research was to evaluate the effect of different concentrations of salicylic acid to enhance the characteristics of *Brassica napus L*. under lead stress. Factorial experimental design was arranged in a completely randomized block design with three replications. Treatments were combination of 7 levels application of lead (0, 0.25, 0.5, 0.75, 1, 1.5 & 2 mM) and 2 concentrations of salicylic acid (5 & 10 μM). The results revealed that increasing lead concentration reduced root and shoot length, leaf area, and root and shoot dry weight, root and shoot fresh weight, specific leaf area (SLA) and leaf weight ratio (LWR). Application of salicylic acid on the other hand, significantly increased the studied traits, although specific leaf weight (SLW) and leaf water content area (LWCA) were significantly increased with an increase in the concentration of $Pb(NO3)_2$ ($P<0.01$).Generally salicylic acid applications improved plant growth parameters of *Brassica napus L*

Plasencia *et al.* (2011) reported that in recent years salicylic acid (SA) has been the focus of intensive research due to its function as an endogenous signal mediating local and systemic plant defence responses against pathogens. It has also been found that SA plays a role during the plant response to abiotic stresses such as drought, chilling, heavy metal toxicity, heat, and osmotic stress. In this sense, SA appears to be, just like in mammals, an 'effective therapeutic agent' for plants. Besides this function during biotic and abiotic

stress, SA plays a crucial role in the regulation of physiological and biochemical processes during the entire lifespan of the plant. Here, the evidence that supports the role of SA during plant growth and development is reviewed by comparing experiments performed by exogenous application of SA with analysis of genotypes affected by SA levels and/or perception.

Yildirim *et al*. (2009) conducted a research to determine the effect of foliar salicylic acid (SA) applications on fruit quality, growth and yield of tomato under greenhouse conditions in 2006 and 2007. In the study, fruit diameter, fruit length, fruit weight, fruit number per plant, Vitamin C, pH, Total Soluble Solids (TSS), titratable acidity (TA), stem diameter, leaf dry matter ratio, chlorophyll content, early yield and total yield were determined. Tomato plants were treated with foliar SA applications at different concentrations (0.00, 0.25, 0.50 and 1.00 mM). SA was applied with spraying four times during the vegetation at 10-day intervals two weeks after planting. In the study, it was determined that foliar applications of SA showed positive effect on some fruit characteristics, plant growth, chlorophyll content in leaves, early yield and total yield. SA treatments had no effect on pH, AA and TA of tomato. Total soluble solids (TSS) increased with foliar SA applications. The greatest stem diameter, leaf dry matter and chlorophyll content were obtained from 0.50 mM SA treatment. SA treatments increased the early yield of tomato compared to the control. The yield of tomato was significantly influenced by foliar SA applications. The highest yield occurred in 0.50 mM SA treatment. According to our results, applications of 0.50 mM SA should be recommended in order to improve yield.

Saavedra *et al*. (2007) recorded in their experiment that the fruit yield, in cucumber and tomato, enhanced significantly when the plants were sprayed with lower concentrations of salicylic acid.

Hegazi and El- Shraiy (2007) found that foliar application of salicylic acid generally had a positive effect on vegetative growth parameters (plant height, leaves no., shoots and roots fresh dry weight) of common bean as compared to the control.

Gharib (2006) and Khan *et al*. (2003) reported that application of SA enhanced photosynthesis rate, so that leaf area has been increased.

Hayat *et al*. (2005), the leaf number, fresh and dry mass per plant of wheat seedlings raised from the grains soaked in lower concentration (10^{-5} M) of salicylic acid, increased

significantly.

Korkmaz (2005) reported that SA at low temperatures increased the germination percentageage of *Capsicum annum* seeds.

Larkindale and Huang (2004) pointed out that the enhanced heat tolerance in plants of *Agrostis stolonifera*, pre-treated with salicylic acid was due to the protection of plants from oxidative damage. These authors further reported that the pre-treatment with salicylic acid had no effect on POX activity, whereas, the CAT activity declined, compared to control. However, the treatment enhanced the activity of enzyme ascorbate peroxidase.

In a study, carried out by Chakraborty and Tongden (2005), it was reported that the heat stress induced membrane injury in the plants of *Cicer arietinum* which was significantly reduced by the application of SA, compared to the heat acclimatized and untreated control. The treatment also enhanced the protein and proline contents significantly with a concomitant induction of various stress enzymes viz. POX and APX. However, the CAT activity was found to be reduced.

Khodary (2004) observed a significant increase in growth characteristics, pigment contents and photosynthetic rate in maize, sprayed with SA. He also found that the exogenous SA application also enhanced the carbohydrate content in maize.

Hew (1987) observed that SA, in association with sucrose, enhanced flower opening in *Oncidium*.

Sandoval-Yapiz (2004) said that lower concentrations of salicylic acid enhanced rooting in *Tagetus erecta.* Promotion was generated in shoot system as well, when the plants of *Tagetus erecta* were treated with lower concentrations of salicylic acid, thereby enhancing the productivity of plants.

Herrera-Tuz (2004) and Martin-Mex *et al.* (2005b) observed that promising results were obtained when plants of *Carica papaya* were treated with salicylic acid which showed a significantly higher fruit setting.

Hegazi and El- Shraiy (2007) reported that acetyl salicylic acid treatment promoted plant growth, plant height and no. of leaves per potato plant.

Tasgin *et al*. (2003) reported that exogenous SA not only provided protection against heat and cold stresses, but was equally beneficial in providing tolerance against freezing (Frost) injury to winter wheat.

Fariduddin *et al*. (2003) reported that the dry matter accumulation was significantly enhanced in Brassica juncea, when lower concentrations of salicylic acid were sprayed. However, higher concentrations of SA had an inhibitory effect.

Khan *et al*. (2003) reported that SA and its close analogues enhanced the leaf area and dry mass production in corn and soybean.

Martin-Mex *et al*.(2003, 2005a).found in their observation that different plant species including ornamental plant *Sinningia speciosa* flowered much earlier as compared to the untreated control, when they received an exogenous foliar spray of salicylic acid.

Kang *et al*. (2003b) recorded that the increase in the activities of antioxidant enzymes, SOD, CAT and APX following SA treatment was related to H_2O_2 metabolism produced by chilling, thereby providing tolerance against the stress.

Exogenous salicylic acid potentially alleviates the damaging effects of low temperatures in rice and wheat (Szalai *et al*., 2002; Tasgin *et al*., 2003), bean (Senaratna *et al*., 2000) and banana (Kang *et al*., 2003a). Pre-treatment with salicylic acid activated various antioxidant enzymes in maize (Janda *et al*., 1999, 2000) and banana (Kang *et al*., 2003b) exposed to chilling stress.

Kang and Saltveit (2002) observed that the chilling injury manifested in the form of electrolyte leakage in leaves was significantly reduced following the application of lower concentrations of salicylic acid to maize, cucumber and rice plants.

Rajasekaran *et al*. (2002) observed that SA or acetyl salicylic acid enhanced the germination percentage of seeds.

Shehata *et al*. (2001) and Abdel-Wahed *et al*. (2006) reported that the application of salicylic acid, or acetyl salicylic acid or other analogues of SA, significantly increased yield and its components of maize and wheat plants.

Aldesuquy & Ibrahim (2000) recorded the effect of shikimic acid on growth parameters, total leaf conductivity, transpiration, photosynthetic pigments, 14C assimilation and productivity of Vigna sinensis (Fabaceae - Phaseoleae) plants was studied. Shikimic acid

application led to an increase in fresh and dry weights of cowpea plants and enhances leaf expansion as well as the root length and plant height. Seed pretreatment with shikimic acid at various doses induces a marked increase in total leaf conductivity and transpiration rate at different stages of growth. Shikimic acid at all concentrations was found to stimulate the production of Chl.a, Chl.b, Carotenoids and 14C fixation during leaf growth and development. Furthermore, shikimic acid at various doses applied improved yield and yield components of cowpea plants by increasing the number of pods/plant, length of pod, number of seeds/pod, seed biomass and 100-seed weight. The protein content of yielded seeds was increased in response to shikimic acid treatments. On the other hand, shikimic acid at all concentrations significantly decreased the polysaccharide content of developed cowpea seeds.

Kumar *et al*. (2000) in a comparative analysis studied the cumulative effect of SA with that of GA, Kinetin, NAA, ethral and chloro chloro chloride (CCC), and found a synergistic effect of SA and GA on flowering compared to other combinations of hormones.

Janda *et al*. (1998, 2000) & Horvath *et al*. (2002) opinioned that salicylic acid, its analogues like benzalde-hyde aspirin or coumaric acid also had a protective role against chilling stress in maize plants.

Senaratna *et al*. (1999) investigated that physiologically active concentrations of Salicylic acid (SA) and its derivatives can confer stress tolerance in plants was evaluated using bean and tomato. Plants grown from seeds imbibed in aqueous solutions (0.1- 0.5 mM) of Salicylic Acid or acetyl salicylic acid (ASA) displayed enhanced tolerance to heat, chilling and drought stresses.

Kumar *et al*. (1999) observed that foliar application of salicylic acid to soybean enhanced the flowering and pod formation.

Dat *et al*. (1998) observed that foliar spray of lower concentrations of salicylic acid conferred heat tolerance to mustard. Salicylic acid treatment, accompanied with hardening at 45^0C for 1 h enhanced the H_2O_2 level and also reduced the CAT activity, thereby increasing the potential of plants to withstand the heat stress. A similar response was observed in potato plantlets, raised from the cultures, supplemented with lower concentrations of acetyl salicylic acid.

Janda *et al*. (1997, 1999) reported that maize plants showed an enhanced cold tolerance when grown in hydroponic solutions & supplemented with 0.5 mM of salicylic acid. The treatment positively affected various parameters of fluorescence and lowered those associated with electrolyte leakage. A decline in CAT activity with a concomitant enhancement in the activities of glutathione reductase and guaiacol peroxidase was also observed.

Gutierrez-Coronado *et al*. (1998) in their experiment found that foliar application of salicylic acid significantly increased the length of roots in soybean.

Aristeo-Cortes (1998) observed and expressed his opinion that salicylic acid is one of the most important, effective and cost beneficial phytohormone that has the potential to enhance the root growth in economically important vegetables and salads like *Daucus carota, Raphanus sativus and Beta vulgaris.*

Pancheva *et al*. (1996) reported that growth promoting response was generated in barley seedlings when sprayed with salicylic acid.

Khurana *et al*. (1987) reported that exogenous application of aspirin (a close analogue of SA) enhanced flowering in *Spirodela & Wolfia microscopica*).

In a study carried out by Saavedra *et al*. (1975), treatment of bean explants with aspirin, which is a close analogue of salicylic acid, enhanced rooting. Since then a lot of work was carried out to elucidate the effect of exogenous SA and other salicylates on rooting and thereby productivity in plants.

Cleland and Ajami (1974) reported that salicylic acid has been found to induce flowering in a number of plants, including Lemna.

Basu *et al*. (1969) observed that the rooting was enhanced in mungbean plants, following the treatment of salicylates.

Rab and Haq (2012) investigated the influence of $CaCl_2$ and borax on growth, yield, and quality of tomato 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 per plant, flowers per cluster, fruits per plant, 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 per plant and decreased the incidence of blossom end rot. Borax alone significantly enhanced the number of branches per plant, number of flowers per cluster, fruits per cluster, fruits per plant, 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 ern), branches per plant (7.21), flowers per cluster (32.36), fruits per plant (96.37), fruit weight (96.33 g), yield (21.33 t/ha), fruit firmness (3.46 $kgcm^{-2}$), 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 non-significant.

Hao *et al.* (2003) carried out an experiment on Tomato (*Lycopersicon esculentum* Mill.) with two concentrations of calcium (150 and 300 mg/L) in combination with four concentrations of magnesium (20, 50, 80 and 110 mg/L) 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) 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). High Ca concentration reduced fruit firmness but did not affect fruit size and leaf photosynthesis. Plants grown at 20 mg/L 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 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 Mg was higher than at 50 mg/L Mg concentration. Fruit resetting in mid-season was affected by nutrient treatments, being the least at 300/50 mg/L 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. The Mg concentration may be started at 50 mg/L and gradually increased to 80 mg/L 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). 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.

A study was conducted 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 per plant, flowers per cluster, fruits per plant, fruit weight, fruit firmness and total soluble solid content of the fruit. Results showed that humic acid (30 ppm) 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), 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.

An experiment was set 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 flowers $cluster^{-I}$, 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 (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 conducted 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 NaCI concentration (75 mM). The treatments were: (i) control (C), nutrient solution alone; (ii) salt treatment (C + S), 75 rnM NaCI; (iii) salt plus calcium treatment 1 (C+S+Cal), 75 mM NaCI plus additional mixture of 2.5 rnM $CaS0_4$ in nutrient solution; (iv) salt plus calcium treatment 2 ($C+S+Ca^{2+}$), 75 mM NaCI plus additional mixture of 5 rnM $CaS0_4$ 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 $CaS0_4$ 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 held 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, zincidence 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 (l %) 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.

White *et al.* (2003) observed 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 00ra 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 specificity 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.

Islam *et al.* (1987) found 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.

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.

Arshi *et al.* (2006) reported that 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

Yaseen *et* al. (2006) 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_4 (up to 23700 nmol/kg 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_4 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.

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

Adams *et al.* (1988 and 1992) suggested 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 presented under the following headings.

3.1 Experimental site

The experiment 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 $90^0$35'E longitude at an altitude of 8.6 meter above the sea level (Anonymous, 2004), which have been shown in the Appendix I.

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 II.

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 collected from the weather station of the Sher-e-Bangla Agricultural University regarding temperature prevailed at the experimental site during the cropping season November 2013 to April 2014 have been presented in Appendix III.

3.4 Planting materials

Seedlings of 25 days of BARl 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)
(The lowest temperature 19^0C)

20 December 2013
Second transplanting time (T_2)
(The lowest temperature 15^0C)

30 December 2013
Third transplanting time (T_3)
(The lowest temperature 10^0C)

Factor (B): Different combination of salicylic acid (SA) and calcium (Ca^{2+}) as alleviating agent of low temperature injury

SA= 0 mM and Ca^{2+}= 0 mM marked as A_0

SA = 0.25 mM and Ca^{2+}= 0 mM marked as A_1

SA = 0 mM and Ca^{2+}= 5 mM marked as A_2

SA = 0.25 mM and Ca^{2+}= 5 mM marked as A_3

SA = 0 mM and Ca^{2+}= 10 mM marked as A_4

SA = 0.25 mM and Ca^{2+}= 10 mM marked as A_5

Total 18 treatment combinations were as follows:

T_1A_0	T_2A_0	T_3A_0
T_1A_1	T_2A_1	T_3A_1
T_1A_2	T_2A_2	T_3A_2
T_1A_3	T_2A_3	T_3A_3
T_1A_4	T_2A_4	T_3A_4
T_1A_5	T_2A_5	T_3A_5

3.6 Design and layout of the experiment

The two factorial experiment was laid out in Randomized Complete Block Design (RCBD) with three transplanting time and six different combinations of SA and Ca^{2+}. Three replications were maintained in this experiment. The total number of unit plots was 54 (3x18). Each plot was 1.8 m x 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 x 60 cm. The layout of the experiment is presented in Appendix IV.

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 x 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. Seeds were sown in the seedbed at 15 November, 25 November and 05 December, 2013 respectively to 25 days old seedlings for T_1, T_2, T_3 treatment time respectively 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 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. Before transplanting a light irrigation was done to moisture the dry soil and to adjust the seedlings properly to the soil. 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 SA and Ca^{2+}

Salicylic acid acid (SA) and calcium (Ca^{2+}) were applied to the tomato plant according to the treatments. As treatment combinations, different concentration of SA and Ca^{2+} were sprayed exogenously with 0.01% of Tween 20 by a hand sprayer in the early morning at 15, 30 and 45 DAT. The Ca^{2+} was used in the form of $CaSO_4.5H_2O$ of Merck India and SA of Merck Specialties Private Limited, India. Before using SA and Ca^{2+} detergent powder solution were used as an adhesive material.

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 and rope 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 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 l0G 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 @2gm/L of water at the early vegetative stage. Ridomil gold was also applied @ 2 gm/L 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 10 March 2014 and was continued up to 16 April 2014.

3.14 Recording of Data

Experimental data were recorded at 20, 40 and 60 days after transplanting (DAT) 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 flower clusters plant^{-1}
6. Number of flowers plant^{-1}
7. Number of fruits plant^{-1}
8. Fruit diameter (cm)
9. Fruit length (cm)
10. Fruit weight plot^{-1}(kg)
11. Yield (t/ha)
12. Low temperature injury alleviation (%)

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 20, 40 and 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 20, 40 and 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 chlorophyll content SPAD meter (CCM-200, Opti-Science, USA). It was done at 40 DAT. At each evaluation the content 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 flower clusters plant^{-1}

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

3.15.6 Number of flowers plant^{-1}

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

3.15.7 Number of fruits plant^{-1}

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

3.15.8 Fruit diameter (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 Fruit length (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.

3.15.10 Fruit weight (kg plot^{-1})

Fruit weight of tomato plot^{-1} was calculated by the following formula:

$$\text{Weight of individual fruit (gm)} = \frac{\text{Total weight of fruits}}{\text{Total number of fruits}}$$

and expressed in kilogram (kg) from gram (gm).

3.15.11 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.

3.15.12 Low temperature injury alleviation (%)

Alleviation percentage was calculated by multiplying each Yield (t ha^{-1}) one by one with 100 divided by Yield (t ha^{-1}) of control (T_1A_0).The formula was–

$$\text{Alleviation (\%)} = \frac{\text{Yield (t ha}^{-1}\text{) of treatment combination x 100}}{\text{Yield (t ha}^{-1}\text{) of control } (T_1A_0)}$$

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

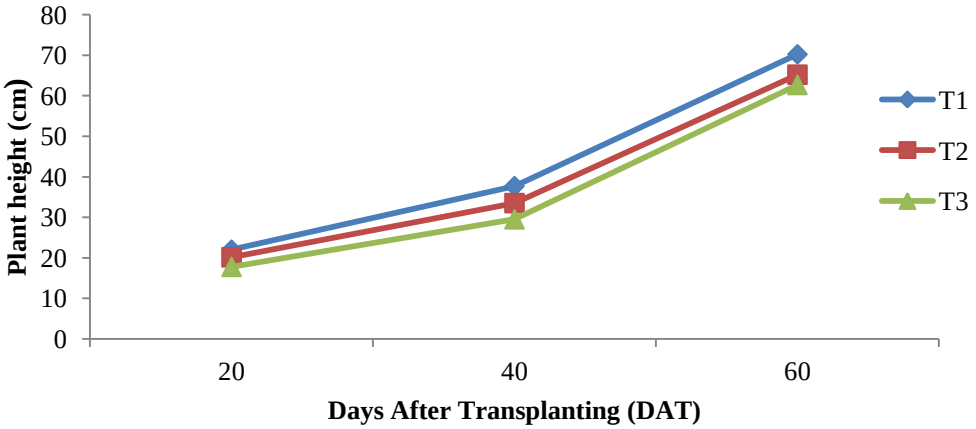
CHAPTER 4

RESULTS AND DISCUSSION

The present experimental results along and discussion in relation to induction of cold stress with different date of transplanting and sole or together foliar application of Salicylic acid (SA) and calcium (Ca^{2+}) have been illustrated and discussed in this chapter. Different morpho-physiology, yield contributing characters and yield of tomato responded with the corresponding treatments. The data about morpho-physiological parameters, yield contributing characters and yield of tomato have been presented in both Figures and Tables. The analysis of variance and corresponding degrees of freedom have been shown in Appendix.

4.1 Plant height (cm)

In this study, the effect of transplanting time of tomato in relation to decline of temperature reduced the plant height. The plant height varied significantly due to the effect of decrease of temperature; observed at 20, 40 and 60 DAT (Figure 1 and Appendix V). At 20 days after transplanting (DAT), the highest plant height (22.01cm) was recorded from the T_1 (10 December, 2013) and the lowest (17.78 cm) was recorded from T_3 (30 December, 2013). At 40 DAT, the highest plant height (37.70 cm) was recorded from the T_1 (10 December, 2013) and the lowest (29.52 cm) was recorded from T_3 (30 December, 2013). At 60 DAT, the highest plant height (70.22 cm) was recorded from the T_1 (10 December, 2013) and the lowest value (62.66 cm) was recorded from T_3 (30 December, 2013). These data showed that plant heights are gradually increasing at all transplanting time where as early transplanting shows maximum plant height than late transplanting. Here it is noted that, the seedlings height was gradually short to late showing (data not shown). Both the plants height and seedlings height were decreased due to decline the environmental temperature is shown in Appendix III. Previous results showed that late transplanting induced cold condition reduced the length of plant height (Chen *et al.,* 1999). These results are consistent with the finding of Lwahori *et al.* (1963) who stated that plant height decreased with decreasing trend of temperature. Recently, Srivastava *et al.* (2007) and More *et al*. (2014) reported that transplanting time has 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 transplanting.

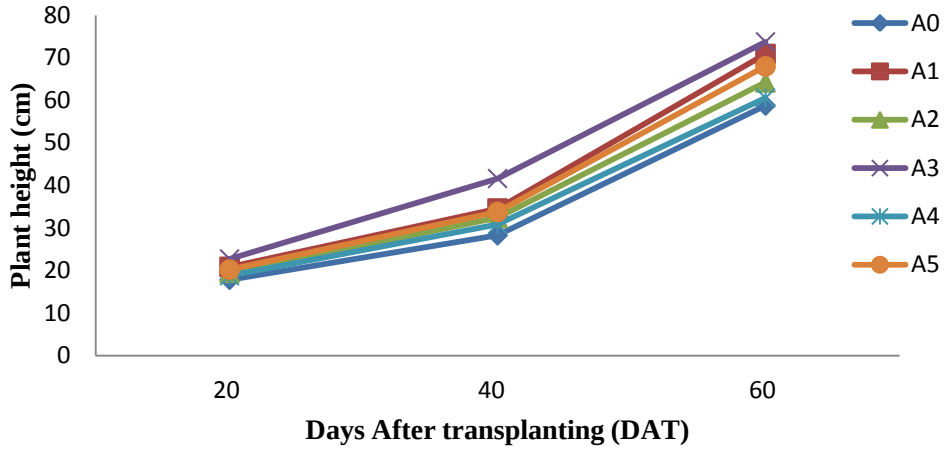


T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

Figure 1. Effect of transplanting time on plant height of tomato at different days after transplanting, $LSD_{0.05}$ = 0.6472, 1.816 and 1.714 for 20 DAT, 40 DAT and 60 DAT, respectively.

In this study, different combinations of SA and Ca^{2+} as 0 mM SA, 0.25 mM SA and 0 mM Ca^{2+}, 5 mM Ca^{2+}, 10 mM Ca^{2+} were used as an alleviating agent of cold injury to examine the adverse effect of late transplanting-induced low temperature injury on regulation of plant height of tomato at 20, 40 and 60 days after transplanting (DAT). The SA and Ca^{2+} showed a significant effect on the improvement of plant height to late planting induced low temperature injury (Figure 2 and Appendix V). At 20 DAT the highest plant height (22.71 cm) was recorded from A_3 (0.25 mM SA + 5 mM Ca^{2+}) and the lowest value (17.83 cm) was recorded from A_0 (0 mM SA + 0 mM Ca^{2+}). At 40 DAT, the plant height (41.62 cm) was found from A_3 whereas the lowest value (28.28 cm) was recorded from A_0. At 60 DAT, the highest plant height (73.74 cm) was recorded from A_3 and the lowest value (58.77 cm) was found from A_0 levels, this one was statistically identical with A_4 (60.55 cm). These experimental results of plant height showed that together application of SA and Ca^{2+} (A_3) increased the plant height of tomato compared to sole application of either SA or Ca^{2+} at early induction of cold injury (Appendix III). In contrast, 0.25 mM SA along with higher amount of Ca^{2+} 10 mM i.e. A_5 significantly produced lower plant height as compared to A_3 levels of SA and Ca^{2+}. These results suggest that higher doses of foliar application of Ca^{2+} inhibits the plant height whereas lower concentration of Ca^{2+} promotes the plant height with SA because the rate of photosynthesis may be reduced by higher cellular concentration of Ca^{2+} by regulating stomatal movement**.** These findings are in agreement with those of Javaheri *et al*. (2014),

Salem (2013), Kazemi (2012), Rab and Haq (2012) and Ilyas *et al*. (2014) who reported that both SA and Ca^{2+} independently increased the plant height of tomato.



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 2. Effect of different levels of salicylic acid and calcium on plant height of tomato at different days after transplanting, $LSD_{0.05}$ = 0.9152, 2.568 and 2.424 for 20 DAT, 40 DAT and 60 DAT, respectively.

The results of the combined effect between late transplanting induced low temperature injury and different does of SA and Ca^{2+} used as an alleviating agent of cold stress showed significant effect on plant height of tomato at 40 and 60 DAT except 20 DAT (Table 1 Appendix V). At 40 DAT, the plant height (45.61 cm) was found from T_1A_3 where the lowest value (33.46 cm) was recorded from T_1A_0. At 60 DAT, the highest plant height (75.66 cm) was recorded from T_1A_3 which was statistically similar with T_1A_1 (73.41 cm) & T_1A_5 (72.41 cm) and the lowest value (64.06 cm) was recorded from T_1A_0. The first transplanting date (T_1) along with 0.25 mM SA 5 mM Ca^{2+} (A_3) i.e. T_1A_3 produced the highest value of plant height and the minimum value were found in third transplanting date (T_3) along with no SA and Ca^{2+} (T_3A_0) treatment combinations and suggest that the plant height failed to increase under cold stress because the temperature was comparatively lower during T_3 planting date (Appendix III). In addition, simultaneous application of SA and Ca^{2+} successfully improve the plant height at all days of transplanting including late transplanting. These findings are partially agreed with the findings of Chen *et al.* (1999) where they reported that plant height decreased with cold stress. Many authors like Kazemi & Salem (2013), Sadra *et al.* (2013), Jazi *et al*. (2011)

and Yildirim *et al*. (2009) also reported that SA and Ca^{2+} increased plant height in different crops such as strawberry, coriander, *Brassica napus* and cucumber as tomato.

Table 1: Combined effect of transplanting time and different combination of SA and Ca^{2+} levels on plant height of tomato at different days after transplanting

Treatment combination	Plant height (cm) at different days after transplanting (DAT)		
	20 DAT	40 DAT	60 DAT
T_1A_0	19.35 ef	33.46 defgh	64.06 ef
T_1A_1	22.87 ab	38.22 bc	73.41 ab
T_1A_2	22.12 bc	35.71 cdef	68.26 cd
T_1A_3	24.03 a	45.61 a	75.66 a
T_1A_4	21.14 cd	35.39 cdef	67.53 de
T_1A_5	22.53 abc	37.82 bcd	72.41 abc
T_2A_0	18.37 fg	29.36 hi	57.17 gh
T_2A_1	21.56 bcd	33.89 cdefg	69.85 bcd
T_2A_2	19.08 ef	32.23 fgh	63.39 ef
T_2A_3	23.11 ab	41.80 ab	73.44 ab
T_2A_4	18.64 efg	30.72 ghi	58.10 gh
T_2A_5	20.11 de	33.02 efgh	69.19 cd
T_3A_0	15.77 i	22.00 j	55.08 h
T_3A_1	18.18 fg	31.55 fgh	69.34 bcd
T_3A_2	17.21 ghi	29.22 hi	61.03 fg
T_3A_3	20.98 cd	37.45 bcde	72.13 abc
T_3A_4	16.56 hi	26.50 i	56.04 h
T_3A_5	17.98 fgh	30.43 ghi	62.36 f
LSD $_{(0.05)}$	1.585	4.449	4.198
Significant level	NS	*	*
CV (%)	4.85%	8.09%	4.88%

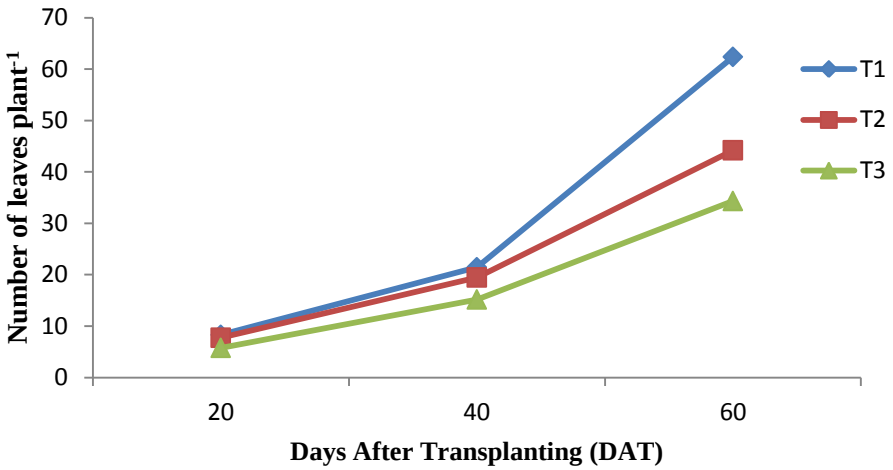
T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

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

4.2 Number of leaves plant^{-1}

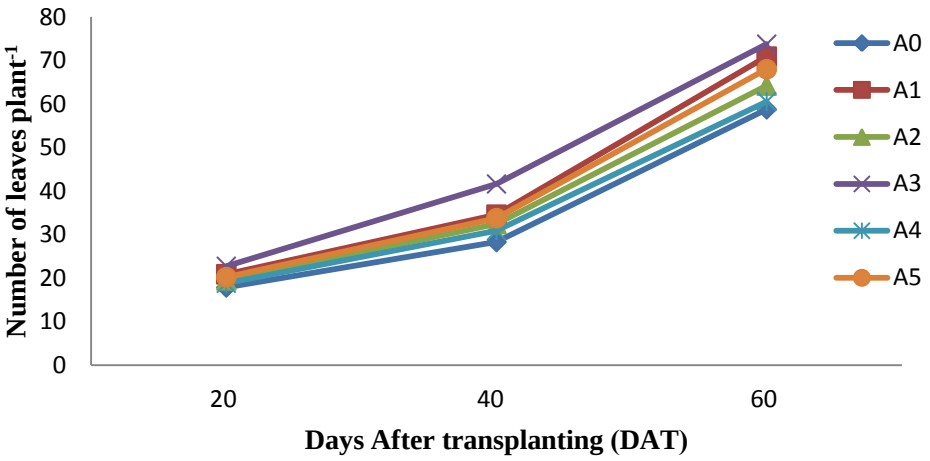
It is known to all that leaf is the main photosynthetic organ of plant along with the leaf number is a fundamental morphological character for plant growth and development. The number of leaves was counted to investigate the effect of different transplanting time on changes in the leaf number plant^{-1} of tomato at 20 DAT, 40 DAT and 60 DAT. Different days of transplanting showed a significant influence on the formation of leaves per tomato plant (Figure 3 and Appendix VI). In this experiment the number of leaves in each plant was decreased due to increasing level of low temperature stress. At 20 DAT, maximum number of leaves plant^{-1} (8.31) was found from T_1 and the lowest value (5.79) from T_3 transplanting time. At 40 DAT, the highest number of leaves (21.46) was recorded from T_1 and the lowest value (15.20) was found from T_3. At 60 DAT, the highest number of leaves (62.39) was recorded from T_1 whereas the lowest value (34.35) was recorded from T_3. These results indicated that highest number of leaves plant^{-1} found from early transplanting time whereas minimum number of leaves was produced to late transplanting time which created cold injury to plants (Appendix III). Hossain *et al.* (1986) reported that total number of leaves plant^{-1} was enhanced by early showing. Therefore, altogether the experimental results indicate that late planting-induced cold environmental conditions decreased the number of leaves plant^{-1} of tomato.



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

Figure 3. Effect of transplanting time on the number of leaves plant^{-1} of tomato at different days after transplanting, $LSD_{0.05}$ = 0.3610, 0.752 and 2.859 for 20 DAT, 40 DAT and 60 DAT, respectively.

Numerous authors reported that cell division promoted with plant growth regulators which influenced considerable stem elongation, bud and leaf formation etc. As an alleviator of cold stress, I used SA and Ca^{2+} which played a significant effect on the number of leaves plant^{-1} of tomato. Figure 4 and Appendix VI showed that number of leaves increased significantly with SA and Ca^{2+}. At 40 DAT, the highest number of leaves plant^{-1}10.78 was found from M_3 (0.25 mM SA + 5 mM Ca^{2+}), which was statistically similar with A_1 (10.19) & A_5 (9.92) where the lowest value 8.70 was recorded from A_0 (0 mM SA + 0 mM Ca^{2+}) which was statistically similar with A_2 (9.37) & A_4 (9.33). At 60 DAT, the highest number of leaves plant^{-1}(54.04) was recorded from A_3 was statistically similar with A_1 (51.11) and the lowest value (41.30) was found from A_0 levels and this one is also statistically similar with A_2 (44.74) & M_4 (43.00). Thus these results suggest that simultaneous application of SA and Ca^{2+} produced higher number of tomato leaves. This fact was supported by many authors like Kazemi (2013), Salem (2013), Zamaninejad *et al.* (2013), Boroumand *et al.*, El-Zohiri *et al*. (2009) and Sahu *et al*. (2007).



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2}

Figure 4. Effect of different levels of SA and calcium on leaves number plant^{-1} of tomato at different days after transplanting, $LSD_{0.05}$ = 0.5105, 1.064 and 4.043 for 20DAT, 40 DAT and 60 DAT, respectively.

The results of the present study showed that, the interaction effect between three planting time and different compositions of SA and Ca^{2+} showed significant variation on leaf number plant^{-1} of tomato at 40 DAT and 60 DAT. At 20 DAT, the combination treatment showed insignificant effect on the formation of leaves (Table 2 and Appendix VI). At 40 DAT, the highest number of leaves plant^{-1} (12.66) was found from T_1A_3 and the lowest value (6.887) was found from T_3A_0. At 60 DAT, the highest number of leaves plant^{-1} (73.78) was found from T_1A_3 which was statistically similar to T_1A_1 (69.89) and the lowest value (30.22) was found from T_3A_0 which was statistically similar with T_3A_1 (36.33), T_3A_2 (33.11), T_3A_4 (31.67) and T_3A_5 (34.22). The first transplanting (T_1) along with 0.25 mM SA & 5 mM Ca^{2+} (A_3) i.e. (T_1A_3) produced the highest value of leaf number plant^{-1} and the minimum value were recorded from third transplanting date (T_3) without SA and Ca^{2+} (T_3A_0) treatment combination and suggesting that the late transplanting planting time (T_3) failed to produce enough leaf as T_1 planting time. Therefore, the leaf number plant^{-1} increased with the combined application of SA and Ca^{2+} rather than SA and Ca^{2+} alone in response to late transplanting-induced cold injury in tomato plant. Kazemi (2013) reported that low SA and Ca^{2+} concentration improved the vegetative growth including leaf number of strawberry. Altogether, it is indicating that SA and Ca^{2+} promote leaf number of tomato to cold injury.

Table 2. Combined effect of transplanting time and different composition of SA and Ca^{2+} on the number of leaves $plant^{-1}$ of tomato

Treatment combination	Number of leaves $plant^{-1}$ at different days after transplanting		
	20 DAT	40 DAT	60 DAT
T_1A_0	5.77 bcd	10.67 bcdef	52.89 de
T_1A_1	6.67 a	11.89 ab	69.89 ab
T_1A_2	6.23 abc	11.11 abcd	58.11 cd
T_1A_3	6.33 abc	12.66 a	73.78 a
T_1A_4	6.55 ab	10.89 abcde	56.67 cd
T_1A_5	6.33 abc	11.56 abc	63.00 bc
T_2A_0	5.77 bcd	8.55 hij	40.78 fghi
T_2A_1	6.33 abc	9.89 cdefgh	47.11 efg
T_2A_2	5.66 cde	9.00 fghi	43.00 fgh
T_2A_3	5.10 def	10.56 bcdefg	47.78 ef
T_2A_4	5.88 abcd	9.22 efghi	40.67 ghi
T_2A_5	5.77 bcd	9.66 defghi	46.00 efg
T_3A_0	4.88 ef	6.88 j	30.22 j
T_3A_1	4.77 f	8.78 ghi	36.33 hij
T_3A_2	4.33 f	8.00 ij	33.11 j
T_3A_3	5.10 def	9.11 efghi	40.56 ghi
T_3A_4	4.88 ef	7.88 ij	31.67 j
T_3A_5	4.77 f	8.55 hij	34.22 ij
LSD $_{(0.05)}$	0.884	1.843	7.003
Significant level	NS	*	*
CV (%)	9.47%	11.43%	9.10%

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

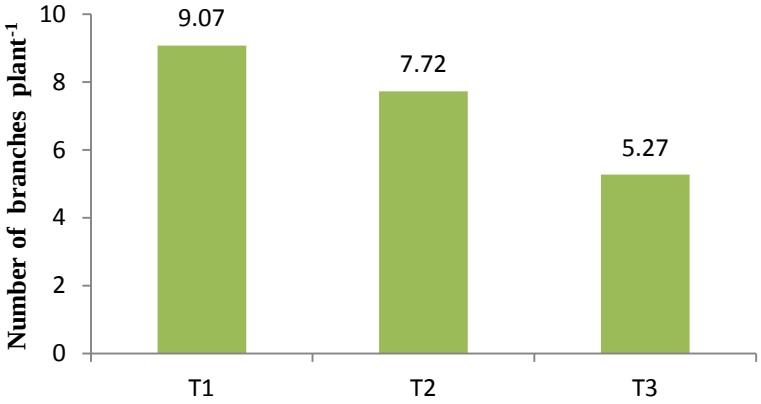
A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

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

4.3 Number of branches $plant^{-1}$

Tomato branches number $plant^{-1}$ was significantly influenced by transplanting times (Figure 5 and Appendix VII). The highest number of branch $plant^{-1}$ (9.07) was observed from the T_1 and the lowest value (5.27) was observed from T_3. Among dates of planting, early planting recorded the highest vegetative growth in tomato which was reported by

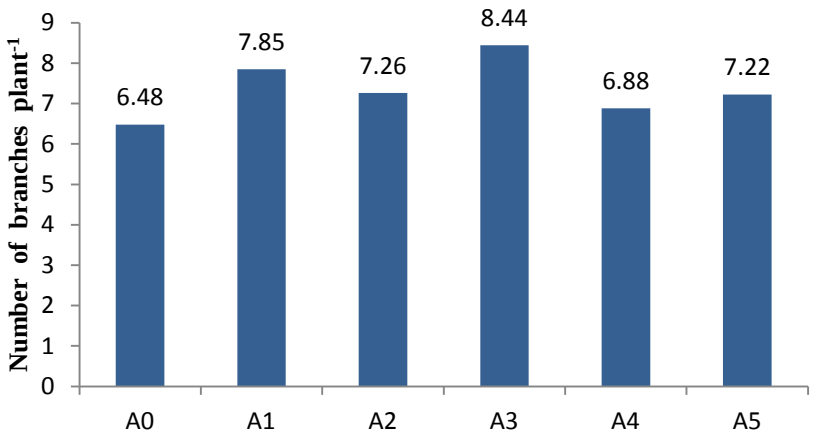
Sing *et.al.* (2005). Mira *et al*. (2011) observed that number of branches plant^{-1} was found to be gradually decreased with the late transplanting dates in same plant. Hence, the obtained results are consistent with many other previous published findings.



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

Figure 5. Effect of transplanting time on the number of branches plant^{-1} of tomato at 60 DAT, $LSD_{0.05}$ = 0.575.

Application of different SA and Ca^{2+} composition showed statistically significant effect on branches number of tomato plant (Figure 6 and Appendix VII). The highest number of branches plant^{-1} (8.44) was observed from the A_3, it was statistically similar with A_3 (7.85) and the lowest (6.48) was observed from A_0 which was statistically similar with A_2 (7.26), A_4 (6.88) & A_5 (7.22). In contrast, Kazemi (2012), Sandoval-Yapiz (2004) and Yildirim *et al*. (2009) observed significant effect of SA and Ca^{2+} in increasing the number of branches in plant. Therefore, variety and environmental factors may have some influence on considerable branches production which also response to the late planting of my research.



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 6. Effect of different composition of SA and Ca^{2+} on the number of branches plant^{-1} of tomato at 60 DAT, $LSD_{0.05}$ = 0.814.

There were significant interaction effects between transplanting time and different composition of SA and Ca^{2+} on number of branches plant^{-1} of tomato (Table 3 and Appendix VII). The highest number of branches plant^{-1} (10.00) was observed from the T_1A_3 treatment which was statistically similar with T_1A_1 (9.55), T_1A_2 (8.77), T_1A_4 (8.66), T_1A_5 (9.22) and T_2A_3 (9.11) whereas, the lowest (4.22) was observed from T_3A_0 treatment which was statistically similar with T_3A_2 (5.55), T_3A_4 (4.77) and T_3A_5 (5.22). The first transplanting (T_1) along with 0.25mM SA 5 mM Ca^{2+} (A_3) i.e. (T_1A_3) produced the highest value of branch number and the minimum value were found in third transplanting time (T_3) without SA and Ca^{2+} (T_3A_0) treatment combinations. These finding are partially agreed with the findings of Plasencia *et al.* (2011), Hayat *et al.* (2009), Ilyas *et al* (2014), Rab and Haq (2012). From these results, it suggested that plant growth as well as branching was decreasing trend which is consistent with the growing temperature in the tomato field (Table 3 and Appendix III). The application of SA and Ca^{2+} at the rate of 0.25mM and 5mM, respectively minimize the adverse effects of cold stress by forming more branches in response to late transplanting (Appendix III).

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

Treatment Combination	Number of branches $plant^{-1}$
T_1A_0	8.22 bcd
T_1A_1	9.55 ab
T_1A_2	8.77 abc
T_1A_3	10.00 a
T_1A_4	8.66 abc
T_1A_5	9.22 ab
T_2A_0	7.00 def
T_2A_1	8.33 bcd
T_2A_2	7.44 cde
T_2A_3	9.11 ab
T_2A_4	7.22 de
T_2A_5	7.22 de
T_3A_0	4.22 i
T_3A_1	5.66 fgh
T_3A_2	5.55 ghi
T_3A_3	6.22 efg
T_3A_4	4.77 hi
T_3A_5	5.22 ghi
$LSD_{(0.05)}$	1.411
Significant level	*
CV (%)	11.71

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

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

4.4 SPAD value

There was a clear effect of transplanting time on the leaf SPAD value of tomato plant for T_1, T_2 and T_3 (Figure 7 and Appendix VIII). The SPAD value was measured in SPAD value in leaves of tomato which was 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 (59.91) and the lowest (54.28) 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.

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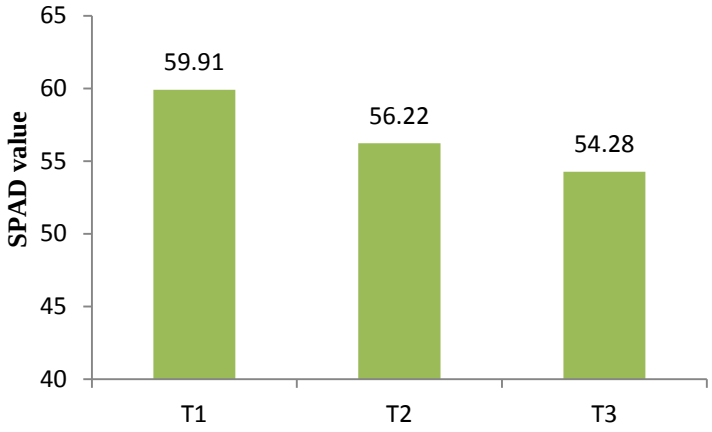
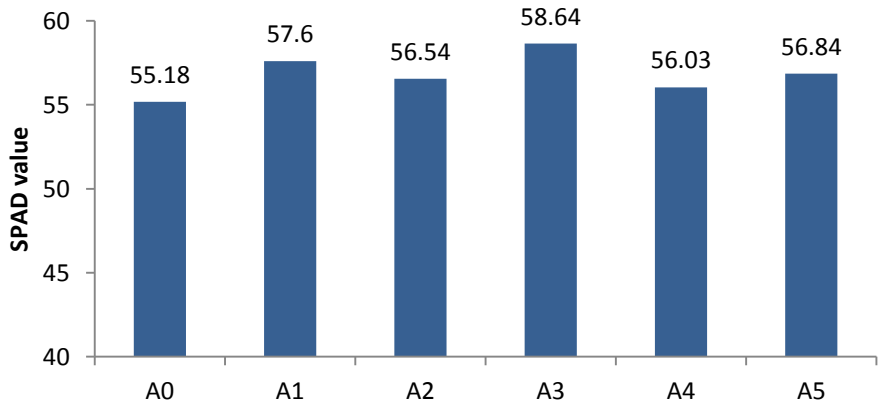


T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3 – Third transplanting time, 30 December 2013

Figure 7. Effect of transplanting time on the SPAD value of tomato plant leaf at 40 DAT, $LSD_{0.05}$ = 1.307.

Doses of SA and Ca^{2+} had insignificant effect on SPAD value of tomato (Figure 8 and Appendix VIII). The highest SPAD value (58.64) was observed from the A_3 and the lowest (55.18) was observed from A_0.



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 8. Effect of different composition of SA and Ca^{2+} on the SPAD value of tomato plant leaf at 40 DAT, $LSD_{0.05}$ = 1.849.

Interaction of different planting time and composition of SA and Ca^{2+} showed significant variation on SPAD value of tomato (Table 4 and Appendix VIII). The highest SPAD value (61.63) was observed from the T_1M_3 treatment which was statistically similar with T_1A_0 (58.50), T_1A_1 (60.43), T_1A_2 (59.37), T_1A_4 (59.32) and T_1A_5 (60.33) whereas, the lowest (52.60) was observed from T_3A_0 treatment which was statistically similar with T_2A_0 (54.43), T_2A_4 (55.13), T_3A_1 (55.27), T_3A_2 (53.89), T_3A_4 (53.70) and T_3A_5 (53.91). From my experiment it has been observed that cold stress reduce the SPAD value and SA and Ca^{2+} with 0.25 mM and 5mM respectively alleviate the effect of cold stress partially when the lowest temperature were recorded during T_3 planting date (Appendix III).The previous literature such as Zhao-Min *et al.* (2013), Aldesuquy & Ibrahim (2000) recorded the effect of SA on photosynthesis pigments. They reported that salicylic acid at all concentrations was found to stimulate the production of Chl.a, Chl.b, Carotenoids and 14C fixation during leaf growth and development. This study suggests that, exogenous SA and Ca^{2+} supply improves the total SPAD value in tomato plant.

Table 4. Combined effect of transplanting time and different composition of SA and Ca^{2+} on the SPAD value of tomato plant leaf at 40 DAT

Treatment Combination	SPAD value
T_1A_0	58.50 abc
T_1A_1	60.43 ab
T_1A_2	59.32 abc
T_1A_3	61.63 a
T_1A_4	59.27 abc
T_1A_5	60.33 ab
T_2A_0	54.43 efg
T_2A_1	57.11 cde
T_2A_2	56.41 cdef
T_2A_3	57.94 bcd
T_2A_4	55.13 defg
T_2A_5	56.28 cdef
T_3A_0	52.60 g
T_3A_1	55.27 defg
T_3A_2	53.89 fg
T_3A_3	56.33 cdef
T_3A_4	53.70 fg
T_3A_5	53.91 fg
LSD $_{(0.05)}$	3.202
Significant level	*
CV (%)	3.44

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

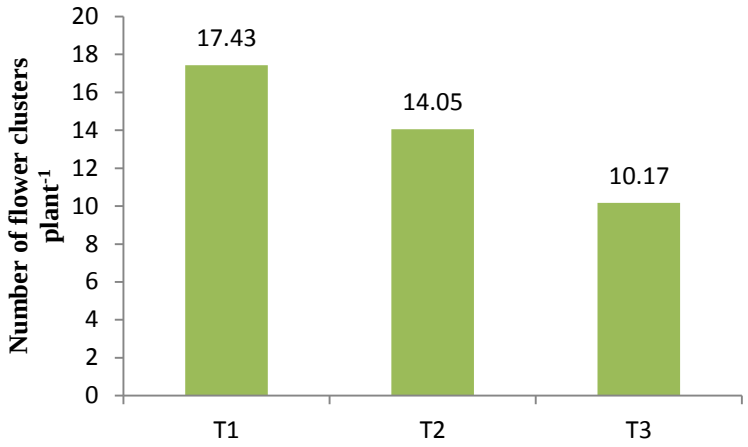
CV = Co-efficient of variance
LSD = Least significant Difference
* = Significant at 5% level

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

4.5 Number of flower clusters plant^{-1}

In this experiment, there was a significant difference in number of flower clusters plant^{-1} at different transplanting time (Figure 9 and Appendix IX). The highest number of flower clusters plant^{-1} (17.43) of tomato was found from T_1 and the lowest number of cluster (10.17) was recorded from T_2. Early planting induced early and more flower clusters initiation plant^{-1} of tomato than late transplanting-induced cold injury. These results indicate that lower temperature reduces the formation of number of flower clusters plant^{-1}

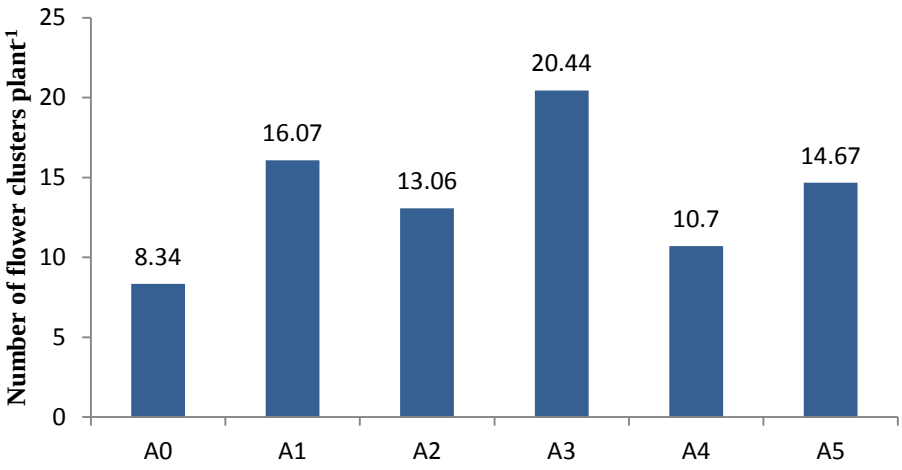
as other above mentioned morphological characters (Fig.1, 2 and 3, Appendix III). Therefore, it is suggesting that low temperature stress significantly affect the both vegetative and reproductive development in tomato.



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

Figure 9. Effect of transplanting time on the number of flower clusters plant^{-1} of tomato, $LSD_{0.05}$ = 1.477.

Significant variation was observed from flower clusters number plant^{-1} of tomato for different levels of SA and calcium, in sole or combination (Figure 10 and Appendix IX). The highest flower clusters plant^{-1} (20.44) was found in A_3 treated plants and control (A_0) plants showed the lowest flower clusters plant^{-1} (8.34). Javaheri *et al.* (2014) reported that SA promoted the number of flower clusters in tomato plant.



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 10. Effect of different composition of SA and Ca^{2+} on the number of flower clusters plant^{-1} of tomato, $LSD_{0.05}$ = 2.089.

Due to the interaction effect of transplanting time and different combination of SA and Ca^{2+} number of flower clusters plant^{-1} varied significantly (Table 5 and Appendix IX). The highest number of flower clusters plant^{-1} (24.44) was found from T_1A_3 which was statistically similar to T_2A_3 (21.44), while the lowest number (5.81) was obtained from T_3A_0, it was statistically similar with T_3A_2 (9.33) & T_3A_4 (7.11). Many authors like Martin-Mex *et al.* (2003, 2005a), Kumar *et al.* (2000) and Janda *et al.*(1997,1999) reported that they had found cumulative effect of SA on flower cluster of plants such as *Sinningia Speciosa* and maize in sole or in combination by reducing the injury.

Table 5. Combined effect of transplanting time and different composition of SA and Ca^{2+} on the number of flower clusters plant^{-1} of tomato

Treatment Combination	Number of flower clusters plant^{-1}
T_1A_0	10.56 ghij
T_1A_1	19.78 bc
T_1A_2	17.44 cde
T_1A_3	24.44 a
T_1A_4	13.78 fg
T_1A_5	18.56 bcd
T_2A_0	8.66 ijk
T_2A_1	15.67 def
T_2A_2	12.41 fgh
T_2A_3	21.44 ab
T_2A_4	11.22 ghi
T_2A_5	14.89 ef
T_3A_0	5.81 k
T_3A_1	12.78 fgh
T_3A_2	9.33 hijk
T_3A_3	15.45 def
T_3A_4	7.11 jk
T_3A_5	10.55 ghij
LSD $_{(0.05)}$	3.618
Significant level	*
CV (%)	15.91

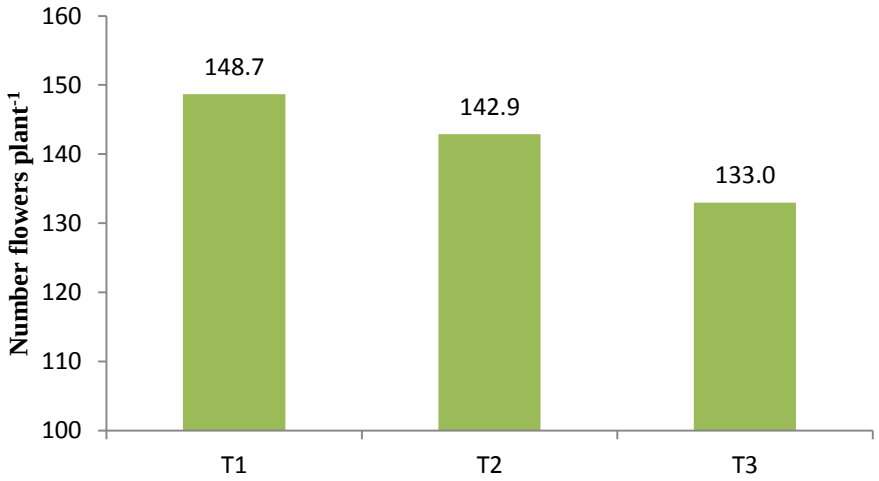
T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

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

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

4.6 Number of flowers plant^{-1}

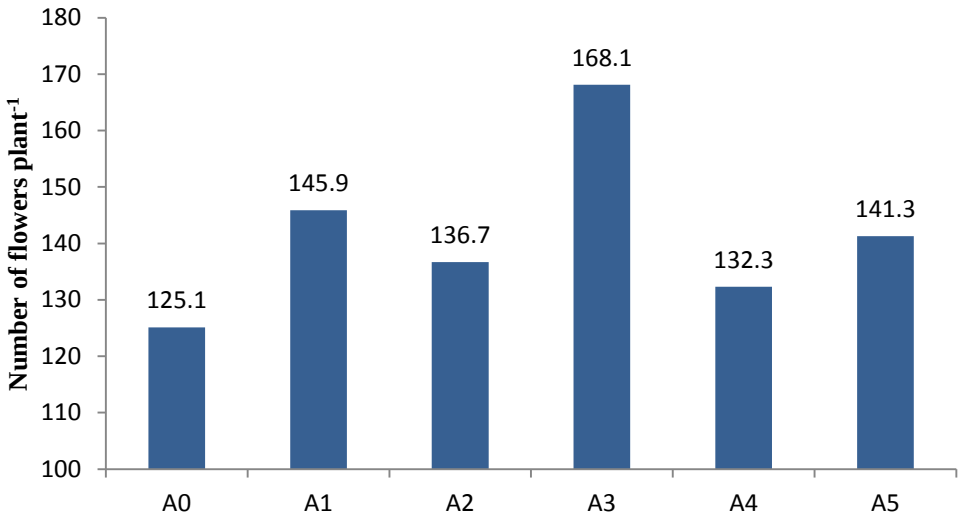
Planting time had significant effect on number of flowers plant^{-1} of tomato (Figure 11 and Appendix IX). The highest number of flower plant^{-1} was observed from the T_1 (148.7) and the lowest (133.0) was observed from T_3 treatment. T_1 was statistically similar with T_2 (142.9). Hossain *et al.* (1986) reported that early sowing enhanced total number of flowers plant^{-1}. From these results, it was found that more flowers were produced from the early transplanted tomato seedlings than lately transplanted seedlings.



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

Figure 11. Effect of transplanting time on the number of flowers plant^{-1} of tomato, $LSD_{0.05}$ = 6.24.

Statistically significant variation was recorded for the number of flowers plant^{-1} of tomato for different composition of SA and Ca^{2+} (Figure 12 and Appendix IX). The highest number of flowers plant^{-1} (168.1) was observed from the A_3 and the lowest (125.1) was observed from A_0 which was statistically identical with A_4 (132.3). These results are in consistency with the findings of Martin-Mex *et al.* (2003), Khurana *et al*. (1987), Janda *et al.* (1997 and 1999), Hew (1987) and Kumar *et al.* (2000) found separately that Salicylic acid (SA) induced flowering in plants such soybean, *Wolffia microscopia* and *Oncidium openin* flower.



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 12. Effect of different composition of SA and Ca^{2+} on the number of flowers plant^{-1} of tomato, $LSD_{0.05}$ = 8.83.

Significant variation in terms of number of flowers plant^{-1} (Table 6 and Appendix IX) was observed through the interaction effect of planting time and different composition of SA and Ca^{2+}.The highest number of flower plant^{-1} (180.8) was observed from the T_1A_3 treatment which was statistically similar with T_2A_3 (173.0) whereas, the lowest (118.6) was observed from T_3A_0 treatment which was statistically similar with T_1A_0 (132.2), T_2A_0 (124.4), T_2A_4 (133.1), T_3A_2 (128.7), T_3A_4 (126.8) and T_3A_5 (133.2). Many authors like Cleland and Ajami (1974) , Kumar *et al*. (1999), Khurana *et al*. (1987), Hew (1987), Martin-Mex *et al*.(2003, 2005a), Ilyas *et al.* (2014), Kazemi (2012) and Rab and Haq (2012) mentioned that SA and Ca^{2+} individually increased the number of flower per plant. So it can be suggested that early transplanting at T_1 increase the flower number and late transplanting at T_3 time decrease flower number due to temperature condition (Appendix III) but partially overcome the unfavorable temperature condition of late transplanting along with SA and Ca^{2+}.

Table 6. Combined effect of transplanting time and different composition of SA and Ca^{2+} on the number of flowers $plant^{-1}$ of tomato

Treatment Combination	Number of flowers $plant^{-1}$
T_1A_0	132.2 cdefg
T_1A_1	151.0 c
T_1A_2	144.6 bc
T_1A_3	180.8 a
T_1A_4	137.0 bcdef
T_1A_5	146.8 bc
T_2A_0	124.4 fg
T_2A_1	146.2 bc
T_2A_2	137.0 bcdef
T_2A_3	173.0 ab
T_2A_4	133.1 cdefg
T_2A_5	143.9 bcd
T_3A_0	118.6 g
T_3A_1	140.6 bcde
T_3A_2	128.7 defg
T_3A_3	150.4 b
T_3A_4	126.8 efg
T_3A_5	133.2 cdefg
LSD $_{(0.05)}$	15.3
Significant level	*
CV (%)	6.60

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

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

4.7 Number of fruits $plant^{-1}$

Number of fruits $plant^{-1}$ of tomato showed significant differences in response to transplanting time (Figure 13 and Appendix IX). The highest fruits number $plant^{-1}$ (86.38) was observed from the T_1 which was statistically similar to T_2 (82.11) and the lowest (73.33) was observed from T_3. Maximum number of fruit $plant^{-1}$ was recorded from early transplanting and the minimum from late, due to low temperature (BARI, 1989). Adelana (1976) and Drost and Price (1991) had also reported that late transplanting reduced fruit 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 highest number of fruit and in unfavorable temperature condition decreased the number of fruit plant^{-1}.

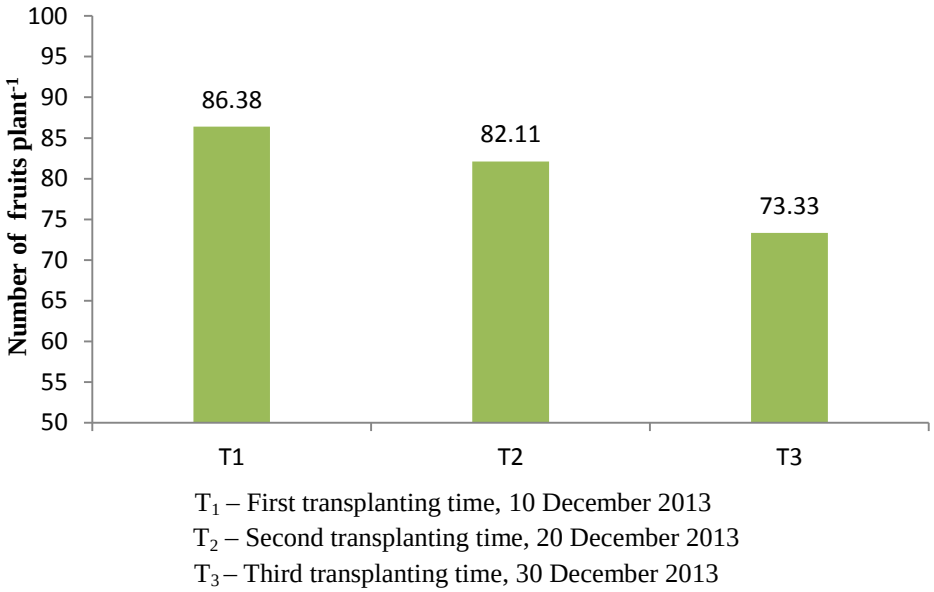
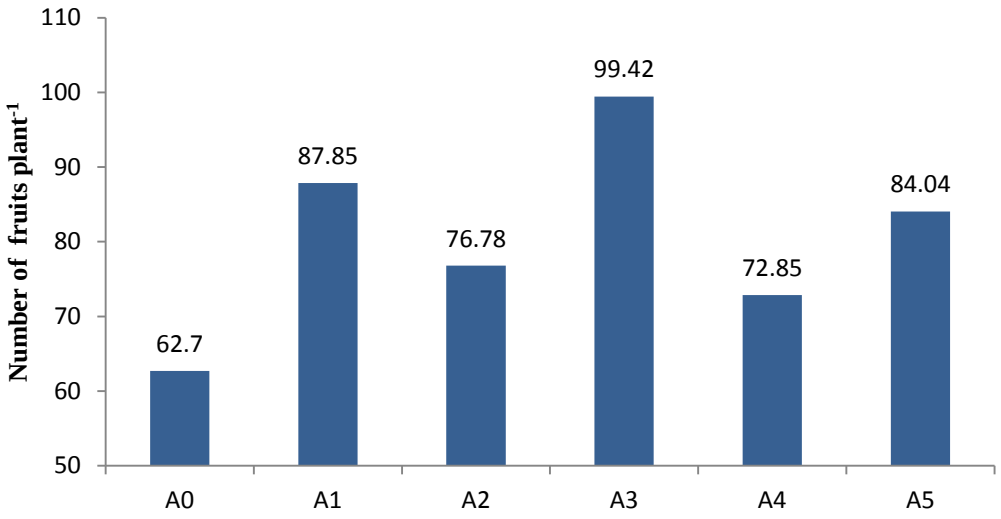


Figure 13. Effect of transplanting time on the number of fruits plant^{-1} of tomato, $LSD_{0.05}$ = 5.886.

Statistically significant variation was recorded for number of fruits plant^{-1} of tomato after the application of different composition of SA and Ca^{2+} (Figure 14 and Appendix XI). The highest fruits number plant^{-1} (99.42) was observed from the A_3 and the lowest (62.70) was observed from A_0. The spraying of concentrations of SA and Ca^{2+} had a great regulatory effect on number of fruits plant^{-1} and increased the fruit yield as suggested by Javaheri *et al.* (2011), Yildirim *et al*. (2009), Herrera-Tuz (2004); Martin-Mex *et al.* (2005b), Sahu *et al.* (2007), Hayat and Ahmad (2007), Plasencia *et.al* (2011), Sandoval-Yapiz (2004), Ho *et al*. (1999), Ilyas *et al.* (2014) and Kazemi (2012).



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 14. Effect of different composition of SA and Ca^{2+} on the number of fruits plant^{-1} of tomato, $LSD_{0.05}$ = 8.324.

Number of fruits plant^{-1} varied significantly with the interaction effect of different transplanting time and composition of SA and Ca^{2+} (Table 7 and Appendix IX). The highest fruits number plant^{-1} (110.5) was observed from the T_1A_3 treatment whereas, the lowest (56.00) was observed from T_3A_0 treatment which was statistically similar with T_1A_0 (68.89), T_2A_0 (63.22), T_3A_1 (67.56) and T_3A_4 (64.78).

Table 7. Combined effect of transplanting time and different composition of SA and Ca^{2+} on the number of fruits $plant^{-1}$ of tomato

Treatment Combination	Number of fruits $plant^{-1}$
T_1A_0	68.89 efghi
T_1A_1	91.56 bc
T_1A_2	82.57 bcde
T_1A_3	110.5 a
T_1A_4	78.11 cdefg
T_1A_5	86.66 bcd
T_2A_0	63.22 hi
T_2A_1	89.66 bcd
T_2A_2	80.22 cdef
T_2A_3	96.44 ab
T_2A_4	75.67 defgh
T_2A_5	87.44 bcd
T_3A_0	56.00 i
T_3A_1	82.33 bcde
T_3A_2	67.56 fghi
T_3A_3	91.33 bc
T_3A_4	64.78 ghi
T_3A_5	78.00 cdefg
LSD $_{(0.05)}$	14.42
Significant level	*
CV (%)	10.92

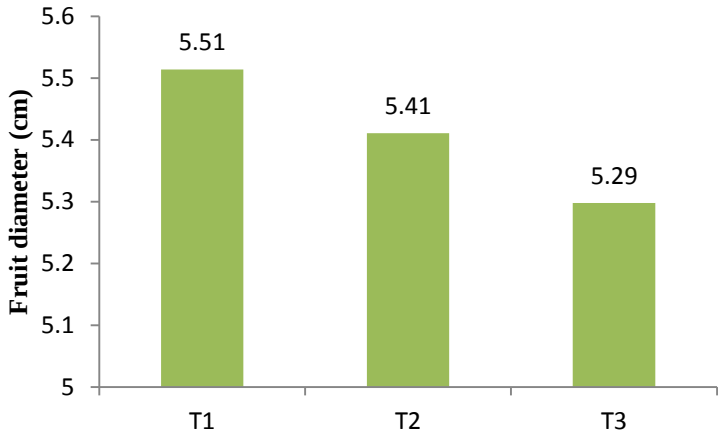
T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

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

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

4.8 Fruit diameter (cm)

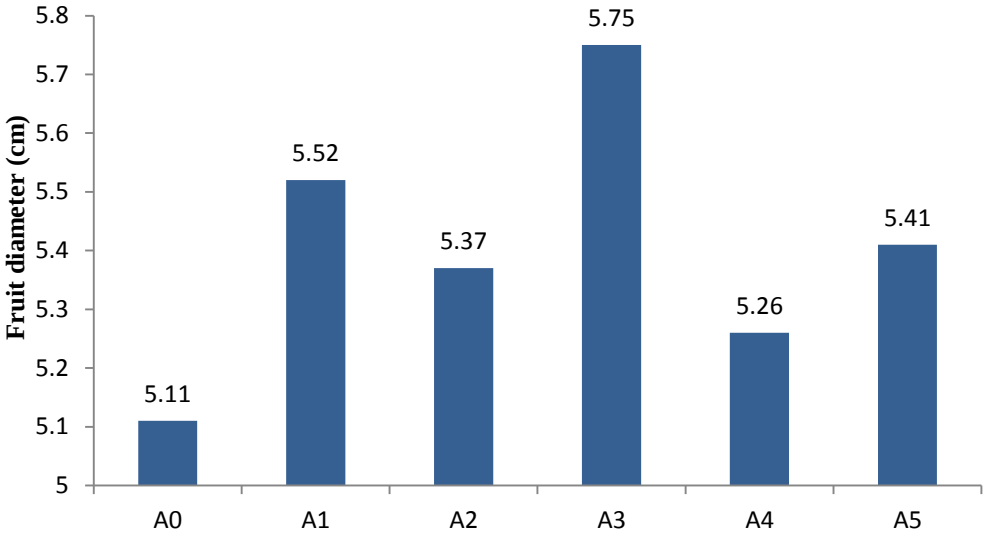
In this study, fruit diameter (cm) of tomato showed significant variation in response to transplanting time (Figure 15 and Appendix IX). The highest fruit diameter (5.51) was recorded from the T_1 which is statistically similar with T_2 (5.41) and the lowest (5.29) was observed from T_3 treatment. Madhumathi 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 provided higher fruit diameter than the late transplanted tomato one and it was also reported by Javaheri *et.al*. (2014).



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

Figure 15. Effect of transplanting time on the fruit diameter (cm) of tomato, $LSD_{0.05}$ = 0.175.

A significant variation was recorded due to the different composition of SA and Ca^{2+} on fruit diameter of tomato (Figure 16 and Appendix IX). The highest fruit diameter (5.75 cm) was observed from the A_3 and the lowest (5.11 cm) was observed from A_0. Previous many authors reported that SA and Ca^{2+} played an important role on the fruit development and setting in many crops. All together the presented data suggest that SA and Ca^{2+} has positive functions on fruit diameter (cm) as well as fruit yield of tomato as supported by Javaheri *et al*. (2014), Herrera-Tuz *et al.*(2004), Martin-Mex *et al.* (2005b), Rab and Haq (2012).



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 16. Effect of different composition of SA and Ca^{2+} on the fruit diameter (cm) of tomato, $LSD_{0.05}$ = 0.2484.

Fruit diameter (cm) showed significant variation due to the interaction between the different transplanting time and application of different composition of SA and Ca^{2+}on tomato (Table 8). The highest fruit diameter (5.92) was observed from the T_1A_3 which was statistically identical with T_1A_1 (5.61), T_1A_5 (5.50), T_2A_1 (5.55), T_2A_3 (5.70) and T_3A_3 (5.65) whereas, the lowest (5.04) was observed from T_3A_0 treatment which was statistically similar with all except T_1A_0 (5.11), T_1A_2 (5.46), T_1A_4 (5.40), T_2A_0 (5.11), T_2A_2 (5.39), T_2A_4 (5.23), T_2A_5 (5.47), T_3A_1 (5.39), T_3A_2 (5.26), T_3A_4 (5.15) and T_3A_5 (5.28). Javaheri *et al*. (2011) and Yildirim *et al*. (2009) reported significant increase of fruit diameter of tomato.

Table 8. Combined effect of transplanting time and different composition of SA and Ca^{2+} on the fruit diameter (cm) of tomato

Treatment Combination	Fruit diameter (cm)
T_1A_0	5.17 def
T_1A_1	5.61 abc
T_1A_2	5.46 bcdef
T_1A_3	5.92 a
T_1A_4	5.40 bcdef
T_1A_5	5.50 abcde
T_2A_0	5.11 ef
T_2A_1	5.55 abcd
T_2A_2	5.39 bcdef
T_2A_3	5.70 ab
T_2A_4	5.23 cdef
T_2A_5	5.47 bcdef
T_3A_0	5.04 f
T_3A_1	5.39 bcdef
T_3A_2	5.26 cdef
T_3A_3	5.65 abc
T_3A_4	5.15 def
T_3A_5	5.28 bcdef
LSD $_{(0.05)}$	0.4302
Significant level	*
CV (%)	4.86

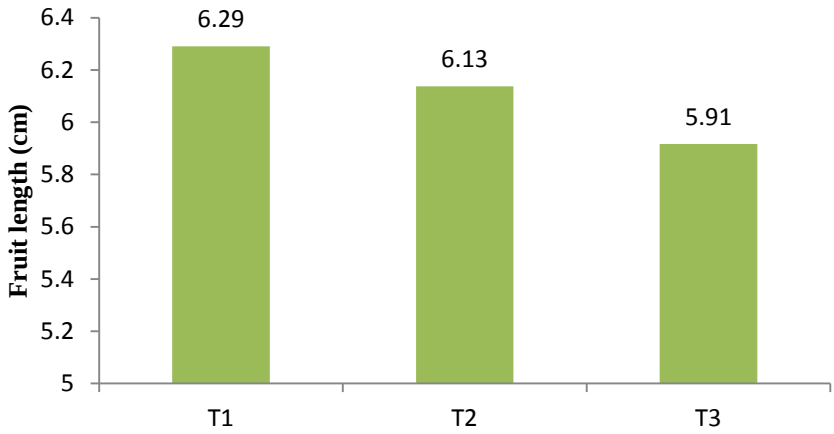
T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

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

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

4.9 Fruit length (cm)

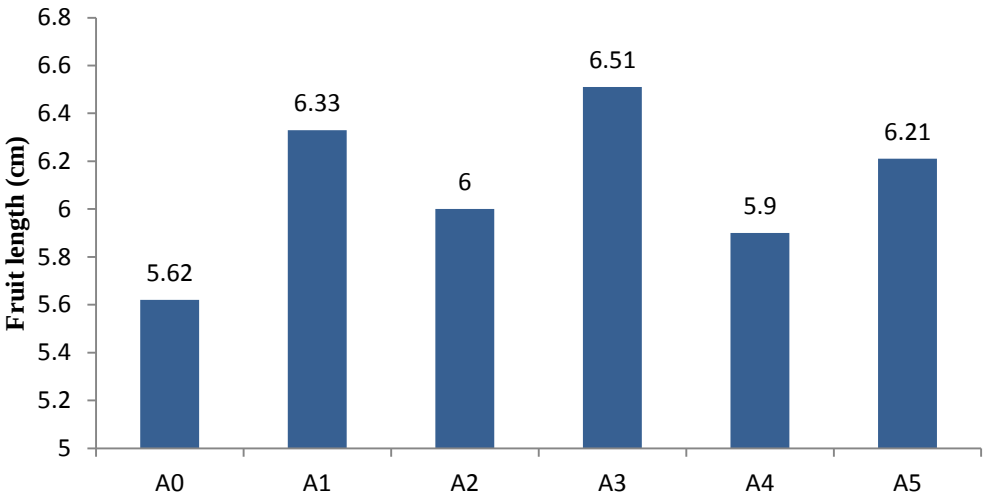
As consistent to fruit diameter planting time had significant influence on fruit length (cm) of tomato (Figure 17 and Appendix IX).The highest fruit length (6.29) was observed from the T_1 and the lowest (5.91) was observed from T_3. These data resulted that early transplanting time increased fruit length (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.



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

Figure 17. Effect of transplanting time on the fruit length (cm) of tomato, $LSD_{0.05}$ = 0.0791.

Different combination of SA and Ca^{2+} had significant effect on fruit length of tomato (Figure 18, Appendix IX). The highest fruit length (6.51) was observed from the A_3 and the lowest (5.62) was observed from A_0. Here results showed that SA and Ca^{2+} increased fruit length as reported by Javaheri *et al*. (2011), Salem (2013) that application of SA increased the fruit length of tomato. Previous many authors reported that SA and Ca^{2+} play an important role on the fruit development and setting in many crops. All together, the presented data suggest that SA and Ca^{2+} had positive function on fruit length of tomato.



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 18. Effect of different composition of SA and Ca^{2+} on the fruit length (cm of tomato, $LSD_{0.05}$ = 0.1119.

Interaction of planting time and different composition of SA and Ca^{2+} showed significant variation on fruit length of tomato. The highest fruit length (6.70) was observed from the T_1A_3 treatment which was statistically similar with T_2A_3 (6.58) whereas, the lowest (5.48) was observed from T_3A_0 treatment which was statistically similar with T_2A_0 (5.65). Results showed that best combination (T_1A_3) increased fruit length at 0.25 mM SA and 5 mM Ca^{2+} as consistent with fruit diameter of tomato.

Table 9. Combined effect of transplanting time and different composition of SA and Ca^{2+} on the fruit length of tomato

Treatment Combination	Fruit length (cm)
T_1A_0	5.73 g
T_1A_1	6.49 bc
T_1A_2	6.30 cde
T_1A_3	6.70 a
T_1A_4	6.11 ef
T_1A_5	6.40 bcd
T_2A_0	5.65 gh
T_2A_1	6.39 bcd
T_2A_2	6.16 ef
T_2A_3	6.58 ab
T_2A_4	5.81 g
T_2A_5	6.22 def
T_3A_0	5.48 h
T_3A_1	6.12 ef
T_3A_2	5.83 g
T_3A_3	6.26 de
T_3A_4	5.77 g
T_3A_5	6.03 f
LSD $_{(0.05)}$	0.1938
Significant level	*
CV (%)	1.94

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

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

4.10 Yield in (kg $plot^{-1}$) and (t ha^{-1})

As morphological characters the yield of tomato was also significantly reduced to low temperature injury that was induced by changing transplanting time (Figure 19.A and 19.B, Appendix X). The highest yield (17.94 kg $plot^{-1}$) and yield (66.46 t ha^{-1}) were observed from the T_1 and the lowest yield (12.32 kg $plot^{-1}$) and yield (45.62 t ha^{-1}) 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 present morpho-physiological and yield contributing characters such as plant height (Figure 1); leaf number $plant^{-1}$ (Figure 3); branch number $plant^{-1}$ (Figure 5); SPAD

value (Figure 7); number of flower clusters plant^{-1} (Figure 9), number of flowers plant^{-1} (Figure 11), fruit number (Figure 13), fruit diameter (Figure 15) and fruit length (Figure 17). This experimental data were consistent with the growing temperature of tomato which clearly indicates that late planting-induced cold injury partially hinder the crop growth and productivity (Appendix III). 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 give increased yield. Previous authors as well reported that early transplanting of tomato give increased fruit weight and yield of tomato (Adelana 1976). In addition, last transplanting time was at low temperature injury whereas these plants were at higher temperature during reproduction stage. Therefore, these plants produce lower fruit yield in comparison with first and second transplanting. These results are in consistent with the other studied both morpho-physiological and yield contributing parameters of this study. Curme (1992) reported that lower temperature failed to give better yield of tomato. As lower temperature, higher temperature did not give better yield of tomato in relation to optimum temperature reported by Lawhori *et al.* (1963). All together, these results suggest that suitable transplanting time is more favorable to produce highest plant height, leaf number, branch number as a result higher flower cluster, 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 acclimation.

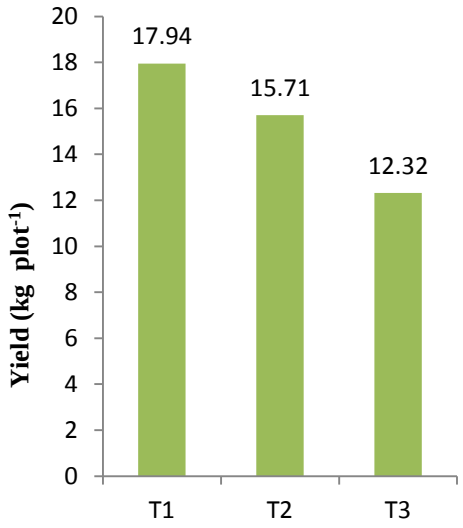


Figure: 19. A

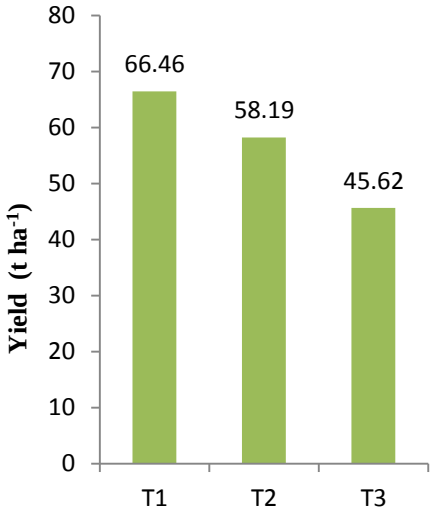
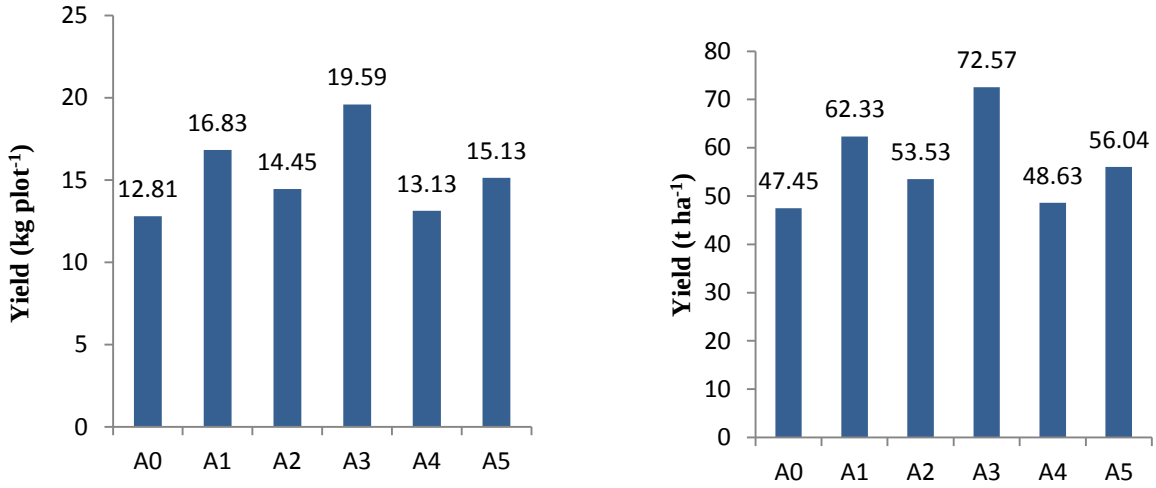


Figure: 19. B

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

Figure 19. Effect of transplanting time on the yield in kg plot^{-1} (A) and yield in t ha^{-1} (B) of tomato, $LSD_{0.05}$ = 1.326 and 4.911 respectively.

In this study, different composition of SA and Ca^{2+} such as 0 mM SA, 0.25 mM SA and 0 mM Ca^{2+}, 5 mM Ca^{2+}, 10 mM Ca^{2+} was used as alleviating agent of cold injury in tomato to improve the tomato fruit to cold injury. The SA and Ca^{2+} showed a significant effect to promote the fruit yield tomato under late planting-induced cold stress (Fig.20.A and B; Appendix X). The highest yield 19.59 kg plot^{-1} and yield 72.57 t ha^{-1} were observed from the A_3 and the lowest 12.81 kg plot^{-1} and 47.45 t ha^{-1} were observed from A_0 which was statistically similar with A_2 (14.45 kg), (53.53 t ha^{-1}) and A_4 (13.13 kg), (48.63 t ha^{-1}). These results are consistent with the present morpho-physiological and yield contributing characters such as plant height (Figure 2); leaf number plant^{-1} (Figure 4); branch number plant^{-1} (Figure 6); SPAD value (Figure 8); number of flower clusters plant^{-1} (Figure 10), number of flowers plant^{-1} (Figure 12), fruit number (Figure 14), fruit diameter (Figure 16) and fruit length (Figure 18). Shehata *et al.* (2001), Lolaei (2012), Saavedra *et al.* (1975) and Kazemi (2013) reported that separately SA and Ca^{2+} increase the yield of tomato. Hossain (1974) testified that SA increased fruit set. Therefore, altogether these results suggest that together application of SA and Ca^{2+} increased the fruit yield of tomato.



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 20. Effect of different composition of SA and Ca^{2+} on the yield in kg $plot^{-1}$ (A) and yield in t ha^{-1} (B) of tomato, $LSD_{0.05}$ = 1.875 and 6.945 respectively.

In this study, the interaction effect between transplanting date and different composition of SA and Ca^{2+} showed a significant positive effect on fruit yield of tomato (Table 11 and Appendix X). The highest yield (23.39 kg $plot^{-1}$) and (86.62 t ha^{-1}) were recorded from the T_1A_3 treatment whereas, the lowest (9.88 kg $plot^{-1}$) and (36.59 t ha^{-1}) was observed from T_3A_0 treatment which was statistically similar with T_2A_0 (12.96 kg $plot^{-1}$, 47.99 t ha^{-1}), T_2A_4 (13.09 kg $plot^{-1}$, 48.49 t ha^{-1}), T_3A_1 (12.59 kg $plot^{-1}$, 46.63 t ha^{-1}), T_3A_2 (12.09 kg $plot^{-1}$, 44.78 t ha^{-1}), T_3A_4 (11.85 kg $plot^{-1}$, 43.89 t ha^{-1}) and T_3A_5 (12.04 kg $plot^{-1}$, 44.59 t ha^{-1}) treatment combinations. These results are consistent with the morpho-physiological data as well as fruit yield contributing characters of tomato (Table 1, 2, 3, 4, 5, 6, 7, 8 and 9). Shehata *et al.* (2001), Abdul (1980), Saavedra *et al.* (1975), Ilyas *et al.* (2014) and Kazemi (2013) reported that early transplanting, SA and Ca^{2+} significantly increased the yield of tomato. Taken together, these results indicate that cold condition decreases the fruit yield and foliar application of plant growth promoter; SA and macronutrient Ca^{2+} enhance the yield of tomato.

Table 10. Combined effect of transplanting time and different composition of SA 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_1A_0	14.44 def	53.49 def
T_1A_1	19.90 b	73.72 b
T_1A_2	16.50 cd	61.10 cd
T_1A_3	23.39 a	86.62 a
T_1A_4	15.60 cde	57.77 cde
T_1A_5	17.83 bc	66.05 bc
T_2A_0	12.96 efg	47.99 efg
T_2A_1	17.99 bc	66.64 bc
T_2A_2	14.77 cdef	54.71 cdef
T_2A_3	19.94 b	73.84 b
T_2A_4	13.09 efg	48.49 efg
T_2A_5	15.52 cde	57.47 cde
T_3A_0	9.88 g	36.59 g
T_3A_1	12.59 efg	46.63 efg
T_3A_2	12.09 fg	44.78 fg
T_3A_3	15.46 cde	57.26 cde
T_3A_4	11.85 fg	43.89 fg
T_3A_5	12.04 fg	44.59 fg
LSD $_{(0.05)}$	3.248	12.03
Significant level	*	*
CV (%)	12.94	12.94

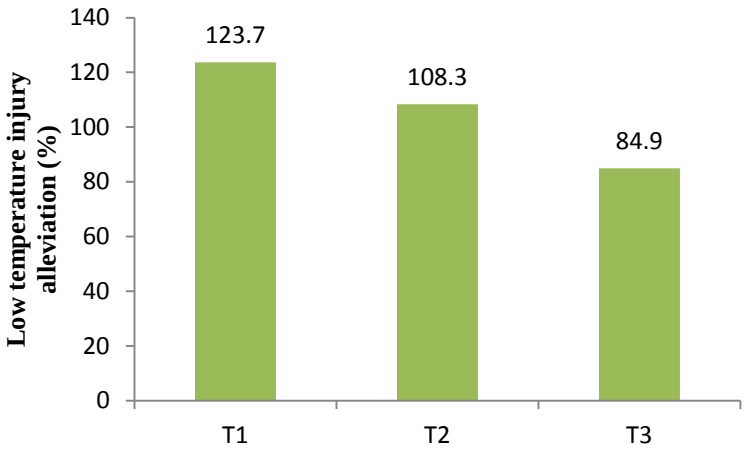
T_1 – First transplanting time, 10 December 2013
T_2 – Second transplanting time, 20 December 2013
T_3– Third transplanting time, 30 December 2013

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

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 10 mM Ca^{2+}

4.11 Low temperature injury alleviation (%)

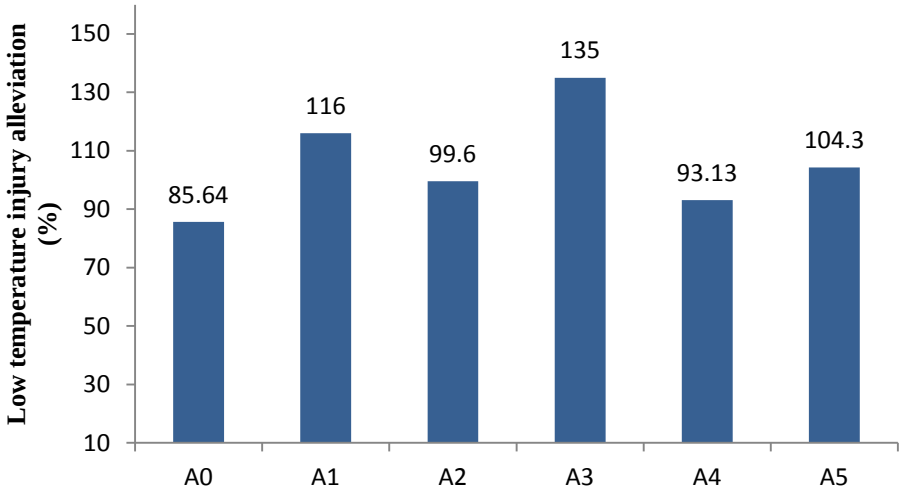
The different transplanting time-induced cold injury showed significant difference on alleviation of cold stress percent in tomato (Figure 21 and Appendix X). The same aged, 25 d seedlings were transplanted three times on 10 December 1013; 20 December 2013 and 30 December 2013 where the all plants were in cold injury at their unlike vegetative phase (Appendix III). The different morphological and yield contributing characters and fruit yield of tomato were decreased more in third transplanting time than first and second transplanting due to the acclimation of cold injury.



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

Figure 21. Effect of transplanting time on low temperature injury alleviation (%) in tomato, $LSD_{0.05}$= 9.26.

Sole or together application of SA and Ca^{2+} in different compositions showed statistically significant difference on alleviation of low temperature injury in tomato (Figure 22, Appendix X). Almost all the studied characters of this experiment were respond positively in presence of SA and Ca^{2+} in tomato. Therefore, the highest low temperature alleviation percentage (135.1) was observed from the A_3, 0.25 mM SA + 5 mM Ca^{2+} whereas the lowest (85.64) was observed from A_0, 0 mM SA + 0 mM Ca^{2+}.



A_0 = 0 mM SA + 0 mM Ca^{2+}, A_1= 0.25 mM SA + 0 mM Ca^{2+},
A_2 = 0 mM SA + 5 mM Ca^{2+}, A_3 = 0.25 mM SA + 5 mM Ca^{2+},
A_4 = 0 mM SA + 10 mM Ca^{2+}, A_5= 0.25 mM SA+ 10 mM Ca^{2+},

Figure 22. Effect of different composition of SA and Ca^{2+} on low temperature injury alleviation (%) in tomato, $LSD_{0.05}$= 13.10.

The significant interaction effects were observed between transplanting time and different composition of SA and Ca^{2+} on cold alleviation percent of tomato (Table 11 and Appendix X). The percentage was highest (161.2) in case of T_1A_3 treatment whereas the lowest (68.09) was found from T_3A_0 which was statistically similar with T_2A_0 (89.30), T_2A_4 (90.24), T_3A_1 (86.77), T_3A_2 (83.32), T_3A_4 (81.67) and T_3A_5 (82.98).Considering the above results, it suggest that the interaction effect of transplanting time and different composition of SA and Ca^{2+} can successfully alleviate the low temperature injury in tomato.

Table 11. Combined effect of transplanting time and different composition of SA and Ca^{2+} on low temperature injury alleviation (%) in tomato

Treatment Combination	Low temperature injury alleviation (%)
T_1A_0	100.0 def
T_1A_1	137.2 b
T_1A_2	113.7 cd
T_1A_3	161.2 a
T_1A_4	107.5 cde
T_1A_5	122.9 bc
T_2A_0	89.30 efg
T_2A_1	124.0 bc
T_2A_2	101.8 cdef
T_2A_3	137.4 b
T_2A_4	90.24 efg
T_2A_5	106.9 cde
T_3A_0	68.09 g
T_3A_1	86.77 efg
T_3A_2	83.32 fg
T_3A_3	106.5 cde
T_3A_4	81.67 fg
T_3A_5	82.98 fg
LSD $_{(0.05)}$	22.68
Significant level	*
CV (%)	12.94

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

A_0 – 0 mM SA + 0 mM Ca^{2+}
A_1 – 0. 25 mM SA + 0 mM Ca^{2+}
A_2 – 0 mM SA + 5 mM Ca^{2+}
A_3 – 0. 25 mM SA + 5 mM Ca^{2+}
A_4 – 0 mM SA + 10 mM Ca^{2+}
A_5 – 0. 25 mM SA + 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 of 15 November 2013 to 15 April 2014 to find out a method to alleviate the late planting-induced low temperature injury with exogenous foliar application of salicylic acid (SA) along with calcium (Ca^{2+}) of 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 salicylic acid and calcium as alleviating agent of low temperature injury *viz*. A_0 = 0 mM of SA and 0 mM Ca^{2+}, A_1= 0.25 mM SA and 0 mM Ca^{2+}, A_2 = 0 mM SA and 5 mM Ca^{2+}, A_3 = 0.25 mM SA and 5 mM Ca^{2+}, A_4 = 0 mM of SA and 10 mM Ca^{2+} and A_5 = 0.25 mM SA 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 salicylic 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. Plant, transplanted on $T_{1=}$ first transplanting time, showed the maximum height more or less over the growth period whereas the lowest height was recorded from late transplanted plants. At 20, 40 and 60 DAT, the tallest plant height were respectively 22.01, 37.70 and 70.22 cm which were recorded from T_1 = First transplanting time whereas the lowest height were respectively 17.78, 29.52 and 62.66 cm which were recorded from T_3 = Third transplanting time. At 20, 40 and 60 DAT, the maximum number of leaves plant^{-1} were respectively 8.31, 21.46 and 62.39 which were recorded from T_1 whereas the minimum number of leaves plant^{-1} were respectively 5.79, 15.20 and 34.35, recorded from T_3. The number of branches plant^{-1} showed significant variation during T_1 and the maximum number of branches plant^{-1} was (9.07).The minimum number of branches plant^{-1} was observed from T_3. Different planting time had also significant effect on SPAD value of tomato leaf, the highest (59.91) and the lowest (54.28) observed respectively from T_1 and $T_{3.}$ The maximum number of flower clusters, flowers and fruits plant^{-1} 17.43, 148.7 & 86.38, respectively were obtained from $T_{1.}$ The highest fruit length and diameter 5.51cm and 6.29cm, respectively were obtained from T_1

= First transplanting time. The highest yield plot^{-1} (17.94 kg) was observed from the T_1. The maximum yield (66.46 t ha^{-1}) was observed from the T_1, whereas the minimum yield (45.62 t ha^{-1}) was obtained from T_3 = Third transplanting time. The highest (123.7) low temperature injury alleviation (%) was recorded from T_1 whereas the lowest (84.9) one was recorded from T_3.

Plant height of tomato showed significant difference in response of exogenous foliar application of salicylic acid (SA) with calcium (Ca^{2+}), where at 20, 40 and 60 DAT the highest plant height 22.71, 41.62 and 73.74 cm, respectively were obtained from A_3 (0.25 mM SA + 5mM Ca^{2+}) over the control plants. At 20 DAT, no significant difference was found with sole or together application of SA and Ca^{2+} on number of leaves plant^{-1} but the results showed significant variation at 40 and 60 DAT. The maximum number of branches plant^{-1} was 8.44 with the A_3 (0.25 mM SA and 5 mM Ca^{2+}). In case of SPAD value, there was a significant variation which affected by different levels of salicylic acid (SA) with calcium (Ca^{2+}) on tomato plant which was highest (58.64) at A_3 (0.25 mM SA and 5 mM Ca^{2+}). The maximum number of flower clusters, flowers and fruits plant^{-1} 20.44,168.1 & 99.42, respectively were obtained from the plants which received 0.25mM SA and 5 mM Ca^{2+} (A_3). The highest fruit length and diameter 6.51 cm and 5.75 cm, respectively were obtained from A_3 (0.25 mM SA and 5 mM Ca^{2+}). The maximum yield plot^{-1} 19.59 kg was recorded from A_3 (0.25 mM SA and 5 mM Ca^{2+}). The maximum yield (72.57 t ha^{-1}) was obtained from A_3 (0.25 mM SA and 5 mM Ca^{2+}), whereas the minimum yield (47.45 t ha^{-1}) was obtained from A_0 (0 mM SA and 0 mM Ca^{2+}). The highest (135) low temperature injury alleviation (%) was recorded from A_3 whereas the lowest (85.64) one was recorded from A_0.

The combinations of transplanting time and salicylic acid with calcium had significant effect on almost all growth and yield contributing parameters. The tallest plant height 24.03, 45.61 and 75.66 cm at 20, 40 and 60 DAT respectively were recorded from T_1A_3 (First transplanting time with 0.25 mM SA and 5 mM Ca^{2+}) treatment combination. The results showed non-significant difference at 20 DAT but significant difference at 40 and 60 DAT, maximum number of leaves plant^{-1} 6.67, 12.66 and 73.78, respectively were recorded from T_1A_1, T_1A_3 and T_1A_3. The maximum number of branch plant^{-1} 10.00 was found in T_1A_3 treatment combination (First transplanting time with 0.25 mM SA and 5 mM Ca^{2+}). Different combinations of time of transplanting and salicylic acid along with calcium also had significant effect on SPAD value of tomato, the highest SPAD value (61.63) was observed from the T_1A_3 treatment. All the yield contributing characters of

tomato showed significant variations with all treatment combinations. The maximum number of flower clusters plant^{-1} (24.44), flowers plant^{-1}(180.8), fruits plant^{-1}(110.5), fruit diameter (5.92 cm) and fruit length (6.70cm) were found in T_1A_3 (First transplanting time with 0.25 mM SA and 5 mM Ca^{2+}) treatment combination. The maximum yield 23.39 kg plot^{-1} was recorded from T_1A_3 treatment. The highest yield 86.62 t ha^{-1} was obtained from T_1A_3 (First transplanting time with 0.25 mM SA and 5 mM Ca^{2+}) treatment, whereas the lowest yield 36.59 t ha^{-1} was obtained from T_3A_0 treatment combination, (third transplanting time and without salicylic acid 0 mM SA and calcium 0 mM Ca^{2+}). The highest low temperature injury alleviation (%) was 161.2 which was recorded from T_1A_3 whereas the lowest one (68.09) was recorded from T_3A_0 treatment combination.

Taking into account 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 salicylic acid (SA) along with calcium (Ca^{2+}).Therefore, the present experimental results suggest that the combined use of 0.25 mM SA with 5 mM Ca^{2+} can alleviate the detrimental effect of late transplanting-induced low temperature injury 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 can be suggested:

1. Such study is needed in different Agro-Ecological Zones (AEZ) of Bangladesh for resemblance the accuracy of the experiment.
2. It needs to conduct more experiments with transplanting time and SA with Ca^{2+} to find out whether these can regulate the morpho-phygiology and yield of tomato var. BARI tomato 15.
3. Needs to conduct related experiments with other tomato varieties.
4. Scope to conduct advance experiments how transplanting time and SA with Ca^{2+} physiologically increase the tomato yield.

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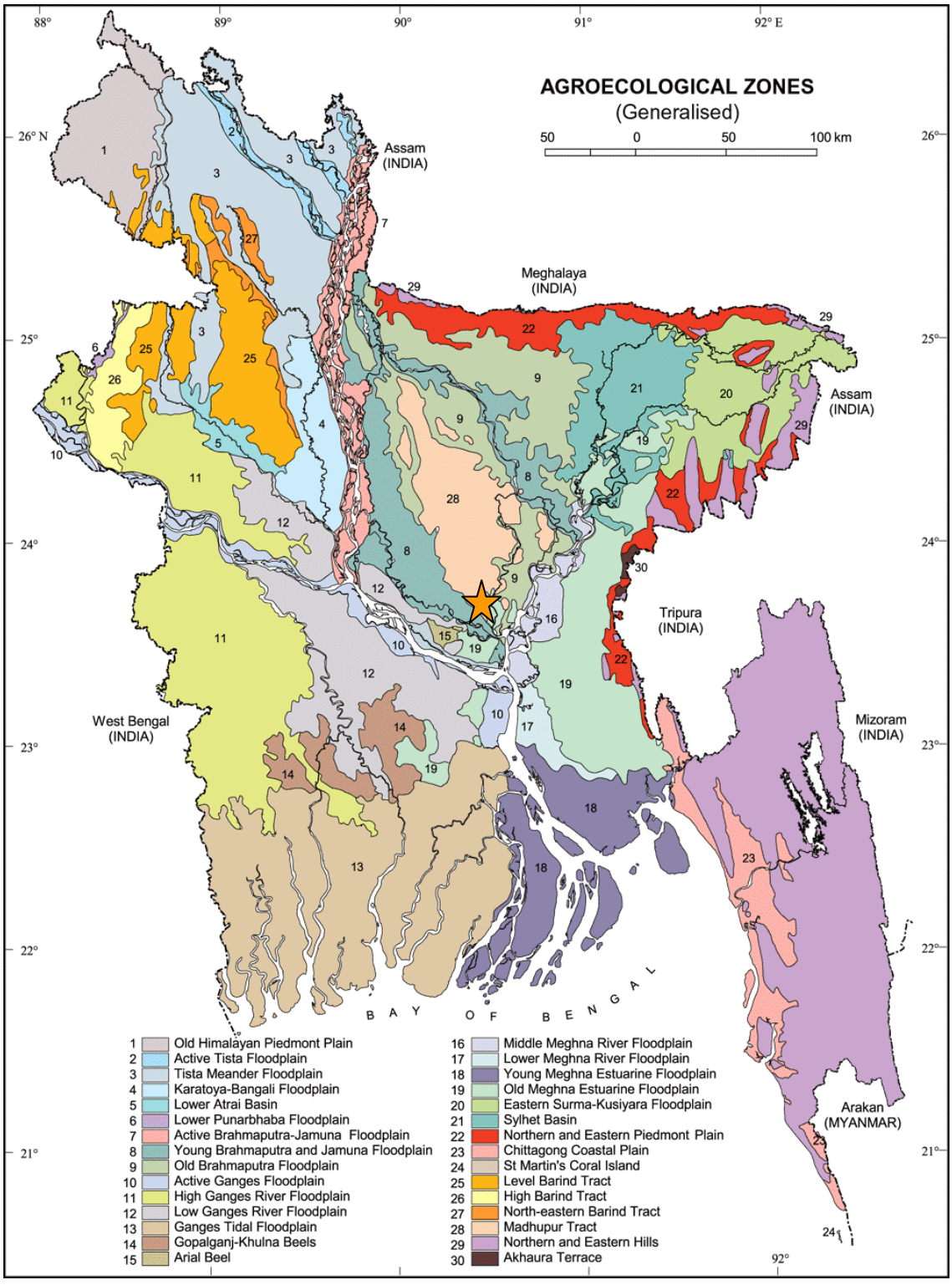
Appendices

APPENDICES

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

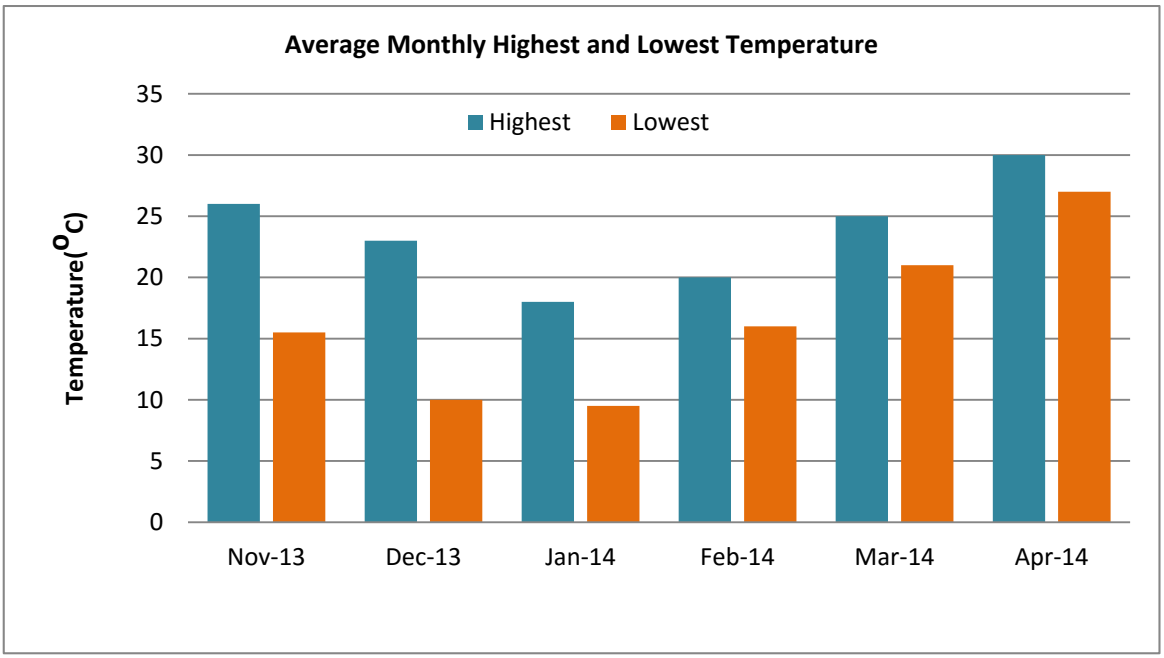


★ **The experimental location under study**

Appendix II: Physical and Chemical composition of soil sample of (0-15) cm depth

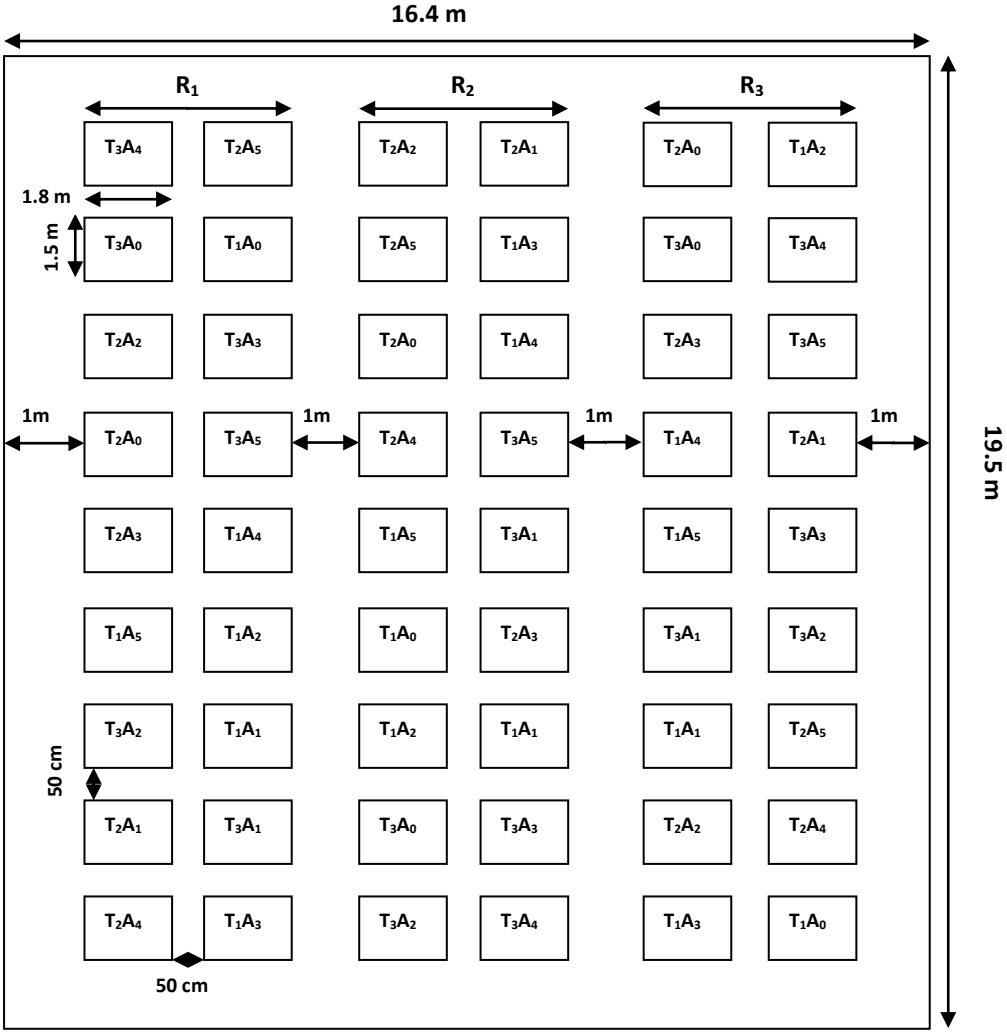
Characteristics of soil	Value	Methods employed
Sand (%)	24.75	Hydrometer Method (Day,1995)
Silt (%)	51.44	-do-
Clay (%)	23.81	-do-
Textural class	Silt loam	-do-
Organic matter (%)	0.82	Walkley and Black, 1947
p^H	6.05	Jackson, 1958
Total N (kg/ha)	1790.00	Bremner and Mulvaney, 1965
Total S (ppm)	225.00	Bardsley and Lancster, 1965
Total P (ppm)	842.00	Olsen and Sommers, 1982
Exchangeable K (kg/ha)	89.60	Pratt, 1965

Appendix III: Monthly averaged highest and lowest temperature of SAU campus during November 2013 to April 2014



Source: Weather station, Sher-e-Bangla Agricultural University, Dhaka-1207.

Appendix IV. Layout of the experimental plot

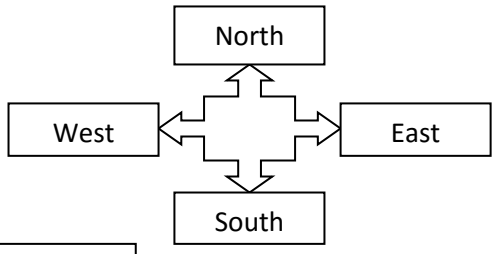


Date of transplanting:

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 composition of SA and Ca^{2+} as alleviating agent of low temperature injury

A_0 – 0 mM SA + 0 mM Ca^{2+}, A_1 – 0.25 mM SA + 0 mM Ca^{2+}

A_2 – 0 mM SA + 5 mM Ca^{2+}, A_3 – 0.25 mM SA + 5 mM Ca^{2+}

A_4 – 0 mM SA + 10 mM Ca^{2+}, A_5 – 0.25 mM SA + 10 mM Ca^{2+}

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

Sources of variation	Degrees of freedom	Mean square of plant height (cm) at		
		20DAT	40DAT	60DAT
Replication	2	0.395	28.341	0.738
Factor A	2	80.788**	301.095 **	266.473**
Factor B	5	26.275**	184.571**	310.728**
Ax B	10	0.829^{NS}	3.553*	11.299 *
Error	34	0.937	7.379	6.571

*significance at 5% level of probability
**significance at 1% level of probability
NS-Non Significant

Appendix VI: Analysis of variance of the data on number of leaves $plant^{-1}$ of tomato as influenced by different transplanting time and SA along with Ca^{2+}

Sources of variation	Degrees of freedom	Mean square of number of leaves $plant^{-1}$ at		
		20DAT	40 DAT	60 DAT
Replication	2	0.636	1.403	46.236
Factor A	2	10.713**	48.511**	3640.652**
Factor B	5	0.346^{NS}	4.828*	217.036**
Ax B	10	0.304^{NS}	0.102*	25.253*
Error	34	0.284	1.234	18.285

*significance at 5% level of probability
**significance at 1% level of probability
NS-non significant

Appendix VII: Analysis of variance of the data on number of branches plant^{-1} of tomato as influenced by different transplanting time and SA along with Ca^{2+}

Sources of variation	Degrees of freedom	Mean square
		Number of branches plant^{-1}
Replication	2	0.213
Factor A (T)	2	66.625**
Factor B (C)	5	4.393*
Ax B	10	0.197*
Error	34	0.742

*significance at 5% level of probability
**significance at 1% level of probability
NS-non significant

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

Sources of variation	Degrees of freedom	Mean square
		SPAD value of tomato leaf
Replication	2	7.398
Factor A (T)	2	147.314**
Factor B (C)	5	13.156**
Ax B	10	0.322*
Error	34	3.823

*significance at 5% level of probability
**significance at 1% level of probability
NS-non significant

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

Source of variation	Degree of freedom	Mean square				
		Number of flower clusters plant^{-1}	Number of flowers plant^{-1}	Number of fruits plant^{-1}	Fruit diameter (cm)	Fruit length (cm)
Replication	2	21.208	16.386	727.403	0.026	0.014
Factor A (T)	2	237.164***	1132.362***	796.322*	0.209*	0.635**
Factor B (C)	5	161.825***	1985.068***	1464.017**	0.439*	0.918**
Ax B	10	2.484*	51.544*	24.846*	0.006*	0.012*
Error	34	4.881	87.330	77.511	0.069	0.014

*significance at 5% level of probability
**significance at 1% level of probability
NS-non significant

Appendix X: Analysis of variance of the data on yield (kg plot^{-1}), yield (t ha^{-1}) and low temperature injury alleviation (%) of tomato as influenced by different transplanting time and SA along with Ca^{2+}

Source of variation	Degrees of freedom	Mean Square		
		Yield (kg plot^{-1})	Yield (t ha^{-1})	Low temperature injury alleviation (%)
Replication	2	47.168	647.105	2240.212
Factor A (T)	2	144.403**	1980.749**	6859.413**
Factor B (C)	5	58.378**	800.745**	2818.936**
Ax B	10	3.211*	44.047*	129.424*
Error	34	3.934	53.947	186.848

*significance at 5% level of probability
**significance at 1% level of probability
NS-non significant