Effect of Different Concentration of 4-Chlorophenoxyacetic Acid on Fruit set of Summer Tomato.

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A Thesis

Submitted to

Biotechnology and Genetic Engineering Discipline, Life Science School, Khulna University, Khulna in partial fulfillment of the requirements for the degree of 1 year

'Master of Science (M.S.) in Biotechnology and Genetic Engineering'

Submitted by

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Examination Roll No: MS-070715
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Declaration

This thesis reports the original research work of the author, except as otherwise stated. The material presented has not been submitted either in whole or in part for a degree from this or any other university. The findings of the research has not been/will not be published anywhere without the concurrence of the concerned supervisor/co-supervisor.

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The Author

ABSTRACT

Effect of 4-chlorophenoxyacetic acid (4-CPA), an artificial plant growth regulator, was studied on fruit-set of summer hybrid tomato line, BARI hybrid tomato-4, at the Horticulture Research Center, (HRC), Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute (BARI), Jessore, during April to October 2008. Five concentrations of 4-CPA viz., 10, 20, 30, 40 and 50 ppm were tested against BARI hybrid tomato-4. Flowers treated with 20 ppm were found suitable to enhance maximum fruit-set. This concentration was applied to evaluate the performance of the hybrid lines FP_5 X MP_5, WP_7 X C-11, WP_8 X C-11 and WP_8 X C-51 and BARI hybrid tomato-4 as check. Individual fruit weight, fruit per plant, as well as yield of hybrid line WP_8 X C-11 were found superior as compared to the check while fruit weight and yield were the lowest in hybrid line FP_5 X MP_5. Early harvest following minimum days to flowering was recorded in BARI-hybrid tomato 4 and FP_5 X MP_5. All the varieties responded positively when flowers treated with 20 ppm 4-CPA at the anthesis.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
^0C	Degree Celsius
2,4-D	2,4-Dichlorophenoxy acetic acid
4-CPA	4-Cholorophenoxyacetic acid
Cm	Centimeter(s)
Conc.	Concentration(s)
CV	Coefficient variant
e.g.	exempli gratia=for example
et al.	*et alii/alia*=and other people
etc.	etcetera=and the others
g	Gram(s)
GA_3	Gibberellic acid
GAs	Gibberellins
i.e.	*id est*=that is
IAA	Indole-3-acetic acid
IBA	Indole-3- butyric acid
Kg	kilograms
L	Liter
mg	Milligram(s)
mg.	L^{-1}Milligram(s)per liter
ml	Milliliter
NAA	B-Napthoxyacetic acid acid
PGR	Plant growth regulator
ppm	parts per million

RCB	Randomized Complete Block Design
t/ha	ton/hactor
Wt.	Weight
X	Times

CHAPTER ONE

INTRODUCTION

INTRODUCTION

Tomato is a very popular consumable vegetable crop in Bangladesh. It is consumed freshly as salad and culinary purposes. There is a high potential of export value of processed products form tomato. Sauce, ketchup, jam, jelly, pickles and other ready to serve foods for the millennium people can be processed form tomato. It ranks high for its nutritive value. It is a good source of vitamin A, B, C and calcium, iron etc (Mohiuddin et al, 2007). It has wonderful medicinal value too. Consumption of tomato is believed to benefit the heart diseases. Lycopen, one of the most powerful natural antioxidants, prevents prostate cancer (Bhutani and Kallo, 1983), reduces blood pressure and improves ability of skin to protect against harmful UV rays. Due to palatability, vitamin content and health benefit, demand of tomato is growing day by day through out the year, while its production is far from the requirements especially in summer season. Temperature, rainfall, humidity and light intensity are the basic limiting factors of tomato production in summer (Abdulla and Verkert, 1968; Villareal, 1980). There is a serious scarcity of vegetables in summer in Bangladesh. As a result the price of vegetables rises during this period. There is a great potentiality to grow tomato in summer through development of adapted varieties as well as exogenous application of some plant growth regulators (Iwahori 1968). Bangladesh Agricultural Research Institute has developed a number of tomato varieties capable to produce flower and fruits under the prevailing climatic condition of Bangladesh. Floral application of 4- chlorophenoxyacetic acid (4-CPA), a synthetic plant growth regulator promotes fruit set in extreme summer climatic conditions of the tropics (AVRDC, 2003).

Therefore, the study was undertaken to evaluate the effect of different concentration 4-CPA on different summer tomato cultivars to increase fruit set in summer and thereby for a successful production tomato in summer.

CHAPTER TWO

REVIEW OF LITERATURE

RIVIEW OF LITERATURE

Considerable interest has developed recently regarding the benefit fro the use of plant growth regulators on fruit set of in fruits tress and vegetable crops. Gustafson (1936) was the first to demonstrate that a specific chemical could be used to achieve fruit set and full organ development with out pollination. In addition, hundreds of studies dealing with the improvement of fruit-set of all kinds of vegetables, berries and fruits have been reported by several scientists. However, some of the important works are reviewed here.

Origin and distribution of tomato:

According to Kaloo (1986) origin of tomato might Peru-Ecuador-Bolivia area of the Andes. Jenkins (1984) mentioned Veracruz-Puebla might be the source of cultivated tomato. Tomato came in the South America from Mexico. The cultivated tomato presumably might have been carried form Andes into Central America and Mexico by the Indian at the time of their migration. In Italy yellow fruit tomato was known since 1544. The red fruited tomato might have been known in 1554 and gradually sprayed to other parts of the world. The Italian grew it in about 1550 and after 25 years it was introduced to the English, Spanish and in mid-Euro. In the USA, the plant was grown in Virginia in 1781 (Muller, 1940 and Jenkins, 1984).

Botany of tomato:

Tomato (*Lycopersicon esculentum*) belongs to the genus *Lycopercicon* of the family *Solanaceae*. Usually it is herbaceous, annual to perennial, prostrate and sexually propagated. Occasionally, however, it can be propagated asexually by cuttings.

Root:

Tomato produce strong tap root and develop profuse adventitious root if main root damage at the time of transplanting. The adventitious roots penetrate 1 m depth and attaining a length of 1.5 m (Ward, 1664).

Stem:

The stem is soft, brittle and hairy when young and hard, woody and copious branched when mature. Its organization is two or three sympodia. It is erect to semi-erect, reaching to 1-3m in height. It has a weak, woody stem that often vines over other plants.. Epidermal hairs with oil glands are present at the early plant growth stage. The plant can be classified into three groups on the basis of their growth habit (Byrne et al., 1975).

Fig. 1. Tomato Plant

1. Determinate self-topping (bush): In this case the lateral axis terminates in blossom which gives self-topping. There appear less than three leaves between the flower cluster
2. Determinate self-topping, dwarf: In this type the development of the growing point is ceased and the plants are upright, the stem is short without a side-shoot and the leaves are thick dark-green.

3. Indeterminate: The growth is continued and there is less initiation of flowers and fruit on the stem, the lateral buds always exits to continue vegetative growth. However, semi-determinate type growth of tomato is not uncommon.

Leaf:

Leaf is alternate, petiolate, leaf-lets are unequal odd pinnate, and the apex is narrowed or acuminated, acute irregularly serrated. Number of leaves varies from 4 to several and the length of leaf ranged from 15-30 cm. On the leaves, har or trichomes are formed by the outer growth of the epidermal cells (Flores and Spinz, 1977).

Inflorescences:

Inflorescence is the extra-auxiliary cymes with dicotomous or polycotomous branching. It is lateral and arises from the stem opposite to the leaf. It may be terminal or may continue with the vegetative shot (Mular, 1940). The early flowers that open in the cluster are large-sized as typical cymes. The number of flower per cluster varies from three to several. Inflorescence apex arises from the terminal directly from the vegetative ape (Sawhney and Greyson, 1972).

Flower:

The flower is cleistogamous and produced in cluster on the stem. Flowers are yellow in color, pentamerous, bisexual, regular, complete, ebracteate and hypogynous. The pistil has two to several carpels. Usually the style is shorter than anther cone which permit high degree of self-pollintion (Calvert, 1965).

Fig. 2. Tomato Flower

The anthesis of flowers starts in the morning at about 6 am with maximum flower opening in the late morning. The stigma become receptive 16 hours before anthesis and remain thus for 5 days after anthesis. The maximum number of anther dehiscence takes place from 8-11 am depending upon the season. The pollen remains viable for a long period 2- 4 days at temperature 20-25 ^{0}C. It remains viable for 6 months at 5 ^{0}C in desiccators. The optimum temperature for pollen germination is 18-25 ^{0}C.

Fig. 3. Fruits of Tomato

Fruit:

The fruit is soft berry of various shapes and possessed five main parts: wall (outer and inner) skin, locular tissue, pulp and seed. The layer of the messocarp increases the size of the cells resulting in the growth of the pericarp. There are variations in the number of

locules (two to several). Each locule has a lot of seeds which surrounded by a jelly – like substance.

Seed:

The seed is oval, flattened, buff of pale brown, 3-5 mm in length. It is developed from the anatropous ovule. A curved filiform embryo and an endosperm are enclosed in the seed. The embryo is surrounded by a small enclose pore (Czaja, 1963).

Climatic Requirements:

Tomato production is highly influenced by environmental factors particularly temperature high light and CO_2. Fruit set and ripening are temperature dependant and when the temperature exceeds 30 ^{0}C or goes below 14 ^{0}C, these parameters are adversely affected (Went, 1944). Under high temperature morphological changes in the flower structure such as style extension out of the antheridial-cone browning and drying of the stigma are observed (Abdulla and Verkerk, 1968). High temperature can also affect the germination and the elongation of the pollen tube into the style and thus inhibit fertilization of the stigma (Iwahori & Takahashi, 1964). High temperature intensity can also affects the internal temperature of the reproductive organ of tomato (Kuo et al., 1978).

Fruit set:

Successful plant reproduction depends entirely on fruit-set, an essential process that can be defined as the activation of a developmental program which converts the pistil into a developing fruit. This transition comprises two different and coordinated processes, namely the fertilization of the ovule and the growth of the surrounding pistil and/or other structures to allocate the developing seeds (Gillaspy et al., 1993). The environmental factors particularly temperature, light and CO_2 highly influence fruit set

in tomato. Subsequent work has demonstrated that fruit set in general is at least in part a function of auxin in the ovary. As a result, the fruit set at high and low temperature is improved by the application of certain groups of growth regulator (Leopold & Scott, 1952). Some of the important literatures are reviewed here.

Effect of Varieties on Fruit set of Tomato:

Due to environmental changes any variety is not always equally superior in respect of yield and resistances to many biotic and abiotic factors. As a results development of varieties is a continuous process to cope with the environmental factors. Bangladesh Agricultural Research Institute (BARI) has released 18 tomato cultivars, out of which 5 are hybrids. Two open pollinated cultivars BARI tomato- and BARI tomato-5 and two hybrids BARI hybrid tomato-3 and BARI hybrid tomato-4 are capable to produce flowers and fruits in summer season. BARI hybrid tomato-4 is most popular varieties due to higher yield, attractive fruit color, size and round shape and adaptive to wide range of agro-climatic condition.

BARI has developed some more hybrid lines of tomato which are highly potential against virus and abiotic stresses e.g., salinity, draught and high temperature as well as light intensity (BARI- Projukti Hat Boi, 2006). Some cultivars capable to fruit-set in the prevailing summer season of Bangladesh during late 90s'. Some of the cultivars were highly responsive to growth regulator regarding fruit set. However, the response of growth regulators in fruit-set was also dependent on temperature. From the experience of Plant growth regulators and fruit-set in summer tomato, BARI realized to invent cultivars suitable to summer season of Bangladesh.

Effect of Temperature on Fruit set of Tomato:

Year round tomato production in Bangladesh is constrained by many factors of which seasonality and multiple disease problems are the two main (Zaman *et al.* 2006). Higher temperature before and after cool season interferes fruit set and its development. So, there is a limitation of growing tomato over a longer period (Adlskaha and Verma, 1965). Fruit setting in tomato is reportedly interrupted at temperature above 26/20 ^{0}C day/night respectively and often completely arrested above 38/27 0C day/night (Stevens and Rudich, 1978; El-Ahmadi and Stevens, 1979; Kuo *et al.* 1979).

Fruit set is usually poor, when the temperature is either relatively low or relatively high. Moore and Thomas (1952) found that when the average maximum temperature was above 90 °F fruit set was low. Ivakin (1977) reported that tomato varieties differed to heat and drought.

In the Garden information series, Dennis R. Pittenger stated in the column entitled "Growing Tomatoes in your Garden" that tomato plants might fail to set fruit for several reasons. The most frequent problems are:

- Cold nights in the spring-several consecutive evenings with temperatures below 55°F.
- High daytime temperatures in the summer.
- Low light intensity created by dense shade from trees, buildings or other sources.
- High applications of nitrogen fertilizer or manure.
- Too much or too little water.
- Smog or ozone.

Fruit set and pollination mainly depend on the sensitivity of temperature. Under high temperatures, morphological changes in the flower structure such as style exertion out of the antheridial-cone, browning and drying of the stigma (Abdalla and Verkerk, 1968) are observed.

High temperatures induce drop of buds and flowers in the tomato (Leopold and Scott, 1952; Leopold and Kriedemann, 1975; Abdalla and Verkerk, 1968). Flower drop is essentially a result of lack of fertilization which, in turn, is affected by a number of factors. Under high temperatures, gametogenesis is disturbed, gamete viability is reduced (Iwahori and Takahashi, 1964; Iwahori, 1965, 1966) and less pollen is produced in the flower (Abdalla and Verkerk, 1968). High temperatures can also affect the germination and the elongation of the pollen tube into the style and thus inhibit fertilization (Iwahori and Takahashi, 1964; Iwahori, 1967). Any of these changes may prevent or reduce self-pollination, resulting in low rates of fruit set.

If the day and night temperature remain cool within the range of 26°C to 12-22°C respectively, the flower initiation set off early and fruit development begins very rapidly (Halder *et al.* 2003). High temperatures have been reported to limit tomato production wherever days are warmer than 32 °C, or nights are warmer than 21°C (Moore and Thomas, 1952). Because of this high temperature sensitivity, poor fruit set is an important limitation to tomato production during summer season (Villareal, 1980). At higher temperature, the probability of floral abscission is high after anthesis (Iwahori 1967). High light intensity accompanied by high temperature is also harmful to fruit-set. High light intensity affects the internal temperature of the reproductive organ of tomato (Kuo *et al.* 1978).

It has been reported that reproductive development is more sensitive to high temperature stress than vegetative development, but the process sensitive to the stress appears to be different for each crop. In tomato (*Lycopersicon esculentum*), temperature increase from 28/22 (day/night)°C to 32/26 °C significantly decreased fruit set, but did not affect photosynthetic rate or night respiration in both high temperature-sensitive and -tolerant cultivars (Sato *et al.*, 2000). The reduction of fruit set under moderately

elevated temperature stress was mostly due to a reduction in pollen release and viability. In groundnut (*Arachis hypogaea*), however, a reduction in pod number by high temperature was caused by a reduction in pollen number and viability (Vara Prasad *et. al.* 1999). Peet *et al.* (1997) reported that the yield of tomato decreases as mean daily temperature increases, and the magnitude of the effect was the same in day and night temperatures. In the experiment under artificial light conditions, a moderate increase of average temperature did not affect vegetative growth of tomato (i.e. photosynthesis, night respiration and biomass) but impaired reproductive development. Specifically, pollen viability and release were affected adversely and became major factors limiting fruit set (Sato *et al.*, 2000, 2005). The narrow window of male reproductive development, 8–13 d prior to anthesis, is the period most sensitive to moderately elevated temperature stress (Sato *et al.*, 2002).

In many crops, not only is the reproductive process more sensitive to high temperature stress than vegetative growth, but within the development of an individual flower, certain stages are also more sensitive. In groundnut, the number of pegs and pods per plant were significantly reduced by high temperature, by duration of exposure, and by timing of exposure. In tomato, it was reported that meiosis in both mega- and microspore mother cells, which took place 8–9 d before anthesis, was very sensitive to high temperature (Iwahori, 1965, 1966). Iwahori also reported that ovules subjected to a temperature of 40 °C 18 h after pollination aborted, perhaps due to inhibition of pollen tube growth and endosperm degeneration (Iwahori, 1966). In wheat, the period of microsporogenesis is also sensitive to other environmental stresses, such as drought (Saini and Aspinall, 1981; Sheoran and Saini, 1996; Lalonde *et al.*, 1997). Kliewer (1977) examined that the percentage of berries set, ovule fertility and berry weight were significantly greater in several grape cultivars at 25°C than at 35 or 40°C.

In experiments with male-sterile tomatoes, it was demonstrated that microsporogenesis was more sensitive to moderately elevated temperatures than megasporogenesis and post-anthesis processes (Peet *et al.*, 1998). Pre-anthesis high temperature treatments to *Brassica napus* was also shown to reduce pollen fertility (Morrison, 1991). A recent experiment with five tomato cultivars differing in temperature sensitivity indicated that when tomato plants grown at 32/26 °C were placed at 28/22 °C for 10 d before anthesis, the number of pollen grains released and germinated increased, which resulted in more fruit set than when plants remained in the high temperature treatment (32/26 °C, Sato *et al.*, 2000). The duration and timing of the sensitive period during microsporogenesis and immediately after pollination was not reported in either of these studies, however. Also, the developmental process leading to the failure of pollen release and the reduction of pollen viability was not described.

Anther indehiscence was reported with other tomato cvs grown under high temperature regimes (Charles and Harris 1972, Ahmadi and Stevens 1979, Rudich *et al.* 1977) and it has been ascribed to abnormal development of the endothecium layer (Rudich *et al.* 1977). Likewise, stigma exertion, a direct cause of flower drop (Rick and Dempsey, 1969), low pollen viability and production (Charles and Harris 1972, Ahmadi and Stevens 1979), low ovule viability (Ahmadi and Stevens 1979) and embryo abortion (Iwahori, 1966.) were also reported to be induced by high temperature. High temperature prevented normal pollen and ovule development and enhanced style elongation resulting from the failure of fertilization for seed production (Ahmedi and Stevens 1979).

In tomatoes, there is little consensus on whether pollen or ovule effects are more important at high temperatures, and none of this work has targeted night temperature effects. Levy *et al.* (1978) used cross-pollination experiments on fertile plants to

compare male and female effects. They reported that overall, with high daytime temperatures, (nighttime temperatures were the same), effects on male gametes seemed more important than those on female gametes. Reduction in pollen fertility at high temperatures have been noted by Dane *et al.*, 1991 and a decline in pollen germination as germination temperatures increased from 20–27°C by Charles and Harris (1972).

High-temperature conditions (39 ± 2°C day and 22 ± 2 °C night) caused deficient fruit set in tomatoes (*Lycopersicum esculentum* Mill.). Fruit set of 12 cultivars from divergent sources ranged from 0% to 22%, while that of `Saladette' was between 56% and 60%. The impaired fruit set of `Roma VF' was found to be associated with pollen viability, style elongation, and lack of formation of the endothecium, which is essential to stamen and pollen thecae opening. In `Saladette,' damage was slight or nonexistent, and the endothecium formed normally under high-temperature conditions (Rudich *et al.* 1977).

Charles and Harris (1972) stated that fruit setting of tomatoes need night temperature of 15 to 20°C, which does not prevail any where in Bangladesh during May to September. Excessive day and night temperatures above 32°C and 21°C, respectively, were reported as limiting fruit-set due to an impaired complex of physiological process in the pistil, which results in floral or fruit abscission (Picken,1984).

Classical studies of Went and co-workers in the 1940 are often cited as the basis for the importance of night temperature effects in tomatoes. Based on studies in growth chambers (Went, 1945) and in air-conditioned greenhouses (Went, 1944; Went and Cosper, 1945) the critical factor in the setting of tomato fruit was reported to be night temperature, the optimum range being 15 to 2°C (Went,1944).

Experiments were carried out to study fruit-set in a John Baer self-sterile strain of tomatoes. Fruit-set was induced either by pollination or by an auxin spray. Experiments with excised flowers have demonstrated that the capacity for fruit-set is dependent upon temperature, and this dependence is inherent to the ovary itself. An optimum range of approximately 18^0C - 22^0C was found (Leopold and Scott, 1952).

The observation that high temperature reduced the fruit set coincides with the results of Iwahori and Takahashi (1964) that the tomato flowers, one to three days after anthesis are especially sensitive to high temperature and fail to set fruits. Abdul-Baki and Stommel (1995) reported that no fruit set occurred in the most heat-sensitive genotypes of tomato under high temperature (35/20°C).

Sato *et al.* (2006) conducted research regarding the averse effect of high temperature on fruit set of tomato. Their research indicated that failure of tomato fruit set under a moderately increased temperature above optimal is due to the disruption of sugar metabolism and proline translocation during the narrow window of male reproductive development.

Effect of Plant Growth Regulators on Fruit-set of Tomato:

Hormones seem to have a prominent role in synchronizing fertilization and fruit growth. As early as the 1930s it was proposed that fruit development would be initiated as a consequence of hormones synthesized in developing seeds (Gustafson, 1936, 1939; Nitsch, 1950, 1952). Later work showed that early abortion of the fertilized ovules prevented fruit development, but fruit growth was restored by application of several plant growth regulators (Eeuwens and Schwabe, 1975). The involvement of hormones in fruit-set is thus supported by the major observation that exogenous application of hormone aids in fruit setting.

Plant hormones are considered to be important mediators of the fruit developmental signal after pollination. The role of phytohormones in tomato (*Solanum lycopersicum*) fruit set was investigated by Vriezen and Co-workers (2007). Transcriptome analysis of ovaries was performed using two complementary approaches: cDNA–amplified fragment length polymorphism (AFLP) and microarray analysis. The gene expression profiles obtained suggest that, in addition to auxin and gibberellin, ethylene and abscisic acid (ABA) are involved in regulating fruit set. Before fruit development, many genes involved in biotic and abiotic responses are active in the ovary. In addition, genes involved in ethylene and ABA biosynthesis were strongly expressed, suggesting relatively high ethylene and ABA concentrations before fruit set. Induction of fruit development, either by pollination or by gibberellin application, attenuated expression of all ethylene and ABA biosynthesis and response genes within 24 h. It is proposed that the function of ABA and ethylene in fruit set might be antagonistic to that of auxin and gibberellin in order to keep the ovary in a temporally protected and dormant state; either to protect the ovary tissue or to prevent fruit development before pollination and fertilization occurs. (Vriezen *et al.* 2007).

The actions of plant regulators in set and development of fruits are well known. However, the presence and function of endogenous hormones in parthenocarpic fruits have still not been sufficiently investigated. A comparison between seeded and seedless fruits makes it possible to obtain a more accurate understanding of some relationships between growth regulators and stages of fruit development. Endogenous auxin and gibberellin activity levels and some growth parameters (fresh and dry weight, cell number and cell volume, DNA content) have been determined in tomato fruits (*Lycopersicon esculentum* Mill.) of the cultivar Ventura and of its isogenic parthenocarpic mutant. In both genotypes, auxin and gibberellin are present in the first

week after anthesis, though at different concentrations and with different patterns. These two activities are involved in fruit setting. The simultaneous occurrence of maximum auxin concentration and of the beginning of cell enlargement, in both genotypes, shows that the activity present at this time starts fruit development and possibly determines the size of the fruits. High auxin activity is observed only in seeded fruits 20–40 days after anthesis, and it is probably synthesized by seeds. Gibberellin activity is present, corresponding to the change in fruit development from the mature green to pink stages (Mapelli *et al.* 1978).

A major contribution to the understanding of fruit set was made by Gustafason (1936) who showed that in some plant the simple application auxins could bring about fruit set even without pollination. It was evident that percent fruit set and subsequent fruit development was directly related to the amount of PGR's (particularly auxin) present in the ovary (Gustafson, 1939, Kim *et al.*, 1992).

Fruit development is under the influence of the plant hormones auxin and gibberellin. Pollen is one of the richest natural sources of auxin, so it is thought that the initial stimulus to fruit growth comes simply from the auxin to which the pistil is exposed as a result of pollination (Sjut and Bangerth, 1981). If pollination and fertilization do not take place, the ovary usually ceases growth and the entire flower abscises. A study by Muir (1947) has shown that diffusible auxin is not present in the ovary of tobacco at anthesis but, following pollination, diffusible auxin appears in the style and later in the ovary. This diffusible auxin is correlated with the growth of the ovary into a fruit. Gustafson (1939) found a higher auxin content in ovaries of certain varieties of oranges, lenmons, and grapes which develop into fruits without pollination and fertilization than was present in the ovaries of the varieties requiring pollination and

fertilization for growth to occur. This finding suggests that perhaps in some instances the auxin in the ovary is not produced there but is derived from the parent plant.

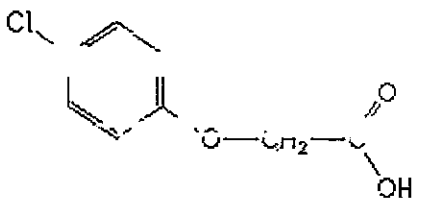
High temperatures reduce fruit set and fruit production in tomato (Peet *et al.* 1997), in eggplant (Sanwal *et al.* 1997) and also in bell pepper (Erickson and Markhart, 2001). High temperature treatment decreased the levels of gibberellin-like substances in developing fruit of tomato (Kuo and Tsai, 1984). Use of growth hormone like Auxin may help to overcome the problem of high temperature barrier of fruit-setting. Homan (1964) and Alabadi *et al.* (1996) reported that auxin is the hormone with a larger capacity to induce fruit-set in tomato. Applying the hormone auxin or gibberellin to the ovaries of flowers can induce a fruit to develop without pollination or fertilization (http://www.crfg.org/tidbits/gibberellic.htm).

Plant growth regulators such as auxin and gibberellins are known to affect parthenocarpy (Matlob and Kelly, 1975) and fruiting (Rappaport, 1957); therefore, synthesized auxin and gibberellins are often used for promotion of fruit set in some fruit vegetable production including tomatoes. A role for auxin in promoting fruit-set and development has been revealed by recent work in Arabidopsis and tomato, species in which key elements in auxin signaling with a function in fruit initiation and development have been identified (Swain and Koltunow, 2006; Pandolfini *et al.*, 2007).

Spray applications of fruit-setting hormone products can improve fruit set (Dennis R. Pittenger, Garden Information Series, 2nd edition). Some of the plant hormones which help fruit setting in the tomato plants in hot climate are 4-chlorphenoxyacetic acid (4-CPA), Gibberellic Acid (GA_3), and B-naphthoxyacetic acid (NAA), (www.tomato-info.com). The active ingredient is the man-made hormone 4-Chlorophenoxyacetic acid (4-CPA) which is well known to promote fruit set commercially.

4-CPA (4-chloro-phenoxyacetic acid) is a synthetic auxin, which has significant physiological activity on many fruits, including tomato. . In food industries 4-CPA is used as a plant growth regulator to restrict root growth during seed germination of mung beans (United States EPA, 1997). 4-CPA was first registered as a pesticide in the U.S. in 1969 for use as a plant growth regulator for mung beans, and later as a fruiting bloom set for tomatoes. 4-CPA did not exhibit mutagenic potential.

Initial bioassays indicated that 4-CPA ensure fruit set by initiating cell division in ovary (Salisbury and Ross, 1992). Molecular formula $C_8H_7ClO_3$ and structural formula



The host molecule of complex *A* is significantly distorted, as a glucosidic residue rotated about the O4'—C1 and C4—O4 bonds forms an aperture where the guest molecule is accommodated. The molecule has to contain a ring system with at least one double bond. The double bond has to be adjacent to a side chain. A carboxyl group that is separated by one or two C-atoms is required.

Auxins play an important role in stimulating cell division and cell enlargement, as well as the delay of tissue senescence and fruit ripening. 4-CPA exhibits auxin-like properties when applied to flower. The primary physiological effects involves the regulation of fruit set, fruit growth and development. When applied immediately prior to anthesis or bloom, 15 to 20 ppm typically increases the set of fruit irrespective of cultivars. 4-CPA stimulates both cell division and cell elongation, resulting in increased berry size (Salisbury and Ross, 1992).

4-CPA is a synthetic hormone and control plant growth by signaling and stimulating the gene. It is not a plant nutrient or vitamin. It triggers signals of competent cells. It does not go into plant tissue as molecules or does not form any structural molecules in plant body. As a signal triggers, the hormone, assumed that it is specifically recognized by competent cells. The hormone achieves this by binding to specific receptors that change into active states

1. Hormone is attached to a binding site of receptor.
2. Binding sites are located on cell membrane and cytoplasm.
3. Binding sites activate a second messenger or chemical signal within the cytoplasm. In plant the best known second messengers are Ca^{++} and phospatidalinositol (PI) system. Ca^{++} regulates the function of hormone by sending signal to the site of action.
4. In cytoplasm second messenger is released by the action of protein kinase (calmudulin).
5. Ca^{++}-calmudulin and PI system activate the second messenger which stimulate gene for enzymatic induction of particular physiological response (Salisbury and Ross, 1992).

HRC/BARI (1996), reported 0.15-0.25% 4-CPA solution ensures cent percent fruit setting. Each flower cluster treated one time after two or three of the flowers are open. Apply two pumps of the solution (about 1 ml) per flower cluster using a handheld mist sprayer after 3:00 PM.

High day (above 32°C) and night (above 21°C) temperatures usually accelerate the abscission of floral organs after anthesis (Iwahori 1967; Picken 1984). At higher temperature, the level of endogenous auxin (IAA-like substance) became low, which arrested the growth of the floral organs and caused abscission (Leopold and

Kriedemann, 1975). The plants treated with hormone reduced flower drop and increased fruit-set, which can be attributed to the preventive effect of Tomato tone (4-CPA) to heat injury. As fruit-setting treatment with 4-CPA caused an increase in GA levels (Sjut and Bangerth, 1981), so exogenous application of the hormone 4-CPA accelerates fruit-set.

Application of GA, IAA or 4-CPA to tomato ovaries induced the development of parthenocarpic fruit, which showed different growth rates. GA_3-induced fruits had considerably less but larger cells than seeded control fruits, IAA treatment resulted in the same number of cells but these were smaller and 4-CPA treatment induced fruits with about 20% more cells. (Kibler and Bangerth, 1982)

(Hidekazu *et al.* 2005) examined the effects of plant growth regulators on fruit set of tomato (*Lycopersicon esculentum* Mill.) under high temperature in a controlled environment and a field under rain shelter. Tomato plants exposed to high temperature (34/20°C) had reduced fruit set. Treatments of plant growth regulators reduced the fruit set inhibition by high temperature to some extent, especially treatment with mixtures of 4-chlorophenoxyacetic acid (4-CPA) and gibberellins (GAs). In the field experiment, tomatoes treated with a mixture of 4-CPA and GAs showed increased fruit set and the numbers of normal fruits (excluding abnormal types such as puffy fruit) were more than the plants treated with 4-CPA alone during summer. These results indicate that high temperature inhibition in fruit set could be reduced by a mixture of 4-CPA and GAs treatment in tomato production.

Halder *et al.* (2003) found that sprays of growth hormone especially tomato tone (4-CPA) on flower cluster effectively increase the fruit set as well as fruit production.

As tomato tone hormone stimulates the normal flow of growth hormone which makes the flower stigma sticky to receive the pollen grain promptly. In their study, the plants sprayed with tomato tone hormone (2% conc.) significantly increased flower cluster, flowers per plants and fruits per cluster than untreated plants. Maximum number of flowers (52.13/plant) and highest number of fruits (4.82) per cluster were recorded by spraying 2% tomato tone hormone. The highest fruit yield (22.26 t/ha) and maximum number of fruits (28.23/plant) were also recorded.

An experiment was carried out at Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh to evaluate the influence of 4-chlorophenoxyacetic acid (4-CPA) on yield of summer tomatoes by Mahmood and Bahar (2006). Two tomato cultivars, namely 'Binatomato-2' and 'Binatomato-3', and four concentrations of 4-CPA viz., 0, 25, 50 and 100 mg L^{-1} were taken for this study. The highest values of plant height, branch number, fruit number, fruit weight and dry matter was obtained with 100 mg L^{-1} 4-CPA but the highest fresh weight and flower numbers were observed with 50 mg L^{-1} 4-CPA. 'Binatomato-3' showed better performance at 100 mg L^{-1} 4-CPA with respect to attaining highest plant height (83.98 cm), maximum branch and fruit numbers per plant (13 and 20), highest fruit weight (844.88 g) and highest accumulation of dry matter (22.51 g).

Unlike in pea, auxin is the hormone with a larger capacity to induce fruit-set in tomato, followed by GAs (Homan, 1964; Alabadi *et al.*, 1996). Adlskaha and Verma (1965) observed 35% higher fruit set and 23% heavier fruits when plants were treated with GA_3 at 100 ppm. Auxin like 2, 4-D is also effective in increasing fruit set, size of fruit and prompting early maturity under low and high temperature (Metha and Mathi, 1975). It was stated that application of 2, 4-D influenced size and yield of tomato (Sharma and Tiwari, 1987).

Watkins and Cantliffe (1980) reported that exogenously applied NAA enhanced fruit set in *Cucumis sativus* L. Application of NAA and IAA on the ovary accumulated auxin in it which triggered fruit set and subsequent growth of *Cucumis sativus* (Kim *et al.* 1992).

Vijay and Jalikop (1980) observed remarkable differences in fruit weight and fruit set with the application of different concentration of growth regulators in teasle gourd. It is likely that fruit size is an important factor to be considered in the determination of hormonal concentration for development of fruits.

Increased concentration of growth hormone sometimes may cause toxicity to the plant. Onofegahara (1981) also confirmed that higher concentration of growth hormone were toxic to tomato plant. Metha and Mathi (1995) and El-Soad *et al.* (1976) reported that lower concentration of 2, 4-D significantly influenced fruit weight.

4-CPA used to initiate fruit set at low concentration and harvested after 4-5 weeks. Chance of exposed to residues of 4-CPA is very low. EPA (1997) has assessed the dietary risk posed by 4-CPA. An acute dietary risk assessment was not required since no acute dietary toxicity end-point of concern was identified. In the chronic exposure analysis, for the overall U.S. population and 22 subgroups, exposure from the current 4-CPA tolerances represents 1.43% of the Reference Dose (RfD), an amount believed not to cause adverse effects if consumed daily over a 70-year lifetime. The exposure levels of the two most highly exposed subgroups, children 1-6 and 7-12 years old, represent 2.62% and 2.24% of the RfD, respectively. Therefore, chronic dietary risk is not of concern for this chemical.

Use of 4-CPA for fruit set in tomato is safer than other PGR (United Stated EPA, 1997). According to EPA (1997) people may be exposed to very low levels of 4-CPA in their diets, this exposure does not pose risks of concern. Since both the pesticide's toxicity and handlers' exposure levels are low, 4-CPA also poses only minimal risks to workers.

CHAPTER THREE

MATERIALS AND METHODS

MATERIALS AND METHODS

Location and duration of the experiment:

The experiments were conducted at the Horticulture Research Center, Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute (BARI), Jessore during 1 April to 30 September 2008.

Planting Material:

Five hybrid summers tomato lines FP_5 X MP_5, WP_7 X C-11, WP_8 X C-11, WP_8 X C-51 and BARI hybrid tomato-4.

Essential Equipments and Chemicals:

Equipments:

1) Scissors.
2) Conical flask.
3) Pipette.
4) Spray machine.
5) Basket.
6) Balance (electronic).
7) Aluminum foil.
8) Measurement flask.
9) Filter paper.

Chemicals

1) 4-chlorophenpxy acetic acid (Source: Sigma company limited)
2) 75% Ethyl alcohol.
3) Distilled water (DW).

Design and layout of the experiment:

Tow experiments were conducted to achieve the goals of the study. Result of first experiment was used in 2nd experiment as tools for proving the hypothesis.

Experiment 1:

The first was conducted to identify the suitable concentration of 4-CPA to set fruit and their subsequent growth from April - August 08.

Materials and methods:

Material: BARI hybrid tomato-4

Treatments: Concentration of 4-CPA (ppm): 5 (10, 20, 30, 40 and 50 ppm).

The experiment was laid out in randomized complete block design (RCB) with three replications. The crop was grown under polyethylene-made-rain shelter in an area of 27.5 m x 10 m. There were two planting times in two separate blocks under the same poly tunnel to repeat the entire experiment twice. Seeds were sown in 1 April 08 for 1st planting and for 2nd planting seeds were sown in 28 April 08. In each treatment, cluster of 20 flowers were sprayed with the assigned concentration of 4-CPA (Fig.4). Data on days to fruit set and percent fruit set (Fig.5) were recorded.

Fig.4. Cluster of flowers subjected to 4-CPA application.

Fig.5. Fruit set after 4-CPA application.

Data recorded:

a. Plant growth parameter

b. Days to flowering from date of seed sowing

c. Days to fruit set

d. Days to first harvest

e. Fruit size

f. Yield

Five plants and fruits were selected randomly for data collection. The experiment was laid out in a randomized complete block design (RCB) with three replications. The whole experimental area was 27.5 m x 10 m which was divided into 3 blocks. Each block was divided into 5 sub block where varieties were allocated at random. Each sub-block was divided again into two to spray PGR; the sub-block size was 2.0m x 2.0 m. Row to row and plant to plant distance were maintained by 50 cm and 40 cm respectively. Seeds were sown in 1st July 08 and seedlings were transplanted in 7 August 08.

Land Preparation:

The selected lands for the experiments were first opened with a power tiller. It was then exposed to the sunlight for seven days prior to the next ploughing. After that, the experimental plot was properly prepared through several ploughing and cross ploughing. All weeds and stubbles were removed from the field and bigger clods were broken into tiny pieces by laddering and mallet during land preparation. The experimental field was leveled and plots were laid out according to the design.

Application of manure and fertilizer:

The land were fertilized with cow dung, urea, TSP (Triple Super Phosphate) MOP (Murate of Potash) and gypsum @ 10000, 550, 450, 250 and 111 kg/ha respectively. The total amount of cow dung, TSP and gypsum and 1/3rd of each of urea and MOP were applied in pit one week before transplanting. The rest of urea and MOP were applied in two equal installments at 21 and 35 days after transplanting.

Intercultural Operation:

The plants were grown with support in polyethylene made rain shelter. Intercultural operations were done as and when necessary.

Thinning out:

Emergence of seedlings started 3 days after sowing of seeds. Small seedlings were transplanted to other seed bed at 5 cm distance for proper growth and development of the seedlings.

Irrigation:

Irrigation was done followed by weeding, as and when necessary, to keep the plots free from weeds and to supply soil moisture.

Preparation of 4-chlorophenoxyacetic Acid Stock Solution:

Cell culture grade of, 4-CPA (4-chloro-phenoxyacetic acid), produced by Sigma-Aldrich, Eschenstra Be 5, 82024, Taufkirchen, German, marketed in Bangladesh by Kuri and Company (Pvt.) Ltd, 78, Motijheel C/A, Dhaka-1000 was collected for the experiment. The stock solution and working solution were prepared in the laboratory of HRC/RARS Jessore for spraying on the experimental tomato lines.

Preparation of 100 ppm stock solution:

Stock solution of 4-CPA was prepared in 100 ppm by dissolving 10 mg of ingredient in 95% ethyl alcohol in a test tube. The mixture was collected in 100 ml measuring flask and the volume was made up to 100 ml by further addition of distilled water. Stock solution was then transferred into a conical flask; opening of the flask was wrapped with aluminum foil and stored at 0°C for further use.

Preparation of working solution:

Required volumes of 4-CPA of different strengths was made from the stock solution by the following formula:

$V_1S_1 = V_2S_2$

Here,

V_1= Volume of stock solution (ml)

S_1= Strength of stock solution (ppm)

V_2= Volume of working solution (ml)

S_2= Strength of working solution (ppm: 10, 20, etc.)

Method of 4-CPA Application:

Each concentration of 4-CPA of 10, 20, 30, 40 and 50 ppm was applied in each set of 10 flowers at full blooming stage. Only one pray was done on each selected flower. Spray was done in the afternoon.

Five plants and fruits were selected randomly for data collection. Following parameters were recorded from the selected plants during the course of experiment and the mean value for every parameter was also collected.

Percentage of fruit set:

After anthesis, fruits were set. Fruit set percentage was find out as follows-

$$\text{Fruit set \%} = \frac{\text{Total Number of Fruit set}}{\text{Total Number of Flowers}} \times 100$$

Plant height:

Plant height was recorded in centimeter from the proximal end of the taproot to the apex of the longest leaf.

Days to 1st harvest: Days to harvest of ripened fruit from the date of seed sowing.

Fruit size: Length and diameter of ten ripe fruits were recorded in centimeter.

Fruit weight:

Individual fruit weight of each genotype was recorded in kilograms using electronic weight machine.

Fruits per plot (number):

Mean fruit number per plot for each variety was recorded. Fruits per each plant was recorded first then multiplied with the total number of plants in a plot to obtain total number of fruit per plot.

Yield:

Weight of individual fruits and fruit per plants was recorded first then multiplied the total number of plants in a plot to obtain total fruit weight per plot and yield per hectare.

Data Collection and Statistical Analysis:

Weekly Visual observation was made showing the effect of 4- CPA on fruit set and yield and yield contributing characteristics (i.e. Fruit length, Fruit diameter Fruits/plant, Individual fruit weight, Fruit wt/plant, Yield/ plot) of various genotypes of summer hybrid tomatoes. The data were analyzed subjected to Duncan's (1995) New Multiple Range Test (DMRT).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

RESULTS AND DISCUSSIONS

Results:

Floral application of different concentration of aqueous solution of 4-CPA as plant growth regulator showed significant influences on fruit-set of summer hybrid tomato cultivar, BARI hybrid tomato-4. The result revealed that 20 ppm concentration of 4-CPA was suitable for fruit-set in summer tomato. Four hybrid summer tomato lines FP_5 x MP_5, WP_7 x C-11, WP_8 x C-11 and WP_8 x C-51 were evaluated against 20 ppm 4-CPA solution in respect of days to first harvest and yield. The data on fruit-set as influenced by 4-CPA are presented in Table 1. Plant height, days to flowering and days to first harvest of the hybrid lines are presented in Table 2. Main effects of 4-CPA and genotypes on fruit characters as well as yield are presented in Table 3 and 4 respectively. Combined effects of 4-CPA and genotypes on yield and yield attributes are presented in Table 5. Analyses of variance for different characters under study are presented in Appendices.

Table 1. Effect of 4-CPA on fruit-set of BARI hybrid tomato-4:

4-CPA (ppm)	Experiment 1		Experiment 2	
	Days to fruit set	Fruit set (%)	Days to fruit set	Fruit set (%)
0	4.00	29.00(31.26)	4.00	76.67(65.83)
10	3.33	70.00(56.98)	3.00	90.00(74.97)
20	2.67	100.00(89.96)	2.33	100.00(89.96)
30	2.93	96.67(83.82)	2.00	96.67(83.82)
40	3.00	100.00(89.96)	2.67	100.00(89.96)
50	3.00	93.33(81.11)	3.00	93.33(81.11)
LSD	0.64	19.11	0.66	23.58
CV (%)	11.64	13.09	12.89	16.01

Days to fruit-set from the anthesis:

Days to fruit-set from the date of anthesis were recorded minimum when flowers were treated with 20 ppm 4-CPA which was statistically similar to that of flowers treated

with 30, 40 and 50 ppm 4-CPA. Maximum times were required when flowers were treated with only water. The result was almost consistent in the 2nd trial (Table 1).

Percent Fruit-set:

Cent percent fruit-set was recorded from the flowers when treated with 20 ppm of 4-CPA (Fig.6) solution which was statistically similar to the percent fruit-set of flowers when treated with 30 and 50 ppm of 4-CPA solution.

Fig.6. Fruit set after application of 4-CPA at 20 ppm.

Fruit-set was significantly reduced when flowers treated with 4-CPA solution at 10 ppm (Fig.7). The lowest percent of fruit-set was recorded from the control.

Fig.7. Fruit set after application of 4-CPA at 10 ppm.

Similar responses were also noticed in the 2nd trial (Table 1). The result was in agreement with Halder et al. (2003) who reported that floral application of 25 ppm 4-CPA solution at the anthesis enhanced fruit-set in summer tomato.

Morphological feature of hybrid tomato lines:

Plant height:

Plant height of tomato is an important character because it influences the yield. In indeterminate type of cultivars, inflorescence arises from the stem, opposite to the leaf and continues the vegetative growth. The extent of production of inflorescence and side-shoots depends on genotypes. Different height of the genotypes might be attributed due to hybrid vigor (Muller, 1940). Plant height of hybrid line FP_5 x MP_5 at the time of last harvest was found inferior to the height of BARI hybrid tomato-4. The highest plant height was recorded in hybrid line WP_8 X C-51 which was statistically similar to the heights of WP_7 X C-11 and WP_8 X C-11 followed by BARI hybrid tomato-4.

Table 2. Plant height, days to flowering and days to first harvest of four summer hybrid lines of tomato and BARI hybrid tomato-4:

Lines	Plant height (cm)	Days to flowering	Days to 1st harvest
BARI hybrid tomato-4	96.44b	26bc	57b
FP_5 x MP_5	93.66c	24c	57b
WP_7 x C-11	97.83ab	29a	58ab
WP_8 x C-11	97.74ab	28ab	61ab
WP_8 x C-51	98.29a	27abc	62a
Level of significant	**	*	*
CV (%)	1.25	8.13	6.90

* = Significant at 5% level of probability, ** = Significant at 1% level of probability

(Different letters within same legend columns are significantly different at 0.05 levels and means with the same letter in a column do not differ significantly.)

Days to first harvest:

Like flowering days to harvest is also an important characters of tomato and emphasis is laid on quick maturity. Days to first harvest of BARI hybrid tomato-4 and hybrid line

FP_5 X MP_5 was recorded similar and earlier than hybrid lines WP_7 X C-11 and WP_8 X C-51. However, hybrid line WP_8 X C-51 took the longest time to harvest (Table 2).

Effect of 4-CPA and genotypes on fruit size and yield:

Fruit size:

Floral application of 4-CPA did not influence the size of fruit (length and diameter) significantly (Table 3). Fruit length of hybrid line FP_5 X MP_5 was similar to that of BARI hybrid tomato-4. The genotypes WP_7 x C-11, WP_8 x C-11 and WP_8 x C-51 were measured superior in respect of fruit length as compared to BARI hybrid tomato-4 (Table 4). Among the genotypes the maximum length of fruit was recorded in hybrid lines WP_8 x C-11 which was identical to that of hybrid lines WP_7 x C-11 and WP_8 x C-51 respectively. In case of fruit diameter, the genotype FP_5 x MP_5 was found inferior while the rest of the genotypes were superior to BARI hybrid tomato-4. The highest and lowest diameters of fruit were measured in hybrid lines WP_8 x C-51 and FP_5 x MP_5 respectively. Combined effect between the genotype and 4-CPA on fruit size did not show any significant effect.

Table 3. Main effect of 4-CPA on fruit size and yield of summer hybrid tomato lines:

Genotypes	Fruit length (cm)	Fruit diameter (cm)	Fruits/ plant (no)	Individual fruit wt (g)	Fruit wt. /plant (kg)	Yield plot (kg)
Hormone	4.21	4.27	42a	50.46	1.55a	44.60a
Without Hormone	4.14	4.23	32b	48.13	1.22b	35.07b
Level of significant)	NS	NS	**	NS	**	**
CV (%)	8.89	7.53	9.25	11.24	14.50	14.48

** = Significant at 1% level of probability.

Number of fruits per plant:

Number of fruits per plant in tomato influences yield directly. The highest number of fruits per plant was recorded when flowers were treated with 4-CPA (Fig.8) as

compared to flower without any application of 4-CPA (Table 3). In case of genotypes, the number of fruits per plant of hybrid line FP_5 x MP_5 was similar to BARI hybrid tomato-4.

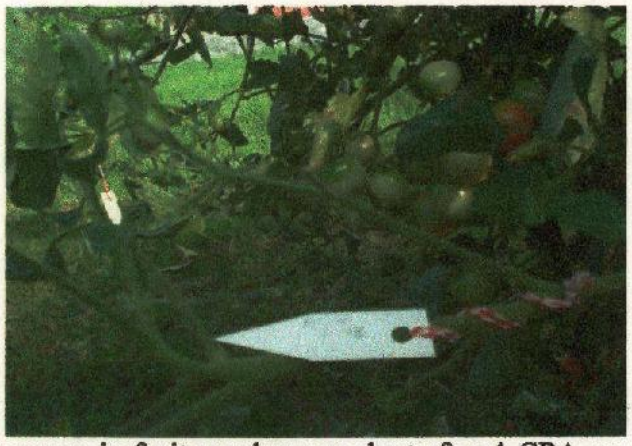


Fig. 8. Increase in fruit number per plant after 4-CPA application.

The number of fruits per plant of rest of the hybrids under study was statistically lower than BARI hybrid tomato-4. However, the lowest number of fruits per plant was recorded in hybrid lines WP_7 x C-11, WP_8 x CFig.4-11 and WP_8 x C-51 respectively (Table 4).

Table 4. Effect of genotypes on yield and yield contributing characteristics of summer tomato:

Genotypes	Fruit length (cm)	Fruit diameter (cm)	Fruits/ plant (no.)	Individual fruit weight (g)	Fruit wt./ plant (kg)	Yield (t/ha)
BARI hybrid tomato-4	3.69b	4.09c	39.50ab	45.00c	1.37b	39.51b
FP_5 x MP_5	3.70b	3.42d	43.18a	29.83d	0.99c	28.82c
WP_7 x C-11	4.36a	4.27bc	32.33c	56.00b	1.40b	40.57b
WP_8 x C-11	4.77a	4.50b	34.33c	45.67c	1.21bc	34.97bc
WP_8 x C-51	4.35a	4.98a	35.67bc	70.00a	1.92a	55.32a
Level of significant	**	**	**	**	**	**
CV (%)	8.89	7.53	9.25	11.24	14.50	14.49

** = Significant at 1% level of probability.

The combined effect between 4-CPA and genotypes in respect of numbers of fruit per plant was also observed significant. Without application of 4-CPA the lowest number of fruit per plant was recorded in BARI hybrid tomato-4 and it was identical to hybrid

line FP_5 x MP_5 (V_2). Number of fruits per plant of BARI hybrid tomato-4 and hybrid line FP_5 x MP_5 increased tremendously and became significantly highest when flowers of the genotypes were treated with 4-CPA solution at 20 ppm.

Individual fruit weight:

Individual fruit weight is another important yield contributing character of tomato. The data in Table 3 indicated that 4-CPA did not influence individual fruit weight. The effect of genotype on individual fruit weight was noteworthy (Table 4). The hybrid line WP_8 x C-51 produced the highest individual fruit weight. The lowest individual fruit weight was measured in hybrid line FP_5 x MP_5 and it also inferior to the fruit weight of BARI hybrid tomato-4. No interaction effect was noticed on individual fruit weight by hybrid lines and 4-CPA.

Fruit weight per plant:

Fruit weight per plant is directly associated with number of fruit per plant as well as the individual fruit weight. When flowers were treated with 4-CPA, fruit weight per plant was significantly higher as compared to the fruit weight harvested from plants without any application of 4-CPA (Table 3). Response of genotype on fruit weight was also observed positive. The highest fruit weight per plant was recorded in the genotype WP_8 x C-51. Fruit weights of rest of the genotypes were either similar or lower than BARI hybrid tomato-4. The lowest fruit per plant was measured in the hybrid FP_5 x MP_5.

Interaction between the genotype and 4-CPA was also significant in this character and highest individual fruit weight was recorded in the hybrid line WP_8 x C-51 when flowers were treated with 4-CPA. On the other hand, the lowest and identical fruit

weights per plant were recorded from the hybrid lines WP_7 x C-11 and WP_8 x C-51 without any application of 4-CPA.

Yield per hectare:

Yield per hectare was measured maximum when flowers were treated with 4-CPA as compared to the yield of genotypes without 4-CPA treatment (Table 3). Significant variations in yield per hectare were recorded in different hybrid lines (Table 4). The genotypes WP_8 x C-51 gave the highest yield per hectare while the lowest yield was obtained in hybrid line FP_5 x MP_5. Yield of hybrid lines WP_8 x C-11 and WP_7 x C-11 were statistically similar to BARI hybrid tomato-4.

The interaction effect between 4-CPA and hybrid lines in respect of yield per hectare was also significant. When the flowers were treated with 4-CPA at 20 ppm, the highest yield was recorded from the hybrid WP_8 x C-51 on the other hand the hybrid FP_5 x MP_5 gave the lowest yield in the absence of 4-CPA.

Interaction effect of 4-CPA and Different Genotypes regarding Yield:

Significant differences were shown in terms of fruits number, fruit weight per plant and yield amongst cultivars while using 4-CPA as growth regulators. Corresponding data are shown in Table 5. Fruits weight per plant was maximum in variety WP_8 x C-51 and lowest in hybrid line FP_5 x MP_5 which was increased to some extent (1.12 kg) with the application of 4-CPA.

Per plot yield of BARI hybrid tomato-4, FP_5 x MP_5, WP_7 x C-11, WP_8 x C-11 and WP_8 x C-51 with 4-CPA application (19.65, 20.28, 27.85, 24.03 and 39.57 kg respectively) was greater than yield without 4-CPA treatment (21.75, 15.75, 22.87, 19.68 and 29.57 kg respectively) that are represented in Table 5.

Table 5. Interaction of 4-CPA and genotypes:

Interaction		Fruit length (cm)	Fruit diameter (cm)	Fruits/ plant (no)	Individual fruit wt.(g)	Fruit wt /plant (kg)	Yield/ plot (kg)
H_0	V_1	3.68	4.02	33c	46.66	1.21cd	21.75cd
	V_2	3.64	3.40	37bc	30.33	0.87d	15.75f
	V_3	4.29	4.25	28d	57.33	1.27c	22.87cd
	V_4	4.71	4.47	30cd	46.33	1.09d	19.68e
	V_5	4.34	4.97	30cd	71.67	1.64b	29.57b
H_1	V1	3.69	4.15	46a	43.33	1.54bc	27.65bc
	V2	3.75	3.43	49a	29.33	1.12cd	20.28d
	V3	4.42	3.28	36bc	54.67	1.55bc	27.85bc
	V4	4.83	4.52	38bc	45.00	1.34c	24.03c
	V5	4.35	5.00	41b	68.33	2.19a	39.57a
Level of significant		NS	NS	**	NS	**	**
CV (%)		8.89	7.53	9.25	11.24	14.50	14.48

V_1= BARI hybrid tomato-4
V_2= FP_5 x MP_5
V_3= WP_7 x C-11
V_4= WP_8 x C-11
V_5= WP_8 x C-51

H_1=Hormone
H_0=Without hormone

** = Significant at 1% level of probability

(Different letters within same legend columns are significantly different at 0.05 levels and means with the same letter in a column do not differ significantly.)

However, the trend of yield of each hybrid was almost similar even after treated the flowers with 4-CPA. Percent increment was found numerically maximum in hybrid line WP_8 x C-51 and lowest in hybrid line WP_7 x C-11.

Discussions:

The results obtained from the present investigation have been discussed in the following paragraphs with logical explanations for the work undertaken.

Use of 4-CPA for fruit set in tomato is safer than other PGR due to it's lack of overt toxicity in sub-chronic dietary studies, the lack of acute dietary exposure concern, and the low volume/minor use status (United Stated EPA, 1997). For the primary introduction of higher percentage of fruit set, establishment of the optimum dose of 4-CPA was essential.

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In the present study, several concentrations of 4-CPA were examined to investigate the fruit setting efficiency of the check variety BARI-4. 4-CPA at both 20 ppm and 40 ppm concentration were found to induce 100% fruit set. The concentration 20 ppm was selected for further application on other varieties as it required less time to set fruit and it is cost effective than that of 40 ppm. Interaction effects of genotypes and hormone 4-CPA were significant in respect of yield contributing characteristics such as fruit number, fruit weight per plant, and yield per hector e.t.c.

Spray applications of 4-chlorphenoxyacetic acid (4-CPA) caused an increase in growth and development of fruit and induced higher fruit set and yield over control. The response was irrespective of varieties. The result coincides with findings of Halder *et al.* (2003) who sprayed 4-CPA on flower cluster and found effectively increase the fruit set. Maximum number of fruits (4.82) per cluster, highest fruit yield (22.26 t/ha) and a maximum number of fruits (28.23/plant) were also recorded with this hormonal application and was recorded by spraying 20 ppm 4-CPA. Similar results were reported by Mahmood and Bahar (2006) on yield contributing characteristics viz. where highest

value of fruit number and fruit weight was obtained with 100 mg L^{-1} 4-CPA but the highest fresh weight and flower numbers were observed with 50 mg L^{-1} 4-CPA.

According to Sjut and Bangerth (1981) plants treated with hormone reduced flower drop and increased fruit-set, which can be attributed to the preventive effect of Tomato tone (4-CPA) to heat injury, so exogenous application of the hormone 4-CPA accelerates fruit-set. HRC/BARI (1996) reported that 0.15-0.25% 4-CPA solution ensures cent percent fruit setting which supports the present study.

Kibler and Bangerth (1982) found 4-CPA treatment induced fruits with about 20% more cells than other plant growth regulators (i.e. GA and IAA). Salisbury and Ross (1992) application of 4-CPA at 15 to 20 ppm prior to anthesis or bloom, typically increases the set of fruit irrespective of cultivars and it stimulates both cell division and cell elongation, resulting in increased berry size. All these results harmonize with the findings of our present study.

CHAPTER FIVE

SUMMARY AND CONCLUTION

SUMMARY AND CONCLUSION

The study was conducted at Horticulture Research Center, Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute (BARI), Jessore, Bangladesh.

Five varieties of summer hybrid tomato i.e. BARI hybrid tomato-4, FP_5 x MP_5, WP_7 x C-11, WP_8 x C-11 and WP_8 x C-51 and plant growth regulator 20 ppm ranging from 10 ppm, to 50 ppm were as experimental materials.

The experiment was laid out in randomized complete block design (RCB) with three replications. The selected lands for the experiments were prepared properly through several ploughing and cross ploughing. Fertilization, weed management and intercultural operations were done to ensure proper growth of the hybrid varieties.

The whole study was divided into two consecutive experiments. In the first experiment, optimum 4-CPA dose for inducing successive fruit set in BARI hybrid tomato-4 was established and in the next, varietal performance of five different summer tomato lines and effect of applying this dose on these varieties in respect of yield and contributing characteristics were recorded.

Cluster of twenty flowers from each plant were sprayed with 4-CPA at the assigned concentration (spraying of water was used as control). Concentration at 20 ppm and 40 ppm sowed 100% fruit set.

In respect of plant characteristics highest plant heights were measured in hybrids WP_8 x C-51 (98.29cm), followed by WP_7 x C-11 (97.83cm), and WP_8 x C-11(97.74cm). Minimum plant height was recorded in hybrid FP_5 x MP_5. Days required for flowering

was the lowest in FP_5 x MP_5 (24d) and highest in WP_7 x C-11 (29d). Hybrid WP_8 x C-51 took longer time (62d) to 1st harvests over other varieties. Regarding yield contributing characteristics, variety WP_8 x C-51 found superior to other varieties (fruit length 4.35cm, diameter 4.98cm, no/plant 35.67, individual weight 70g, weight/plant 1.92kg, and yield/ha 55.32 tons). Highest no. of fruits/plant (43.18) was harvested from FP_5 x MP_5. Yield next to WP_8 x C-51 was observed in WP_7 x C-11 which was 40.57 t/ha.

Almost all the varieties responded positively when treated with 20 ppm 4-CPA irrespective of cultivars. No significant differences were recorded in days to 50% flowering, days to harvest, fruit size (length and breadth) and individual fruit weight as compared to control. Spraying of 4-CPA induced higher number fruit per plant (42) and yield per plot (44.60 ton) over control which was 32 and 36.07 kg respectively.

With the application of 20 ppm 4-CPA, fruit number in BARI hybrid tomato-4 increased from 33 to 46, in WP_7 x C-11 from 28 to 36, in FP_5 x MP_5 from 37 to 49 and in WP_8 x C-51 from 30 to 41. Though maximum no. of fruits were harvested from FP5 x MP_5, radically increase in yield per hector was recorded in WP_8 x C-51 which was uplifted from 73.925 t/ha to 98.925 t/ha with hormonal application as because it's individual fruit weight thus fruit weight/plant (2.19 kg) was also increased than other varieties.

So it may be concluded that spray of 4-CPA of 20 to 50 ppm enhance fruit set of summer tomato irrespective of varieties. From the point of economic view spraying of 20 ppm 4-CPA is suitable for summer tomato cultivation. Further study is needed to find out the effect of 4-CPA on fruit set as well as quality of fruit when the concentrations are above 50 ppm.

Role of environmental temperature was ignored in the study. Therefore, day temperature during the period of study should be included in the future investigation of PGR 4-CPA on summer tomato production particularly on fruit set.

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APPENDICES