EFFECTS OF PRUNING AND GIBBERELLIC ACID ON QUALITY GRAPE (*Vitis vinifera* L.) PRODUCTION

A THESIS
BY

SIRAJUL ISLAM
Examination Roll No. MS 070711
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Approved as to the style and content by

16.08.09

Supervisor

Dr. Md. Nazirul Islam
S.S.O,
Horticulture Division,
Regional Agricultural Research Station (RARS),
Bangladesh Agricultural Research Institute (BARI),
Jessore.

30.8.2010

Co-supervisor

Professor Md. Raihan Ali
Professor,
Biotechnology and Genetic
Engineering Discipline
Life Science School
Khulna University
Khulna – 9208
Bangladesh.

30.8.2010

Professor Md. Raihan Ali
Chairman
Examination Committee
Biotechnology and Genetic Engineering Discipline
Life Science School
Khulna University
Khulna-9208
Bangladesh.

Biotechnology and Genetic Engineering Discipline
Life Science School, Khulna University
Khulna-9208, Bangladesh

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THE AUTHOR

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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 The findings of the research has not been/will not be published anywhere without the concurrence of the concerned supervisor/co-supervisor.

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SIRAJUL ISLAM
Examination Roll No.: MS 070711

ABSTRACT

An experiment was conducted at the Horticulture Research Center, Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute (BARI), Jessore during December 2007 to April 2009 to examine the effect of pruning on vegetative and reproductive bud break and the effects of GA_3 on seedless grape production. Pruning of vine found to influence both vegetative and reproductive bud break, anthesis of flower and harvesting of berries significantly. Vine pruning in January gave earlier harvest as compared to October and December. Flowers sprayed with 100 ppm GA_3 attributed cent percent seedless berries. Taste of berries was sweet and sour when treated with 100 ppm GA_3 while berries harvested from controlled vines were sour. Berries obtained from GA_3 treated flower weighed higher than that of untreated flower. Total Soluble Solid (TSS) was significantly higher in berries obtained from 100 ppm GA_3 treated flower as compared to those of untreated flowers.

LIST OF ABBREVIATIONS

ppm -Part per million

GA- Gibberellic acid

g- Gram

ml- Milliliter

mg/L - Milligram per litter

%-percentage

TSS - Total soluble solid

$\chi 2$ - Chi- square test

O- Observed value

E-Expected value

RARS-Regional Agricultural Research Station

BARI-Bangladesh Agricultural Research Institute

IPA- Isopropyl alcohol

DW-Distilled water

CV -Coefficient of variation

PGR -Plant Growth Regulator

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CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the most delicious, refreshing and nourishing fruits of the world. It is also a popular fruit in Bangladesh. Entire demand of grape in Bangladesh is fulfilled by importing from neighboring countries. Narrow variety base and lack of technologies to achieve high eating quality are the drawbacks of grape cultivation in Bangladesh. However, the prevailing climatic conditions of Bangladesh seem favorable for grape cultivation as to similar climate of other grape cultivated countries of Asia. At least four and a half dry months requires for successful grape cultivation and the fruiting season has to coincide with dry months to avoid rain damage and disease infection (Pandey and Pandey, 1990). Bangladesh enjoys sub-tropical to tropical climate and usually November to May is dry, which seems to coincide with the requirement for grape production. Traditional variety of grape known as "Jhakka" is cultivated in Bangladesh from the time immemorial, which is seeded and sour. Jhakka cultivar flowers round the year. Since, flowering and fruit sets of grape are highly responsive to vine pruning, cropping season in our country can be allowing in their native climatic range. Vine pruning also improves the nutrient uptake and utilization, control vine growth and set crop loads. New shoot after pruning do not compete for nutrition with fruits and increase in the ratio of leaves to crop and thus improved grape quality (Winkler, 1958; Kliever & Weaver, 1971). Presence of seed in the berries is a worrisome to the consumer and thus seedless trait in highly desirable in grapes. There are several sources of seedlessness trait of grapes. Currently more than dozen varieties of seedless grape are available worldwide. Unfortunately none of them is suitable under the climatic conditions of Bangladesh. Consignable interest has developed recently regarding the benefit the use of plant growth regulators of varying concentrations to grow seedless grape. Gibberelic acid, a synthetic plant growth regular is widely used for seedless grape production in the world. Time of application and doses of GA_3, the age of plant, the development of the plant, etc. are important for a particular physiology. As a growth regulator GA_3 also improve mobilization of photosynthetic products of leaves in the berry (Ledbertter and Ramming, 1989; Butler and Rush, 1994; Harrell and Williams, 1987; Varma, 1991;Zabadal and Bordelon, 1993) which ultimately increase sweetness. Therefore, the study was undertaken to investigate the time of pruning of grape vine on bud break and effect of GA_3 on improving the eating quality grape under the climatic condition of Bangladesh. Recommended dose of 100 ppm of GA_3 was used in the experiment as reported by Kajure (1962), Clore, (1965) and Yadav et al. (1977).

1.2 OBJECTIVES:

The purposes of this study were

1) to examine the effect of pruning on vegetative and reproductive bud break
2) To investigate the effects of GA_3 on seediness and sweetness of berry, and cluster size

CHAPTER TWO
REVIEW OF LITERATURE

Numerous scientific works have been carried out in different countries of the world for determining the effect of pruning and application of GA_3, a plant growth regulator for seedlessness of grape. Seedless is a highly desirable trait in table grape selection. There are several sources of seedlessness traits of grapes. Consignable interest has developed recently regarding the benefit the use of plant growth regulators of varying concentration. Gibberelic acid, a synthetic plant growth regular is widely used for seedless grape production. Viticulture researchers around the world reported numerous formulas and claims in this regard. However, some of the most relevant works are reviewed here.

2.1 Plant Review

2.1.1 Origin

According to Bindra (1918) it is anticipated that V*itis*, to which grape belongs, was originated in North America and Europe. Out of 60 species of the genus *Vitis*, 25 have been reported occurring in the Himalayan region of India (Hayes, 1957). Botanists have advocated two origins of grapes. The American grapes consisting of *V. labrusca* and other species of *Euvitis* and *Muscadinia* are considered to have originated in the North American regions. The European grapes *Vitis vinifera* Linn originated primarily in Caucasus region between Caspian Sea and Black Sea (Snyder 1937, Vavilop 1951, Winkler 1962). On the basis of legend and tradition Olmo (1976) considered ancient Armenia as the home of first grape culture. This *V. vinifera* species is considered to have its secondary centre of origin in the north-western Himalayas, south Europe and western Asia. The inference that the Himalayas are secondary centre of origin of *Vitis vinifera* may be true because the genus on arrival here perhaps expanded by finding a new conducive environment. There is, however, no evidence to point out the exact date of the arrival of grape vine in the Himalayas or India. The species of *Vitis* found in India seem to have escaped from the secondary centre of origin based on the different number of chromosomes in *V. Vinifera* (2n=38) and *V. rotundifolia* (2n=40), and a marked sterility in their F_1s, these 2 species are considered distantly related. Hybridization between the cultivated varieties of these is affected by a large number of mutations accumulated and preserved though vegetative propagation since generations in a single clone of the former species (Olmo 1970). It is also possible that *Vitis vinifera* varieties may have arisen from different subspecies having different genomic relations with ancestral *V. rotundifolia* (Olmo 1970).

2.1.2 Grapes in India

Grapes were well known in India since the century BC. But its history is not adequately known before the pointed of the Muslim invasion. Pillay (1968) reported a Chinese Buddhist (629-645 AD), a Moorish Traveler on Ibn Batuta (1403) and French Traveler Thevenot (1667 AD) having seen grapes in India. The commercial varieties of grapes were introduced in India mostly by traders of Iran and Afghanistan (Thaper, 1660). Mohammed Bin Tughlaq introduced Bhokri Farkri and Sahebi cultivar in Aurangabad in 1938 ({Pillay, 1968). Bangladesh was then in the political boundaries of ancient India and it is thought that grape was traveled from Hydrabad to Bengal and other provinces of India (Asham, Meghalay and Tripura) at the beginning of the last century.

2.1.3 Botany

Panday and Pandy (1990) described grape vine comprises roots, trunk, shoots, canes, leaves, buds, tendrils, influence and fruit etc. Different parts are described below.

Root: The grape vine has preceding and descending types root system. The feeders roots are present up to 25 cm depth down and up to 60-120 cm away the trunk.

Trunk: Trunk is a permanent stem of the vine on which the whole framework is bared

Shoot: The succulent current seasons' grape arising from a bud after hurrying is known shoot.

Cane: The woody mature and doormat shoot is called came.

Leaf: Leaf in grape vines is orbicular-cordate mare or less deeply and palmately lobed into 3.5 or 7 lobed irregularly toothed, glabrescent above, often grey torments beneath.

Bud: Grape vine has auxiliary and compound bud, often called 'eye' usually consisting of 3 partially developed shoots with rudimentary leaves or booth rudimentary flower cluster.

Tendril: Tendrils are shoots. In Eustis, tendril is bi-fid or forked bud in Muscadinia it is unified. Tendrils are continuous i.e., found operate every leaf in *Vitis labrusca.*

Arms: The main branches arising from the trunk which produce fruiting shoots.

Spars: Cans cut back to one or two nodes to produce the fruiting canes for the next growing season, located near the bare of each fruiting cane.

Inflorescence: The in florescence is a cyme which is a much branched cluster, each branch ending in a terminal flower (Pratt,1971). The inflorescence born opposite the leaf and they emerge with the leaves when the shoot start growing. *Vitis spp* had been originally a dioecious or bsub-dioecious plant when some male flowers transformed in to a hermaphrodite one (Olmo,1976).

Flowers: Flowers are hermaphrodite, male and female. Hermaphrodite flowers have 5 partly fused sepals, 5 petals united at the top, 5 stamens and a 2 loculed pistil with a short style and a stigma. The pistil has enlarged superior ovary. Each ovary contains 2 ovules and each ovule has 1 embryo sec containing egg, which after fertilization results into seed. The calyptra, or cap is the corolla, in which the petals are fused at the apex; it abscises at the base of the flower and pops off at anthesis. Species in *Euvitis* may have 100+ flowers per cluster, whereas muscadine grapes have only 10-30. Vinifera and concord grapes are perfect-flowered and self-fruitful, whereas some muscadine cultivars have only pistillate flowers. The receptivity of stigma is a varietal character and influnced by climatic factors. Stigma receptivity is on the day of anthesis which disappear on the third day (Alley, 1957).

Pollination: Most grapes are self-fruitful and do not require pollinizers; however, pistillate muscadines (e.g., 'Fry', 'Higgins', 'Jumbo') must be interplanted with perfect-flowered cultivars for pollination. Pollination is accomplished by wind, and to a lesser extent insects.

Fruit: Grapes are true berries; round to oblong, with up to 4 seeds having a fine layer of wax on the surface. Skin is generally thin, and is the source of the anthocyanin compounds giving rise to red, blue, purple, and black (dark purple) colored grapes. The edible part is pericarp and placenta

Seed: Seed with hard testa, copious, ruminate endosperm and small straight embryo.

2.1.4 Classification of grape

Grapes have been classified under the genus *Vitis* belonging to family *Vitaceae.* The genus *Vitis* is broadly distributed, largely between 25° and 50° N latitude in eastern Asia, Europe, the Middle East, and North America. *Vitis* is split into 2 subgenera:

1. ***Euvitis*** - "True grapes"; elongated clusters, berries that adhere to stems at maturity, forked tendrils, loose bark that detaches in long strips, and diaphragms in pith at nodes. *Vitis vinifera*, the European grape, and *V. labrusca*, the Concord grape
2. ***Muscadinia*** - "Muscadine grapes"; small fruit clusters, thick-skinned fruit, berries that detach one-by-one as they mature, simple tendrils, smooth bark with lenticels, and lack diaphragms in pith at nodes. *V. rotundifolia.*

2.1.4.1 Species and Cultivars

The following three important species and one hybrid group comprise most of grape production worldwide:

1. ***V. rotundifolia*** **Michx (muscadine grape).** The species is extremely vigorous and disease tolerant compared to *Vinifera* grapes, and is well-adapted to the southeastern USA. It lacks cold hardiness, so is not grown in the mid-Atlantic, Northeast, or Midwest. Two classes of cultivars occur: 1) pistillate or female, and 2) perfect flowered or hermaphroditic. 'Cowart', 'Hunt', 'Noble', 'Jumbo', 'Nesbitt', and 'Southland' are popular black cultivars, and 'Carlos', 'Higgins', 'Fry', 'Dixieland', and 'Summit' are popular bronze-skinned cultivars other than 'Scuppernong'.
2. ***V. labrusca*** **L. (Concord, American bunch, or Fox grape).** The species is used primarily for sweet grape juice and associated products - jelly, jam, preserves.

'Concord' is the major cultivar, responsible for about 80% of production. Other important cultivars include 'Niagara', `Isabella', 'Delaware', and' Catawba'.

3. ***V. vinifera* L. (European or Wine grape).** *Vitis vinifera* is also called "Old World grape" since most production occurs in Europe and the other species are native to the New World. This species accounts for over 90% of world grape production. Most of the production is used to make wine, but it is also the primary species used for table (fresh eating) and raisin grape production. There are at least 5000 cultivars of Vinifera grapes grown worldwide, and some estimates put the number of known cultivars as high as 14,000. However, less than 100 make up the vast majority of production. Popular cultivars include 'Thompson Seedless' 'Cabernet Sauvignon', 'Merlot', 'Pinot Noir', 'Airen', 'Malbec', 'Chardonnay', 'Sauvignon Blanc', and 'Riesling'.
4. ***French-American Hybrids.*** The introduction of the phylloxera or grape root louse to Europe in 1860 devastated vineyards composed of the susceptible *Vitis vinifera* species, and created a need for resistant rootstocks. *Vitis labrusca* and other species native to the host range of the phylloxera (north-central USA) were hybridized with Vinifera grape to produce a range of rootstocks with resistance. In addition to use as rootstocks, some of the hybrids had both phylloxera resistance and good wine quality attributes. Cultivars include 'Marechal Foch', 'Vidal Blanc', 'Chambourcin', and 'Seyval'.

2.1.4.2 Grape species and cultivars are also classified base on food uses

1. ***Table grapes.*** These are consumed as fresh fruit. 'Thompson seedless' is a major cultivar of table grape. Other seedless cultivars include 'Flame Seedless' and 'Ruby Seedless' (both red), and 'Perlette'; seeded cultivars in the USA include 'Emperor', 'Ribier', and 'Calmeria'
2. ***Raisin grapes.*** 'Thompson seedless' is the major cultivar worldwide, and makes up 90% of raisin production in the USA.
3. ***Sweet juice grapes.*** Traditionally, this classification was primarily dominated by 'Concord', the major American bunch grape. In addition to juice, jelly, jam, preserves, and some wine is produced from sweet juice grapes.

4. ***Wine grapes***. Some wine is produced from all grape species (and many other fruits), but the bulk of commercial production is dominated by *V. vinifera* cultivars. Several French-American hybrids also produce good quality wine.

2.1.4.3 Based on the fertilely due to importance of pollen grapes are classified as:

1. **Self fertile:** Pollen of self-fertile varieties is oblong, blunt at the and quite symmetrical and show good germination
2. **Self sterile:** The stamen are crinkled possess wrinkled and dead pollen-grains
3. **Partly sterile:** Self fertile but sterile pollen sometimes results from, disintegration processes after mitosis in the micro-spore nucleus and occurs associated with reflex type of stamen and the absence of germpore.

Aborted pollen occurs in both sterile and fertile forms and seems to be arrested development son after being liberated from tetrade. Sterile pollen resulted from disintegration processes after mitosis in microspoere nucleus and occurs associated with reflex type of stamen and absence of germpore. The pollen sterile can also be induced artificially with malic hydrazide (500-750 ppm) and GA_3 applied during early period of panicle developed. As stated by Pandey and Pandey (1990) flowering in grape is regulated by proper balance of auxins, gibberellins, cytokines and inhibitor.

2.1.5 Training of vine

The apical dominance presence in grape fine necessitates checking of straight growth of vine to regulate the optimum growth and fruiting. Pandey and Pandey (1990) described following 5 trading system depending on the vigor of canes.

Bowers: A crises-cross, netting is mounted at a height of 180-210 cm on the top of iron poles. This net supports the main framework of older braces scattered well spaced in all direction which bear fruiting canes.

Telephone system: In this method 3 parallel wires are strung 30-45 cm apart through the cross-iron arms welded on the top of angle iron poles at 180-200 cm above the ground level. The arms of the vines are trained along the wires in direction of the row.

Trellis system: In this method two wires are stretched vertically through the holes in angle iron poles at height of 90 and 150 cm and 4 main arms trained along the wires in both directions. The fruiting canes are dispersed on these arms. The wires are connected to the end poles through screw arms which help in further tightening the wires after each pruning operation.

Vertical Cordon System: The vines are allowed to grow 1.5 tall and all sprouts are rubbed off except the topmost 4 buds to develop into new shoots. The shoots are pruned back to 3.4 buds. Such pruning is repeated each year. After 4-5 years the vines become strong and thereafter 4 strong canes of vines are tied to the adjacent vines.

2.1.6 Pruning: Judicious pruning of fruitful cane is essential for proper fruiting. Without pruning grape vines become tangled mass of unproductive shoot, that decline yield and quality (Olmo, 1970). To obtain best results correct timing and techniques are necessary. Selction of fruiting cane, tying the vine, thinning and de-suckering and renewing old or diseased trunks must be properly (PCARRD, 1979). Two kinds of pruning in grape practiced e.g., pruning for fruiting cycle and foundation pruning for vegetative growth (1990). Waqar et al., (2004) evaluated pruning severity with previous year bearing status of grape vine and reported no effect on bud burst, panicle emergence and anthesis date. Ledbetter *et al.* (1996) similarly reported yield and quality of Alacahua Muscadine grape was affected by vine pruning significantly affected fruit

2.1.6.1 Level of pruning: According to PCAARD (1979) pruning consists of cutting the balk of previous season's growth. All the leaves on the leaves on the remaining cares are cut close to the main areas. The weak and old cares are cut close to the main arms. Pruning cuts are made about 2 to 3 cm above the last buds so that the shoot that will develop will have a strong base. The main arms unless badly damaged or diseased are not cut off. The vine is totally bare after pruning. The number of fruiting canes per vine and the number of nodes per fruiting canes per vine and the number of nodes per fruiting cane left at pruning times varies according to the vigor of the vine cultivars as well as support and training system of vines (Sing et. al. 1983, lavar et al. 1978).

2.1.6.2Time of pruning

Das and Melanta (1977) stated that after planting in January, foundation pruning for vegetative growth, leaving 1-2 buds, is done in April while pruning for fruiting cycle is done purposively to control crop season and berry ripening. In north India vine pruning is done during dormant season from late December to end of January. Sometimes for different varieties different dates are found suitable for pruning in the same regions. Hatch and Ruiz (1987) reported that earlier bloom and subsequent harvest coincided with optimum pruning dates and accumulated winter chilling to determine optimum pruning dates was suggested as a means of improving desert table grape production. PCARRD (1979) suggested pruning vine at the onset of dry season particularly in tropical to sub-tropical regions so that the flowers and the fruit develop and ripen during the period of abundant sunshine and vines have full complement of healthy leaves.

2.1.7 Induction of seedless

Seedless is a highly desirable traits in table grape. There are several sources of seedless in table grapes. Currently there are more than dozen varieties of seedless grape cultivated worldwide. Unfortunately none of them is suitable under the climatic conditions of Bangladesh. Seedless grapes can be harvested from the GA_3 treated seeded grapes depending on the cultivars, concentration applied and stage of cluster development. Kajure (1962), Clore, (1965) and Yadav et al. (1977) harvested seedless grape when flower cluster of Diware cultivar treated with 40 and 100 ppm GA_3 at pre-bloom and cluster of Hur cultivar treated with 50 ppm at full bloom followed by 25 ppm at fruit set. Similarly, Rao et. al (1962) induced seedless in Bhokri cultivar with pre-blooming application of GA_3 @ 400 ppm. They explained that pre bloom application of GA_3 dose not affect the morphological development of pollens and their content of amino acid, sugar a starch. However, the respiration rate and oxidative enzyme activities of pollens are much enhanced just after treatment and reduced markedly towards the blooming time, resulting in the failure of pollen germination. Embryo sac in the treated ovary does not fully develop to fertilize at the time bloom. Therefore, it is supposed that seedless berries may be induced by incompletes of both male and fame organs in the cultivar. GA_3 application at pre-bloom stage is given to make pollen non functional. The cultivar 'Hur' being functionally female, does not require pre-blooming application of GA_3 because it poillens are already non-functional. GA_3 application at full bloom gives impetus to the growth of ovary and induce seedless berry.

2.1.8 Use Gibberellins in grape cultivation

Gibberellic acid or gibberellins are isoprene compounds synthesized from mevalonic acid in tissue and seeds. Its chemical formula is $C_{19}H_{22}O_6$, soluble in ethanol and slightly soluble in water. Individually, GAs are usually referred to by their A-number e.g., GA_1, GA_2 ... GAn. They are containing gibbene skeleton which consists of a carbon skeleton with four rings A, B, C and D (Figure 1). Hence, they are designated as tetracarbocyclic compounds. GA_3 has a double bond in ring-A and more unstable thereby more reactive than GA_1, GA_2 or GA_4 (Davies, 1995).

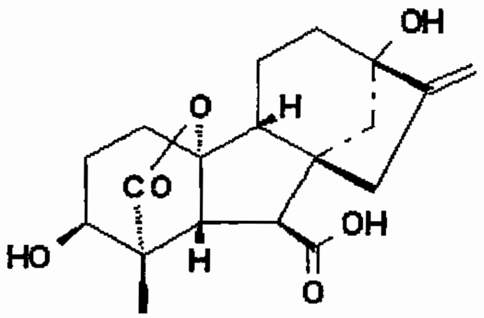

Figure 1. Structure of Gibberellins

Gibberellins (GA) induce cell enlargement of intact plant, promote growth of dormant bud and induce seed germination. Application of very low concentration can have a profound effect while too much will have the opposite effect. It is used in concentration between 0.01 to 10mg/L. They promote flowering, cellular division and reverse the inhibition of shoot growth and dormancy induced by ABA (Tsai et al., 1997). Promote growth of fruit tree crops until required vegetative growth attained.

2.2 REVIEW OF SCIENTIFIC STUDY

Some of the important findings are reviewed and presented below:

Waqar *et al.* (2004) evaluated pruning severity with previous year bearing status for growth behavior of spur and bunch morphology of Grape (*Vitis vinifera* L.) cultivar perlette. They reported no effect of pruning severity and previous year bearing status on bud burst, fruitful buds and TSS of berries were noticed in spurs pruned to six nodes. Pruning of spurs at different nodes affected quality and weight per cane significantly.

Lombard et al. (2006) reported pruning practices modify budburst and reproductive development in table grapes in regions with low and often inadequate winter chilling. They explained that pruning treatments increase endogenous cytokinins level and enhance budburst and opined that availability of more cytokinin in longer canes induce reproductive development. In their investigation Alphonse Lavaleé' (seeded) was sufficiently fruitful and produces adequate yields following spur pruning Long pruning in 'Sultanina' and 'Sunred Seedless' (both parthenocarpic) enhanced fruit set and yields.

Alejar et al. (1999) stated gibberellins (GA_3) as plant growth regulators (PGR), naturally produced within plant body and occurs in extremely low concentration regulate cellular process in targeted cell locally as well as distance. PGR when naturally produced in plant body termed as hormones which shape the plant, affect seed growth, time of flowering, sex of flower, senescence of leaves and determine flower, stems, leaves, the shading of leaves, development and ripening of fruit.

Jiang Lu et al. (1995) studied the effect of GA_3 of varying concentration on leaves and fruit cluster of muscadine cultivars e.g., Carlos, Fry, Higgins and Triumph. The first spray was at late blooming season, followed by a second spray a week later. Effects on fruit and seed development were observed in those sprayed vines and reported early ripening, increased uniformity of ripening, increased berry size and the presence of seedless berries.

Qadir et al. (1989) reported increased bunch and berry weight of Flame seedless cultivar when vines were sprayed with GA_3 at bloom and fruit setting.

Fellman et al. (1991) sprayed GA_3 on the foliage of the seeded grape cultivar 'Swenson Red' before and after anthesis and similarly founded high percentage of seedless berries.

Fukunaga and Kurooka (1988) also demonstrated the effectiveness of GA on production of seedless berries in Kyoho grapes.

Panwar *et al* (1994 a&b) reported that GA_3 stimulated early and even ripening on uneven ripening Beauty seedless grapes (*Vitis vinifera* L.).

Gray and Harris (1996) reported that Pruning significantly affected fruit yield and quality of Alacahua Muscadine grape.

Nick Dokoozlian (1998) studied the effect of vine pruning and application of GA_3 on fruit quality and productivity of spar of Crimson Seedless grapes. Application of GA_3 at bloom increased fruit size, reduced berry color, increased berry shatter and decreased return fruitfulness. Both spur and cane pruned vines responded similarly to the GA_3 treatments evaluated in this experiment.

Iknur Korkutal et al. (2008) reported the effect of Gibberellic in viticulture and stated that GA_3 affects grape berry by means of different ways. Some of these effects include formation of flower cluster, berry set, berry enlargement, cluster length elongation, berry thin in cluster, prevention of berry cracking, pollinicide effect of Gibberellic acid in seeded grape varieties to create seedless berry. He opined that since Gibberellic acid is not harmful for human health, it can be suggested for different purposes. The application time, the application dose, the age of the plant, the development of the plant, etc. are important.

Jiang Lu (1996) explained that Gibberellic acid, a plant growth regulator commonly used for berry enlargement of seedless grapes, was used to spray on Orlando seedless, the only seedlessness bunch grape cultivar with Pierce's disease resistance grown in Florida. 50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l and 300 mg/l were sprayed on the leaves and flower as well as fruit clusters. For each treatment, GA_3 was applied twice with the first spray at full bloom and spray again a week later. Water was sprayed to serve as a control. The average cluster and berry weight of the sprayed vines increased significantly over the non-sprayed vines, and the largest berries were found in the treatment with the highest concentration of GA_3 (300 mg/l). On the other hand, weight of seed trace decreased as the GA concentration increased, and no difference was observed for the number of seed traces per berry. Based on this result, 100 mg/l GA_3 was recommended for spraying on the Orlando seedless.

Korkutal *et al.* (2007) and Lu *et al* (1997) reported Gibberellin alerted parthenocarpic fruit formation and improved seedless berry formation 20-25%.

Coombe *et al* (1970) demonstration that Gibberellic acid causes seeded grape varieties seedless. It was stated that when Gibberellic acid applied to the grapevine before the inflorescence, pollens germination ability lessens, the seed tracing degenerates and fruit flesh become abnormally thick.

Motomura (1998) reported 100 ppm Gibberellic acid when applied before the inflorescence period and on the full bloom caused some grape varieties seedless. Because of the reason that the Gibberellic acid regulates growth, it caused big differences although applied in small amounts. He opined that high doses of Gibberellic concentrations might result in unexpected results.

Ben *et al.* (1990) noticed no responses effect of Gibberellic acid on Thompson Seedless grape variety applied after the harvest.

Teryl *et al.* (1989) reported that Gibberellic acid mitigated the depressing effect of girdling on grape during the same diumal measurements. The concentrations of foliar carbohydrates were greatest for the girdled vines, followed by the combination treatment were lowest for the control vines. Foliar carbohydrates were greater for girdled vines 4 weeks after the treatments were imposed, however, by this time there was no significant difference in between the control and girdled vines. Two and 4 weeks after the experiment was initiated root carbohydrate concentrations were less for the girdled vines when compared to the control vines. The data indicated that the reduction of girdled grape vines is not associated with the accumulation of leaf nonstructural carbohydrates following the girdling treatment.

Mander (1994) reported that among Gibberellic acid, GA_3 is widely used in the enlargement of grape berries in seedless grape varieties in practice. As stated by Celik (1998) GA started cell growth and enlargement showed a raped growth in the growth period just after berry set and reached the highest level towards the middle of this period. At the end of the researches, he conceded that Gibberellic acid was active on the point of the full bloom of stamens and kinds of grapes such as seeded and seedless types.

Okamoto and Miura (2005) reported that the applications of GA developed the largeness of fruit and balanced the structure of bunches. He also added that the combination between the time of application, the dosage and the open air temperature was directly related to the expected utility.

Gokcay (2005) showed that effect of GA_3 on production of grape was noticed with the application before inflorescence. The best effect could be obtained from the applications done 3 or 4 weeks before the beginning of inflorescence. The formation of small grains started when GA_3 applied before the inflorescence, but this happens rarely with the applications done with lesser than 10 ppm dose.

Aguero *et al.* (2000) hypothesized that high gibberellins levels in the seed during the first stages of its development caused abortion and resulted seedless grape in stenospermocarpic grapes (*Vitis vinifera* L.).

Fukunaga and Kurooka (1988) demonstrated that the effectiveness of GA on production of seedless berries in Kyoho grapes.

Panwar *et al.* (1994 a&b) reported that GA_3 has also proven to be useful for stimulating early and even ripening on uneven ripening Beauty seedless grapes (*Vitis vinifera* L.).

2.3 RELATED WORK DONE IN BANGLADESH

No report has been found on the pruning and spraying of GA_3 on grape variety.

CHAPTER THREE

MATERIALS AND METHODS

The experiments reported in this were conducted in Horticulture Research Centre, Regional Agricultural Research Station (RARS) of Bangladesh Agricultural Research Institute (BARI) Jessore during December 2007 to April 2009.

3.1. Plant Material

Four year old grape vines of Jhakka cultivar were used in this study. The plants were grown on a single wire telephone line system.

3.2 Essential Equipments and Chemicals

Equipments

1. Scissors
2. Conical flask
3. Pipette
4. Spray machine/Automizer
5. Basket
6. Balance (electronic)
7. Aluminum foil
8. Measuring flask
9. Test tube
10. Filter paper

Chemicals

1. Gibberellic acid (Source: Sigma company limited)
2. Isopropyl alcohol (IPA)
3. Distilled water (DW)
4. Washing detergents
5. Tween-20

3.3 Pruning

Before pruning, bearing and non-bearing canes were tagged for the study. Vine was pruned on 1st December, 2007; 7the October, 2008. All the bulk of the previous year season's growth was cut. The leaves on the remaining canes were cut close to the main arms. The weak and old canes were cut close to the main arms. Pruning cut were made about 2 to 3 cm above the last buds so that the shoots that would develop would have a strong base. The vine was totally bare after pruning. The main arms unless badly damaged or diseased were not cut.

3.4 Preparation of 1000 ppm GA_3 stock solution (1000 mg/L)

1 gm (1000 mg) GA_3 was dissolved in 20 ml Isopropyl alcohol in a test tube. Then the alcoholic solution was made 1000 ml with distilled water to prepare 1000 ppm stock solution of GA_3.

Preparation of working solution

100 ppm working solution of GA_3 was prepared from the stock solution of GA_3 by the following formula as follows:

$$S_1V_1 = S_2V_2$$

Here, S_1= Strength of stock solution

V_1 = Volume of stock solution

S_2 = Required strength of solution

3.5 Method of GA_3 Application

After preparation of working solution of 100 ppm GA_3, the required sample solution was poured into an automizer and sprayed on floral buds at least 1-2 weeks before anthesis. Water was sprayed on flower to serve as a control. Spray was done early morning before scorching of full sun shining. The growing berries were sprayed with 5 ppm GA_3 after 4 weeks of fruit set.

3.6 Data collection

1. Date of pruning
2. Date of bud break
3. Days of bud break
4. Berry size (length x breadth) (cm)
5. Berry weight (g)
6. Bunch weight (g)

7. Total Soluble Solid of berries (%): Determine by refracto-meter (Brix Meter).
8. Presence of absence of seed

3.7 Data analysis

Student's t-test was done to compare the means of two groups. Chi-square (I) test was used to determine whether there was any significant difference between the expected frequencies and the observed frequencies in one or more categories.

Chi-square test:

$$X^2 = \frac{(O-E)^2}{E}$$

Here, O = Observed value
E = Expected value

CHAPTER FOUR

RESULTS AND DISCUSSION

Time of pruning of vines influences bud break, anthesis of flower and harvesting significantly. Application of GA_3 at 100 ppm also influences significantly both qualitative and quantitative characters of berries. The data on different characters are presented in Table 1 and 2. The results of the study are discussed below.

4.1 Reproductive bud break

Days to reproductive bud break was significantly influenced by the date of pruning. When vines were pruned in 1st Dealer 2007 days to bud break was recorded maximum from the date of pruning while days to bud break was significantly reduced is the vine was pruned in 1st week of October 2008. (Table 1).

4.2 Anthesis of flower

The days to anthesis of flower for the date of spray of GA_3 were not influenced significantly by the date of pruning of vine. Days to anthesis of flower was the longest when vine were pruned in December 2007 and the lowest in January (2008) pruning. An thesis time in October (2008) pruning was logs the jamming paring but lower than Dee pruning (Table-1).

4.3 Days to harvest

Days to harvest were recorded minimum from the vine pruned in January and maximum in December (2007). Pruning which was very close to October pruning (Table-1).

4.4 Seedlessness

All burins obtained from various date of vine pruning were seedless when treated with GA_3 while berries were always seeded from the control. Similarly GA_3 influenced the sweetness of berries. Berries treated with GA_3 were sweet and sour and only sour berries were harvested from the control. (Table-2).

4.5 Individual berries

Time of pruning as well as GA_3 treatment showed significant variation in individual fruit of berries. January pruning produced individual height berry weight as con pare to December and October pruning. No significant variation in individual berry weight was noticed from control plants. However, paired test indicated that GA_3 individual berry weight was many or as compared to control (Table 3).

4.6 Individual berry weight.

Individual berry weight was significantly higher when flowers were treated with 100 ppm GA_3 as compared to control. However, individual berry weight was statistically similar among the GA_3 treated flowers as will in control obtained form different pruning time.

4.7 Total soluble solid (TSS)

The effect of GA_3 on TSS of berries was note wary. TSS of berries obtained from flowers treated with GA_3 was significantly higher then control. But there were no significantly among the berries obtained from different pruning times. TSS ranged from -14% in berries obtained from flowers when treated with GA_3 while the range of TSS was 5-14% in control.

4.8 DISCUSSION

Grape vine normally have more branches than necessary for reproductive purpose. Removal of unnecessary branches has an advantage of receiving of nutrients of remaining brunches. Pruning does not disturb was done without disturbing root and destroys apical auxine manufacturing mechanism which suppresses lateral bud growth. More nutrients and water become available for the growth of new lateral shoots of cane. Wounding tissues of pounced vines enhanced endogenous ethylene, a gaseous autocatalytic hormone. High concentration of ethylene opposes action of GA_3 and auxin and elevate ratio of cytokine: auxin which breaks down auxiliary bud dormancy and stimulates bud initiation. Davies (1995) stated that in plant leaves there is interaction between flower stimulator and an inhibitor. This inhibitor interferes with the expression of floral stimulus resulting in regular growth. High level of floral stimulus led to flowering while low level to vegetative growth. The floral stimulus remains unidentified, but evidences indicate that the inhibitor may be GA_3. Alejer *et al.* (1999) explained that the new shoots after pruning do not compete for photosynthetic products and plant attains an autonomous promotion of vegetative growth as well as flowering. Similarly Lombard *et al.* (2000), Hatch and Ruiz (1987) and Gray *et al.* (1996) reported vines pruning initiated vigorous vegetative growth along with reproductive buds break. Accumulation of winter chilling during December-January and subsequent mild temperature afterward favored the growth and development of berries gave advantage of early harvest when pruning was done in January (Hatch and Ruiz, 1987). Fruit is a mature or ripened ovary and seed formation begins with the combination of male and female gamest. A weak grade of femaleness is responsible for the development of seedless grape by simulative parthenocarpy. The female flowers fertilized with sterile pollen stimulate ovary to grow, GA_3 application at problem stage is given to pollen non functional. On the other hand embryo sac in the treated ovary does not fully develop to fertilize at the tine of anthesis. Applied GA_3 promoted pericarp growth through well enlarging. Therefore it is supped that buries may be induced by incompletes of both male and organs.

Table-1: Effect of pruning on bud break and anthesis of flower and harvesting

Trial	Date of pruning	Date of reproductive bud break	Days to reproductive bud break	Date of spraying of GA_3	Date of anthesis	Days to anthesis	Days to anthesis from bud break	Days to 1st harvest from bud break
Dec ,2007	1Dec/07	3 Feb/08	64	2 Mar/08	12 Mar/08	10	37	98
January, 2008	7 Jan/08	20 Feb/08	43	3 Mar/08	10 Mar/08	7	18	71
October,2008	7 Oct/08	5 Nov/08	28	19 Nov/08	30 Nov/08	11	25	96
χ^2 Value			14.533			.929	6.925	4.725
Chi test			0.001			.629	0.03	0.094
Level of significant			**			ns		**

** indicates significant at 1% level; * indicates significant at 5% level; ns indicates non-significant

Table 2: Effect of GA_3 on qualitative character of grape

Trial	Seediness		Taste	
	Treated with GA_3	Control	Treated with GA_3	Control
December, 2007	All berries Seedless	Seeded berries	Sour and Sweet	Sour
January, 2008	All berries Seedless	Seeded berries	Sour and Sweet	Sour
October, 2008	All berries Seedless	Seeded berries	Sour and Sweet	Sour

Table 3: Effect of GA_3 on quantitative character of grape

Trial	Individual berry weight (g)		TSS (%)	
	Treated with GA_3	Control	Treated with GA_3	Control
December, 2007	1.3	0.87	11 (6-16)	5 (4-6)
January, 2008	1.5	0.87	12 (9-14)	9 (4-14)
October, 2008	1.2	0.69	10 (6-14)	6 (4-8)
Mean	1.33	0.87	11.00	6.67
Standard deviation	0.152	0.10	1.00	2.08
CV(%)	11.45	12.82	9.09	31.22
χ^2 Value	0.03	0.98	0.91	0.52
t-test	9.00*		4.91*	
Probability	0.012		0.039	
* indicates significant at 5% level				

Fig-1: Grape Orchard

Fig2: Grape vaines

Fig-3: Infloresence of grape

Fig-4: Anthesis of flower

Fig-5: Grape on vaines

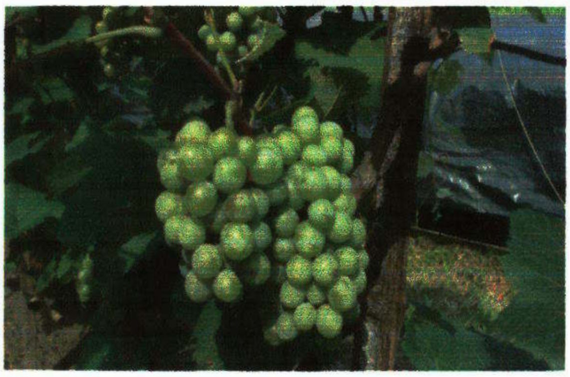

Fig-6: Grape of control vines

Fig-7: Seedless grape

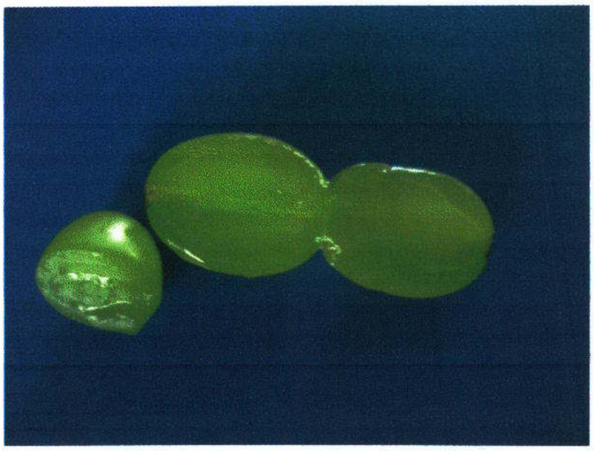

Fig-8: Seedless grape

Fig-9: Seedless grape

Fig-10 Seedless grape

Fig-11: Control and GA treated grape

CHAPTER FIVE

CONCLUSION

It was found that the application of GA_3 at the rate of 100 ppm as well as pruning of vines were highly potential in production of high quality grape in Bangladesh. Pruning practice of local might be followed to allow Jhakka cultivar flowering in late winter to induce growth of fruit in dry and mild weather of early summer. It was also observed in the study that the ripening of the berries were not synchronized. As a result the berries showed sour to sweet tested. Further studies are essential on floral morphology and doses of GA_3 to recommend the stage/age of and concentration of GA_3 to develop sweetness and synchronizing of ripening of berries.

CHAPTER SIX
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APPENDICES

Appendix 1: Days to reproductive bud break

	Observed N	Expected N	Residual
1 Dec/07 -3 Feb/08	64	45.0	19.0
7 Jan/08 -20 Feb/08	43	45.0	-2.0
7 Oct/08 -5 Nov/08	28	45.0	-17.0
Total	135		

Test Statistics

	Days to reproductive bud break
Chi-Square(a)	14.533
df	2
Asymp. Sig.	.001

a 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 45.0.

Appendix 2: Days to anthesis

	Observed N	Expected N	Residual
2 Mar/08 -12 Mar/08	10	9.3	.7
3 Mar/08 -10 Mar/08	7	9.3	-2.3
19 Nov/08-30 Nov/08	11	9.3	1.7
Total	28		

Test Statistics

	Days to anthesis
Chi-Square(a)	.929
df	2
Asymp. Sig.	.629

a 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 9.3.

Appendix 3: Days to anthesis from bud break

	Observed N	Expected N	Residual
3 Feb/08 -12 Mar/08	37	26.7	10.3
20 Feb/08 -10 Mar/08	18	26.7	-8.7
5 Nov/08 -30 Nov/08	25	26.7	-1.7
Total	80		

Test Statistics

	Days to anthesis from bud break
Chi-Square(a)	6.925
df	2
Asymp. Sig.	.031

a 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 26.7.

Appendix 4: Days to 1st hervest from bud break

	Observed N	Expected N	Residual
1	98	87.3	10.7
2	71	87.3	-16.3
3	93	87.3	5.7
Total	262		

Test Statistics

	Days to 1st hervest from bud break
Chi-Square(a)	4.725
df	2
Asymp. Sig.	.094

a 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 87.3.

T-Test

Appendix 5: Paired Samples Statistics for Individual berry weight (g)

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	treated	1.3333	3	.15275	.08819
	Control	.8100	3	.10392	.06000

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	treated & Control	3	.756	.454

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	treated - Control	.52333	.10066	.05812	.27327	.77340	9.005	2	.012