CHAPTER 1

INTRODUCTION

The wax apple or locally known as *jambu air*, is a non-climacteric tropical fruit which belongs to the family Myrtaceae and is botanically identified as *Syzygium samarangense* (Morton, 1987). Among its various vernacular names are: wax apple, wax jambu, samarang rose apple, and water apple. The waxy fruit is pear shaped, usually pink, light red, red, sometimes greenish- white or cream-colored, often crisp, with a subtle sweet taste and an aromatic flavor (Morton, 1987). The species presumably is a true indigene of in Malaysia and other South-East Asian countries (Nakason and Paul, 1998). In Malaysia, there are about three species which bear edible fruits, namely the water apple *(Syzygium aquem),* Malay apple (*Syzygium malaccense*) and wax apple or *jambu air* (*Syzygium samaragense*). *Syzygium samarangense* is the most popular of the three in South-East Asia and the trees are cultivated in home gardens, often planted along driveways and paths.

The wax apple is widely cultivated and grown throughout Malaysia and neighboring countries such Thailand, Indonesia and Taiwan on a small scale where the climate is suitable for its production all year round. Wax apple is popular fruit in Malaysia and other South East Asian countries. Currently in Malaysia, it is cultivated mainly as smallholdings ranging from 1 to 5 ha with its hectarage estimated at about 2000 ha in 2005 (Zen-hong *et al.,* 2006). The area cultivated and production for the year 2003 in Indonesia were 13,454 ha and 239,108 tons, respectively. However, fruit production is non seasonal and the peak periods are in March to April and November to December. Relatively unknown outside South Eastern Asia, wax apple is an economically important fruit crop in Taiwan (Wang, 1991, Shu *et al*.1996). The area, total production and production value for the wax apple industry in Taiwan were 7302 ha, 84,991 tons and $ 182 million US dollars, respectively,

in 2005 (Zen-hong *et al.,* 2006). The pink varitey is the leading cultivar in Taiwan. Due to successful off-season production techniques, fruits can be harvested all-year-round in Taiwan. In Thailand there were 10,240 ha planted area and 69,608 tons fruits produced with a production value of $ 26.5 million US dollars in Thailand in 2004 (Zen-hong *et al.,* 2006). There is a great scope to develop wax jambu industry in Malaysia and other tropical countries. The Malaysian climate is suitable for the wax jambu production and fruits can be harvested all the year round. It has become increasingly popular in this region and has the potential to bring great benefit to local farmers and the country's economy.

The fruits are pear shaped, often juicy, refreshing, with a subtle sweet taste and has an aromatic flavour. The quality of the best variety is not surpassed by the other species. The wax jambu is glossy indeed wax-like and the flesh is rather dry compared to other species of *Syzygium*. In Malaysia, the fruits of *jambu air* are eaten raw with salt or cooked as a sauce. Ninety per cent or more of the fruit is edible and the fruit pulp is a rich sources of phenolics, flavonoids and several antioxidant compounds (Rivera and Obón, 1995). In addition to its use as food, it has also been used in traditional medicine for a variety of illnesses and conditions. The wax jambu has great potential benefits for human health because it is a rich source of polyphenolic antioxidants (Rivera and Obón, 1995).

Manipulating the vegetative and reproductive growths of fruit trees has long been the goal of growers and researchers in agriculture. There are several agricultural techniques have been used for improved yield of horticultural products. Mutation breeding, genetic engineering, ionizing radiation, cross breeding, tissue culture and biochemical and DNA markers are important methods and tools for crop improvement that have been employed over the years and recently (Novak and Brunner, 1992). The combination of *in vitro* systems, molecular biology and genetic transformation also contribute to an ultimate

strategy for crop breeding and improvement (Wagner and Hazel, 2005). The basic cultural managements like fertilizer application, pruning, thinning and irrigation are also important factors to increase the yield of vegetables and fruits (Gunes *et al.,* 2010). Foliar application of various macro and micronutrients has also been shown to be beneficial for plant production improvement (Baloch *et al.,* 2008). Soil and foliar applications of urea, potassium and zinc has been reported to increase yield in avocado (Nevin *et al.,* 1990), lemon and orange (Jones and Embleton, 1969). Foliar application of balanced fertilizer and zinc increased the fruit growth, yield and quality of apple (Malakouti, 2007). Pruning of fruit trees has been shown to provide early bearing of fruit trees, lengthen productivity periods and eliminate alternate bearing (Westwood, 1993). Thinning of leaf, flower bud and branches improve the size, yield and quality of fruits (Boler, 1998). Irrigation management is another powerful tool to manipulate tree growth for greater fruit-fullness and less vegetative growth (Ruiz-Sanchez *et al.,* 2010). It has been reported to regulate drymatter accumulation, fruit growth and development and soluble solids, peel color as well as nutritional quality of fruits (Girona *et al.,* 2004; Perez-pastor *et al.,* 2007). In addition to the above, every agricultural production in the tropics has to control pest and diseases which are important for both productivity and quality of horticultural crops. Pesticide applications are one of the major fossil fuel energybased inputs used to increased plant productivity. Insecticide and herbicide applications can prevent yield losses due to insect and weed infestation (Erdal and Clive, 2003)

Plant growth regulators are organic compounds, either natural or synthetic, that modifies or controls one or more specific physiological processes within a plant. Plant growth regulators are substances intended, through physiological action, to accelerate or retard the rate of growth or maturation, or otherwise alter the behavior of plants or their produce.

There are five groups of plant-growth-regulating compounds: auxin, gibberellin (GA), cytokinin, ethylene, and abscisic acid (ABA). For the most part, each group contains both naturally occurring hormones and synthetic substances.

In the new millennium, plant growth regulators are perhaps one of the most powerful tools available for achieving this goal (Salazar-Garcia and Lovatt, 2000). It has been well documented in the literature that the size and quality of the fruits can be affected by certain horticultural practices, such as the application of plant growth regulators, growth promoting chemicals and growth manipulation of trees (Guardiola, 1992). Physiological parameters, namely, leaf chlorophyll content, CO_2 assimilation rate, stomatal conductance, and dry matter accumulation in the leaf are greatly influenced by growth regulating substances (Grewal *et al.,* 1986; Nahar and Takeshi, 2002). Plant growth regulation chemicals have been used extensively in fruit research over the years, for a wide variety of fruit trees. There have been many examples of the successful use of plant growth regulators (PGR) to enhance flowering time, number of flowering buds, reduce excessive bud dropping, increase fruit set, fruit retention, growth, yield and quality of crops (Kakaoka *et al.,* 2009) .Gibberellic acid (GA) has been shown to increase fruit set and growth in clementine orange**,** fruit size and firmness in cherry fruits and increased the yield of Balady mandarin (Van Rensburg *et al.,* 1996; Choi *et al*., 2002; El-Sese 2005). GA_3 has been used to increase fruit firmness, fruit weight, and soluble solids in cherry fruits (Basak *et al.,* 1998). It has also been reported that application of GA_3 increased the sugar content in various mandarin orange and the anthocyanin content in strawberry fruit (Wang *et al*., 2004; Roussos *et al.,* 2009). It has also been documented that GA_3 enhanced the peel colour development and stimulate the PAL activities in strawberry fruits (Teresa *et al.,* 1998). Growth regulating chemicals has also been shown to promote the activities of two

key enzymes in CO_2 assimilation, and the expression of *rbcL*, *rbcS*, and *rbcA* in spinach (Yuguan *et al.*, 2009).

Synthetic auxins (NAA and 2,4-D) are well known plant growth regulators that can substitute for pollination and induce fruit setting and growth, development as well as quality of tomato (Kataoka *et al.,* 2009). NAA has been reported to stimulate cell enlargement in orange, enhancing fruit growth in citrus (Agusti *et al.,* 1995; Davis, 2004). More recently, it was observed that NAA significantly increased fruit setting, fruit length, diameter and weight as well as yield in guava (Dutta and Banik, 2007). It was found that application of NAA reduced the fruit drop, increased yield, TSS, total sugar and vitamin-C contents in guava fruits **(**Iqbal *et al.,* 2009**).** In addition to this 2, 4-D is regarded as one of the most effective PGR in preventing fruit drop in citrus (Davies and Zalman, 2006). It has also been reported that 2, 4-D when applied at ultra-low doses significantly improved the fruit size, yield, color and TSS content of Bing Cherry fruit and also increased its total sugar content and enhance activities of antioxidant enzymes (Raphael *et al.,* 2007; Baogang *et al.,* 2008). Synthetic auxin has been shown to increase total antioxidant capacity as well as the nutritional quality in transgenic silcora seedless grape (Elisa *et al.,* 2007).

However although the application of plant growth substances and chemicals has been employed as a practice to affect the size and quality of a fruit, this strategy has become increasingly viewed as inappropriate due to its possible effects on the environment and human health. This has led to the use of hydrogen peroxide as an alternative. Hydrogen peroxide is a stable partially reduced oxygen form and its rapid turnover is characteristically mediated by enzyme action. Hydrogen peroxide plays a dual role in plants. At low concentrations, it acts as a messenger molecule involved in adaptive

signaling, triggering tolerance against various abiotic stresses (Dat *et al.,* 2000), and, at high concentrations it orchestrates programmed cell death (Alvarez *et al.,* 1998). Used in horticulture, hydrogen peroxide provides a host of benefits by cleaning water of harmful substances such as spores, dead organic material and disease causing organisms while preventing new infections from occuring. Hydrogen peroxide is of great use in hydroponics, soilless gardening and root cuttings. Recently Ozaki *et al.* (2009) spraying hydrogen peroxide on melon fruits increases the sugar content and antioxidant levels. They showed that hydrogen peroxide also affected the photosynthetic activity in melon plants, in addition to its effects on selected Calvin cycle enzymes.

Another route to achieve the good food production would be employing the girdling technique, an old practice used to improve crop productivity. Girdling has been, and is still, a worldwide horticultural practice used to manipulate tree growth and development. The removal of a small strip of bark around a branch or trunk will obstruct basipetal phloem transport and make available more photosynthetic metabolites to the growing regions above the strip (Casanova *et al.,* 2009). Although removing the strip of bark wounds the tree, it heals within several weeks. The ringing of trees can bring about an increase in the size and sugar content as well as quality characteristics of fruits and cause them to mature a few days to a week earlier as was shown in apple (Arakawa *et al.,* 1997). The effects of girdling are presumably brought about by accumulation of assimilates above the girdle. It has been suggested that girdling can change the fruit quality, especially increased soluble solids content and reduced acid concentration, by blocking the translocation of sucrose from leaves to the root zone through phloem bundle. It has also been reported that the gibberellin and cytokinin content are also modified by girdling in olive (Barut and Eris, 1994). They reported that girdling maintained the leaf N content,

hormonal balance, C/N ratios and increased the carbohydrate concentration in the region above the girdling point in olive. Girdling has also been shown to enhance flowering, increase the number of buds, fruit set, reduced bud and fruit drop by supplying carbohydrate at the early stage of fruit development in citrus fruits (Rivas *et al.,* 2008).

Research objectives

The objective of this project is to study the effects of selected plant growth substances on the growth, development and quality of the *Syzygium samarangense* fruits with a view to enhance its growth as well as increase its yield and quality. In the present study, the effects of plant growth regulators namely: GA_3, NAA and 2, 4-D, girdling and H_2O_2 on fruit growth, development and physiology of *S. samarangense* were studied under field conditions (Fig.1.1).

The main objectives of the present work were:

1. To assess the effects of GA_3, NAA and 2,4-D on fruit growth, development and biochemical properties of *Syzygium samarangense* fruits.
2. To analyze the influence of hydrogen peroxide and girdling on the biochemical, phytochemical properties and antioxidant capacity of the *S. samarangense* fruits.
3. To examine the effects of GA_3 and NAA on colour development, anthocyanin formation, expression of PAL enzyme and rbcA gene expression

The research questions are posed in this study are:-

(i) Will the application of GA_3, NAA, 2, 4-D, H_2O_2 and girdling affect or promote the growth and development of *S. samarangense* fruits?

(ii) Does GA_3, NAA, 2, 4-D, H_2O_2 application and girdling affect the quality of *S. samarangense* fruits?

(iii) If the answer to (i) and (ii) are positive, how is it brought about?

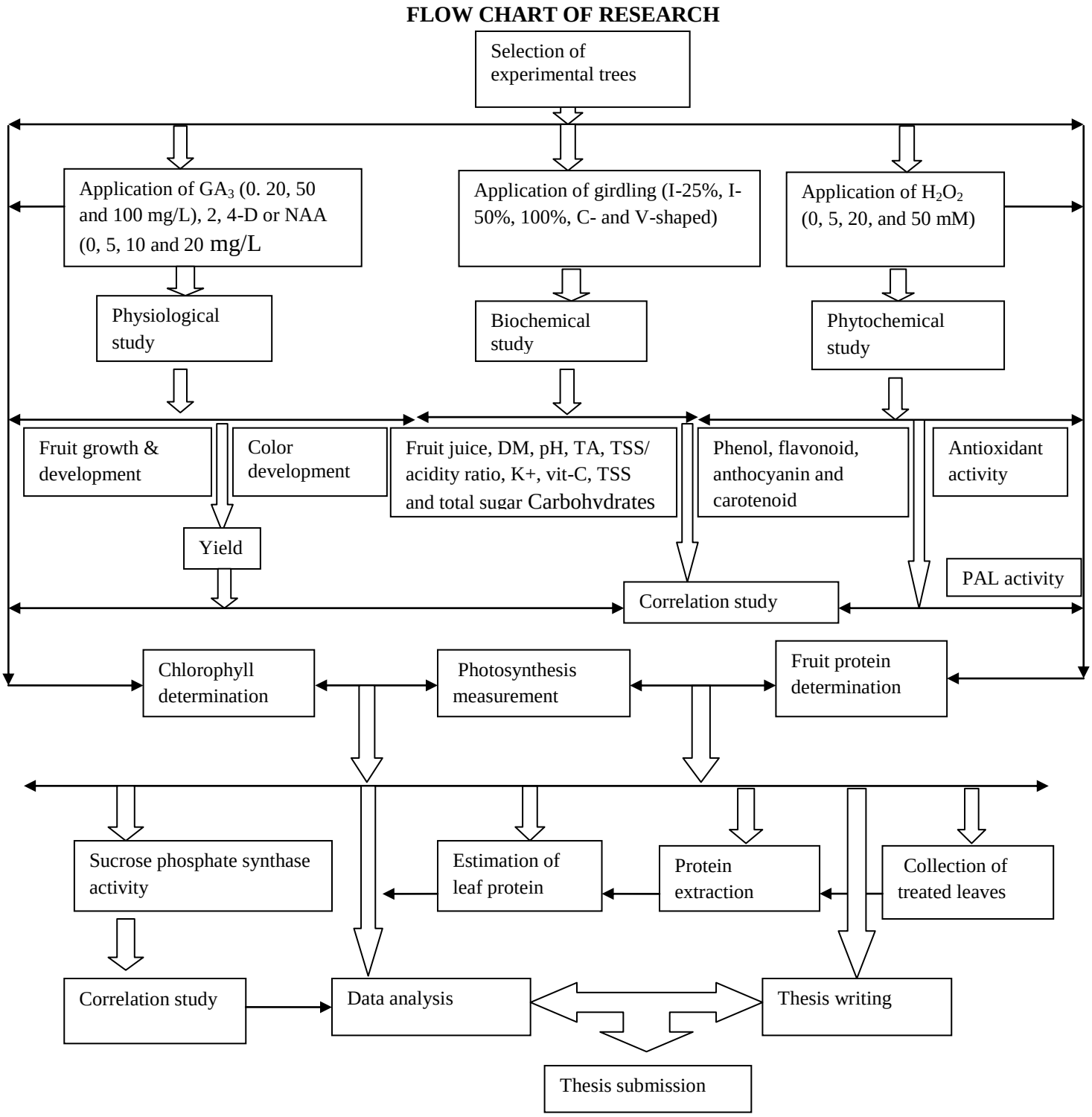


Fig. 1.1. Flow chart of the study from plant selection and treatments to data analysis and thesis writing.

CHAPTER 2

LITERATURE REVIEW

2 .1 The Botany of *Syzygium samaragense*

The tree is commonly cultivated throughout the tropical lowlands in South East Asia where it is believed to originate from. The genus *Syzygium* consists of about 1100 tropical species. The nomenclature of the *jambu air* fruits is as follows (Morton, 1987):

Kingdom: Plantae

Sub Kingdom: Tracheobionta (Vascular plants)

Super Division: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Order: Myrtales

Family: Myrtaceae

Genus: *Syzygium*

Species: *Syzygium samaragense* (Blume) Merr. & Perry

Common names of *S. Samarangense* include wax *jambu*, wax apple, java apple (English); *jambu semarang, jambu klampok* (Indonesian); *jambu air mawar* (Malay); *makopa* (Filipino); *chomphu-kaemmaem, chomphu-khieo* (Thai); *roi* (Vietnamese); *bellfruit* (In Taiwan) (Verheij and Coronel, 1991).

The trees of *S. samaragense* are cultivated in home gardens, often planted along driveways and paths as well as cultivated in small holdings. The trees grow well in fairly moist tropical lowland areas up to 1200 m in elevation. They also grow best in areas with a fairly long dry season (Nakasone and Paull, 1998).

However this does not mean that the tree is drought-resistant. In fact it requires a reliable supply of water and is often planted along small rivers, streams or ponds (Hakim and Panggabean, 1992).

The tree is 3-15 m tall, with short and crooked trunk, 25-50 cm diameter, often branched near the base and with wide, irregular canopy. Leaves are opposite, elliptic to elliptic-oblong, 10-25 cm x 5-12 cm, coriaceous with thin margin, pellucid dotted, rather strongly aromatic when bruised; petiole thick, 3-5 mm long (Fig. 2.1). The wax apple is a heavy producer on wellfertilized good soils, and can produced more than 200 clusters per tree, with 4-5 fruits in each cluster at maturity. Wax apple commonly flowers early or late in the dry season; the flowers appear to be self-compatible and the fruit ripens 40-50 days after anthesis. Inflorescences are terminal and in axils of fallen leaves, 3-30-flowered; flowers 3-4 cm in diameter, calyx-tube ca. 1.5 cm long, ventricose at apex, lobes 3-5 mm long; petals 4, orbicular to spathulate, 10-15 mm long, yellow-white; stamens numerous, up to 3 cm long; style up to 3 cm long (Fig. 2.2).

Fruit is a berry, pear shaped, broadly pyriform, crowned by the fleshy calyx with incurved lobes, 3.5-5.5 cm x 4.5-5.5 cm, light red to white; fruit flesh is white spongy, juicy, aromatic, sweet-sour in taste (Fig. 2.3). Seeds 0-2, mostly suppressed, globose, up to 8 mm in diameter (Morton, 1987). The waxy fruits is peer shaped and the color of the fruits is usually pink, light red, red, sometime time green or cream-colored (Morton, 1987). In Malaysia, jambu madu fruit fruits are eaten with salt or cooked as a sauce. Fruit production is non seasonal and fruits can be harvest three times per year. Almost all of the fruit is edible.

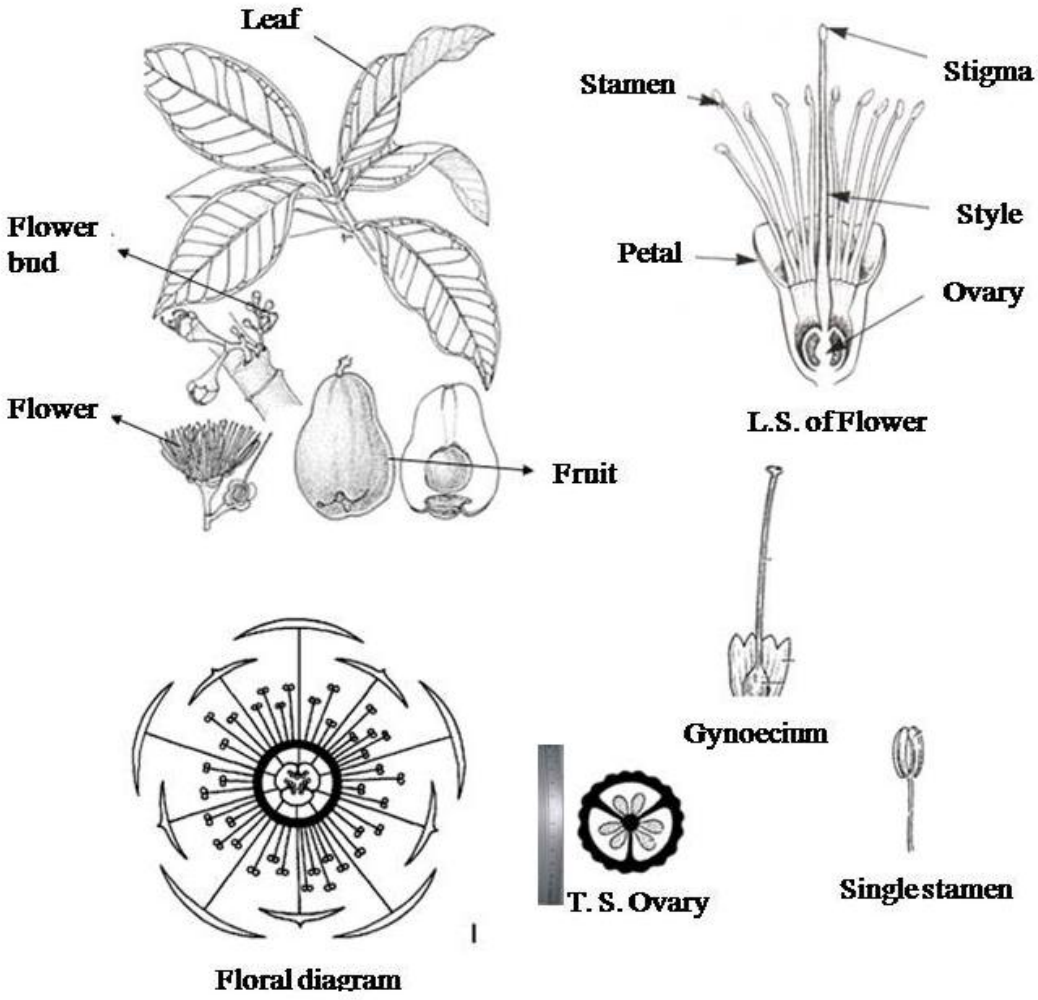


Fig. 2.1. Leaf, flower bud, flower and different part of flower, floral diagram and fruit of wax apple (*Syzygium samarangense*) (Source: Nakasone and Paul, 1998).

Fig. 2.2. Blooming flower of wax apple (*Syzygium samarangense* var. jambu madu).

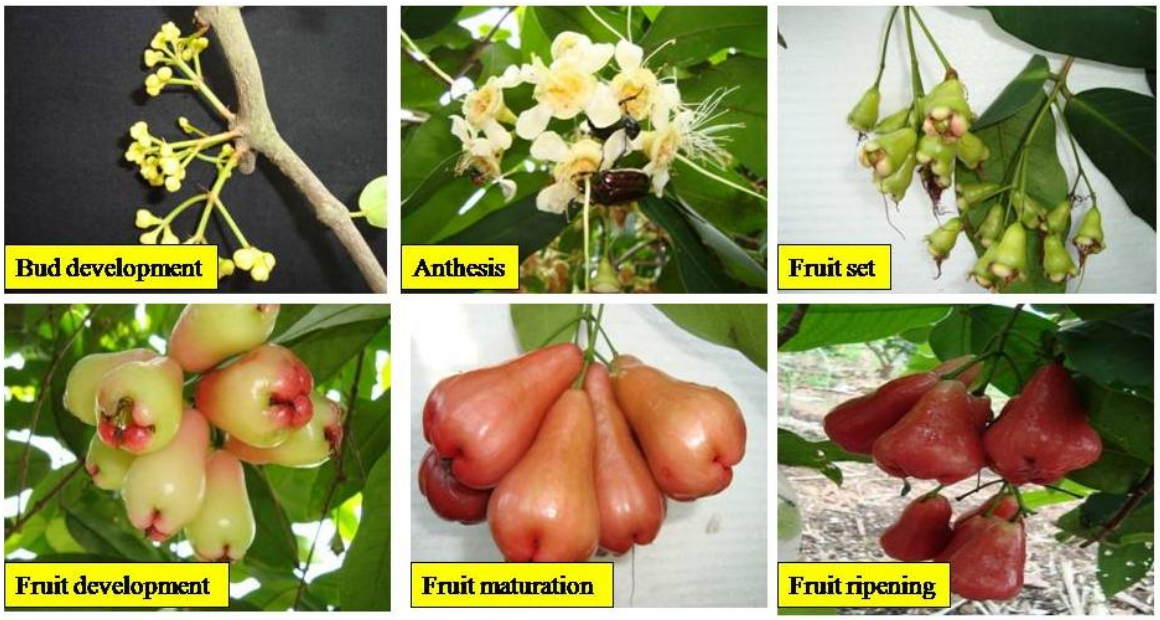


Fig. 2.3. Different stages of fruit growth and development of wax apple (*Syzygium samarangense*).

2.2 Ecology and distribution of *Syzygium* sp

The fruit tree, although almost completely unknown outside southeastern Asia, is an economically important fruit crop in Taiwan (Shu *et al.*, 1996). The 'Pink' ('Nun-Young' in Chinese) cultivar represents 99% of the planted area in Taiwan (Wang, 1991). The regular blooming time for 'Pink' is around March in Taiwan (Young, 1951). However, 'Pink' blooms and sets fruit almost year-round after flowering (Wang, 1991; Shu *et al.*, 1996). As a result, fruits at different growing stages could be found in different orchards, different trees and even on the same tree. Located at the centre of origin, Indonesia has a huge amount of variety with great diversity. Pale Green, Dark Red, Light red and Green are the four major Indonesian varieties. Fruit production is non seasonal, however the peak periods are in March to April and November to December. The major wax apple cultivars in Thailand are Phet Jin Da, Number one, Phet Sam Phran, Dang Indo, Phet Nam Pueng, Thub thim chan. Fruits can be harvested all the year round. Despite its name, this cultivar produces fruits varying from pink to deep red, depending on environmental and cultural conditions (Shu *et al.*, 2001). Wax apple grows best in areas with a fairly long dry season. This does not mean that this species is drought-resistant.

The species require a reliable water supply and are often planted along streams or ponds. Current distribution ranges from India through South-East Asia to the Pacific Islands (the Malay apple features in Fijian mythology). *S. samarangense* is the most popular among the three species grown in South-East Asia (Fig. 2.4) (Chang *et al.*, 2003). Now, the tree is cultivated throughout the tropics as far as east of Hawaii, as well as Central and South America (Whistler and Craig, 2006). Recently, wax apple has become a popular exotic fruit in western countries because the combination of apple-like crispness,

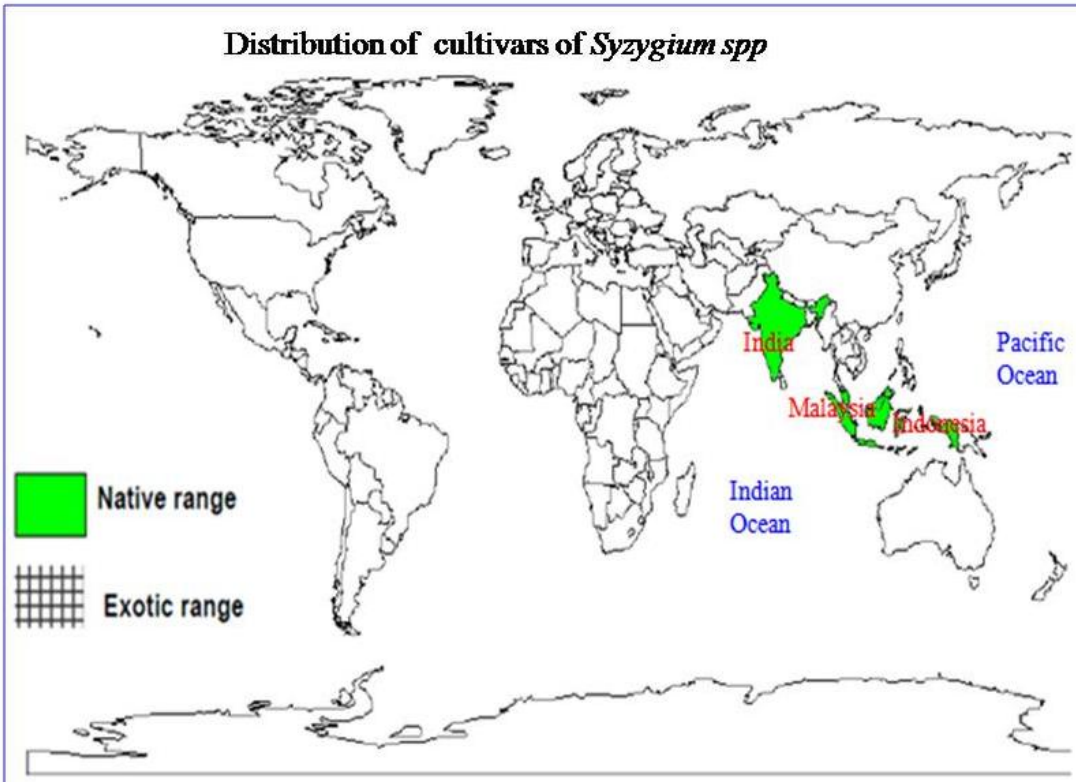


Fig. 2.4. Location of wax apple cultivation in native and exotic range.

watery sweet and low-acid taste and the aroma of roses (FAO, 2005). Presently, wax apple cultivation also spread in Indonesian island of Java, Philippines, Thailand and other Southeast Asian countries. China's Guangdong, Hainan, Fujian, Guangxi, Yunnan and Sichuan provinces (autonomous regions) has a small area of cultivation. Cultivation in Taiwan, the largest species of its greatest economic value for the black pearl wax apples and black diamond wax apple.

2.3 Fruit growth, development and harvest

Fruit growth and development involves changes in its morphology, anatomy and physiology (Chahidi *et al.,* 2008) whilst fruit ripening is associated with dramatic changes in rind texture, color, juice composition, increase in softness due to changes in the cell walls, the metabolism of organic acids and the development of compounds involved in flavor and taste (Davies *et al.,* 1997; Javanmardi and Kubota, 2006). The number of days from bloom to the time for harvest is a useful guide to determine the harvest time where the shape, ground color, size or sheen on the fruit are usually as indicators of maturity.

There are definite flowering seasons, often two, sometimes three in a year, in spring, summer and fall. The biggest crops are produced in the spring and fall flowering seasons. The tree flowers in May and June and the fruits ripen in August and September and the second crop is often in November and December. Wax apple commonly flower early or late in the dry season; The flowers appear to be self-compatible and the fruit ripens around 30-40 days after anthesis. (Morton, 1987).

There are three common types of growth patterns among different fruits including a single, double, or triple sigmoidal curve for growth (Coombe, 1976; Trimble *et al.,* 2006). Many fruits, such as apple (Dennis, 1986) and banana (Israeli and Lahav, 1986) are reported to have a single sigmoidal growth curve in which there is an initial phase of slow growth,

followed by a rapid growth period, and finally a period of declining growth rate when ripening is often initiated.

Other fruits including stone fruits, figs, and grapes show a double sigmoid curve in which there are two rapid growth phases (respectively named as periods I and III) interrupted by one short period of growth, namely period II. The growth pattern of the kiwifruit (*Actinidia chinensis*) can be described as triple sigmoidal (Bollard, 1970). Normally, the fruit is harvested when blossom-end is fully expanded and skin shows desired market colour. The fruit size can be as small as about 4.3 cm long and 4.7 cm wide to more than 5.2 cm long and 5.0 cm wide (bell-shaped) or 7 to 8 cm long and 4 cm wide (elongated) (Fig. 2.5). According to Shu *et al. (1998),* the growth of *jambu* fruits exhibits a sigmoidal growth curve.

Fruit mass ranges from 28 g to 100 g and fruit sized of more than 200 g per fruit (Fig. 2.7 & 2.8) (Shu *et al.*, 2007). Fruit shape ranges from round to bell shaped, oval or elongated and skin color diverges from white to pale green to dark green, pink to red to deep red (Fig. 2.5 & 2.8). Wax apple trees are tropical preferring temperature above 18ºC and cannot tolerate temperatures below 7ºC, (Huang *et al.,* 2005). Fruits of wax apple prefer warm temperatures for normal growth and development. Low temperatures impede fruit growth and red color development, while high temperatures accelerate fruit growth and ripening yet inhibit red color development. The fruits have a thin red skin and are delicate (Fig.2.6); they need to be picked by hand twice a week and handled with care. The fruit should be consumed or preserved within a few days from harvest.

2.4 Growth and ripening of wax jambu

It is a non-climacteric fruit according to (Akamine and Goo, 1979). It has been found that wax apple have a very low respiration rate of 10-20 mg CO_2/kg. h at 20°C,

Fig. 2.5. Ripening fruits of wax apple (*S. samarangense*).

Fig.2.6. Peel color of wax apple (*S. samarangense*).

although they are highly perishable fruits (Akamine and Goo, 1979). Without any preformed injuries, the sound fruit shows a good keeping quality at 2 to 5°C and at 2 to 10°C wax apple produce very low ethylene. However, chilling injury is a problem at these temperatures. Storage at 12 to 14°C (54 to 57°F) with 90 to 95% RH would result in a shelf-life of 10 to 14 days. Fruit peel color of the most popular cultivar in Taiwan, 'Pink', ranges from light-red to deep-red despite of its name, besides the pulp color white creamy to light red (Fig. 2.5 and 2.7a & 2.7b). As more is paid for the deep-colored fruits, factors influencing red color of 'Pink' has attracted much interest. At present, not only does intact wax apple fruit is an important fruit in the markets but fresh-cut wax apple has also become a popular fresh-cut product, especially in Southeast Asia (Worakeeratikul *et al*., 2007).

It has been reported that red color of wax apple is influenced by such factors as: leaf: fruit ratio (Wang, 1991), sugars (Liaw *et al.,* 1999; Shu *et al.,* 2001), position of fruits on the tree (Shu, 1999), fruit development stages (Chang *et al.,* 2003), light and temperature (Shu *et al.,* 2001). The crucial timing for sugar availability (or optimum developmental stages for sucrose utilization) for the development of wax apple fruits especially anthocyanin in fruit skin is not known (Chang *et al.,* 2003).

2. 5 Functional uses of *Syzygium spp*.

The ripe, pink fruits of wax apple are bell-shaped, sweet and can be eaten fresh or cooked, for sauces, jams, jellies desserts, wines, liquors, and vinegars. In Malaysia, the green fruits of wax apple are eaten raw with salt or cooked as a sauce. Some volatile constituents, such as 2-phenylethanol and its derivatives, were found to be the major compounds in the fruit (Pino *et al*., 2004).

Fig.2.7a. Pulp color of wax apple (*Syzygium samarangense*).

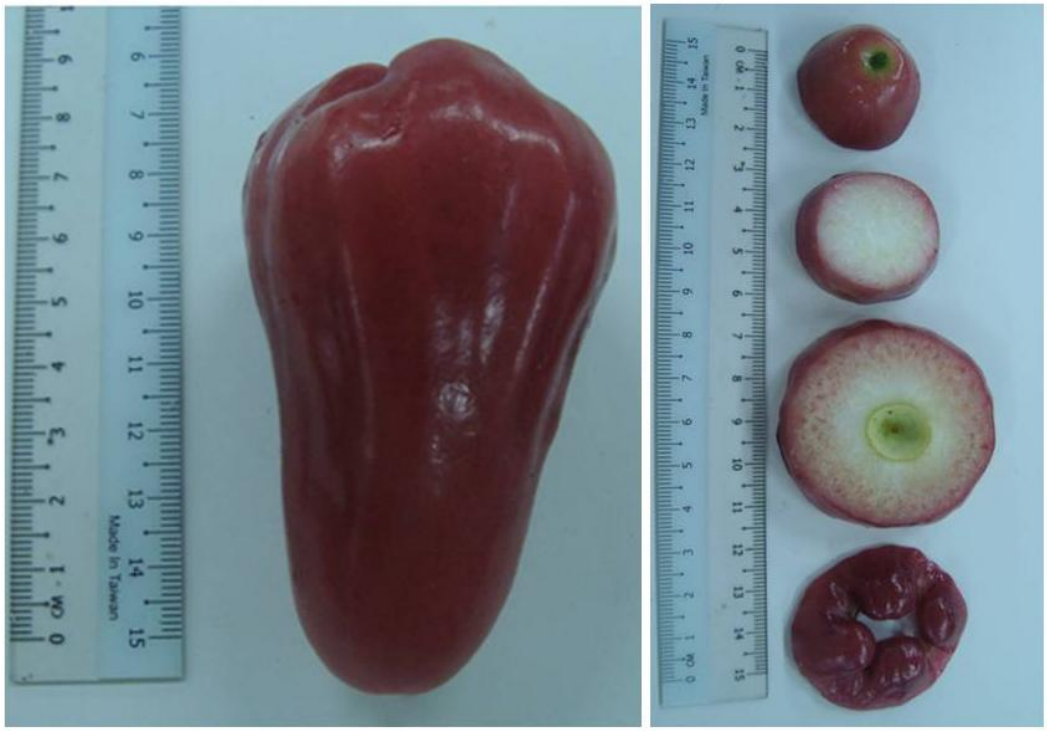


Fig. 2.7b. Fruit size and fruit diameter of distal and proximal ends of *S. samarangense*.

This plant has also been reported as a good xanthine oxidase (Guerrero *et al*., 1998) and an aldose reductase inhibitor (Guzman *et al.,* 2005). The whole plant has a variety of medicinal uses which range from dermatological, digestive, head and throat to endocrine remedy. In Malaysia, powder from the dried leaves is applied on cracked tongue, while a preparation of the roots is used to cure itching, given to alleviate swelling, to treat dysentery and serves as an emmenagogue and abortifacient (Brown, 1935).

The fruits of *S. samarangense* are also have several medicinal properties. In Taiwan, people make wax apple soup with crystal sugar to treat coughing without phlegm. The fruit is also served as a cold dish on banquets to relieve the effects of alcohol. The flowers, which contain tannins, desmethoxymatteucinol, 5-O-methyl-40-desmethoxymatteucinol, oleanic acid, and b-sitosterol, are used in Taiwan to treat fever and halt diarrhea (Morton, 1987). In addition to their use as food; many of these fruits have been used in divergent traditional medical practices for a variety of illnesses and conditions. Most notably, the seeds of the *jamun* (*S. cumini*) are an important ayurvedic medicine for diabetes. The rose apple (*S. jambos*) has been used in India as a tonic for the brain and for liver problems, as an astringent, and digestive (Kirtikar and Basu, 1988), and distilled to make rosewater (Morton, 1987). Other related Myrtoideae fruits can be used for several inflammatory conditions, including sore throat, high blood pressure, ringworm, and as an antimicrobial, antiscorbutic, carminative, diuretic, and astringent (Rivera and Obón, 1995). Ethanolic leaf extract of wax apple has been reported to exhibit immuno stimulant activity (Srivastava *et al.,* 1995), while the hexane extract was found to relax the hyper motility of the gut (Ghayur *et al.,* 2006). The alcoholic extract of the stem bark has shown antibacterial activity (Chattopadhayay *et al.,* 1998). In Brazil, *Eugenia brasiliensis* leaves have been used for gastrointestinal disorders and rheumatism (Morton, 1987), and the

jaboticaba fruit (*Malpighia cauliflora*) has been used as a treatment for hemoptysis, asthma, diarrhea, and chronic inflammation of the tonsils. *Syzygium jambolanum* is well known Indian folk medicine for treatment of diabetes mellitus and heat antidiabetic potential of the fruits and seed extracts (Samba-Murthy and Subrahmanyam, 1989). Methanol leaf extract of *Syzygium Jambolanum* has insulin- like properties and may be useful as potential therapeutic agent in the management of hyperglycemia (Lim *et al.*, 2008).

2.6 Physicochemical changes in fruits during ripening

Fleshy fruits have been classified into two categories: climacteric and non-climacteric (Wills *et al.*, 1986). Non-climacteric fruit will not ripen after harvest whilst climacteric fruits will ripen and get softer and sweeter after harvest. The biochemical process involved is that climacteric fruits give off large amounts of ethylene gas (a natural plant hormone) whereas non-climacteric fruits give little or no ethylene gas. Ethylene, considered as being the ripening hormone, controls ripening by coordinating the timely activation of many genes. Considerable progress has been made in the characterization of the ethylene biosynthetic pathway. Besides ethylene, other hormones and environmental factors affect the ripening process as well (Vendrell *et al*., 2001).

limacteric fruits can be harvested at any time between the mature and ripe stages. If the fruits are harvested as soon as they are mature, the ripening period can be used to transport and market the fruit, and the ‘shelf-life’ of climacteric fruit can be extended for weeks or months, facilitating long distance trade. Climacteric fruits usually undergo dramatic changes during "ripening" and these changes have often been associated with a large increase in respiration and ethylene production (Obando-Ulloa *et al.*, 2009). Avocado, mango and banana are classified as climacteric fruits whereas the pineapple,

strawberry, citrus, rambutan are classified as a non-climacteric fruit. The wax apple fruit (*S. samarangense*) (Fig.2.8) is a non-climacteric tropical fruit according to Morton (1987) and Moneruzzaman *et al*. (2011).

2.7 Postharvest changes in non-climacteric fruits

Non-climacteric fruits do not change significantly after harvest and have mature fruits that ripen gradually, at a steady pace. These fruits should not be harvested before they are ripened because the ripening process stops as soon as they are picked. The taste, flavor and texture of an unripe fruit do not improve after harvest. Harvesting at the ripe stage implies that the fruit should be eaten soon and this means that there is little time for transport, trade and display in the market. On the other hand the harvest time may range widely, depending on the preferred quality.

Non-climacteric fruit do not exhibit the increase in respiration or the rise in ethylene production. The ripening of some non-climacteric fruit may be ethylene-independent although several studies have shown that ethylene has some effects on non-climacteric fruit such as cherries, citrus, and strawberries. Gong *et al.* (2002) reported that exogenous ethylene stimulated respiration and accelerated the development of stem browning in the sweet cherry fruit. Ethylene has also been shown to be involved in the regulation of maturation and senescence in citrus fruits (Porat *et al.,* 1999).

2.7.1 Tissue firmness

Firmness of tissue is another important aspect of fruit quality and this depends on the stages of fruit maturity as the fruit softens when it ripens. This occurs as selected cell wall hydrolytic enzymes such as pectinase, breaks down the cell wall matrix and allows the cells to slide more easily against each other and hence the softness in fruits on ripening

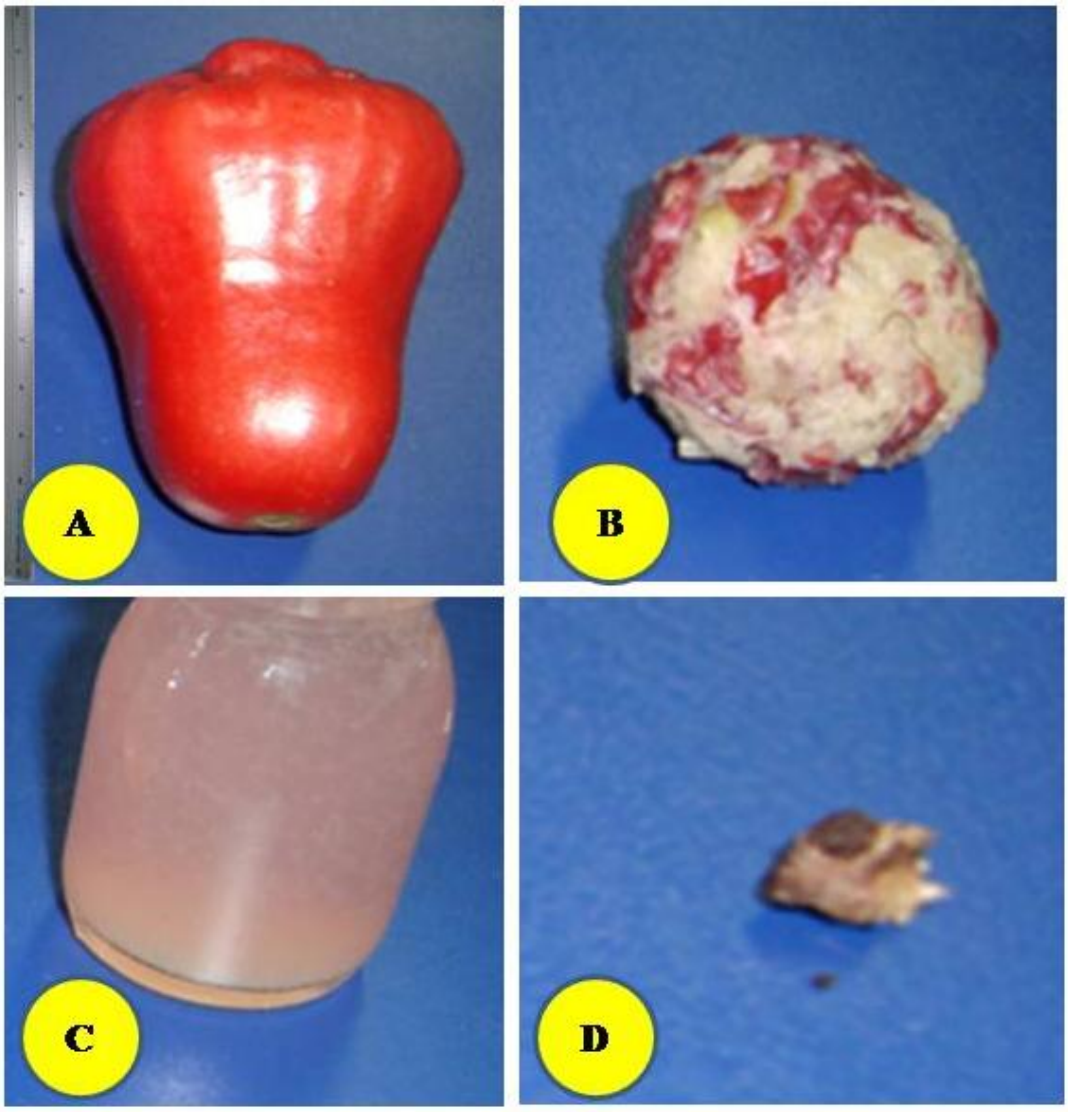


Fig. 2.8. (A): Fruit, (B): Fresh biomass, (C): Fruit juice and (D): Seeds of wax apple.

(Jain *et al.,* 2003). Textural change in ripening fruit is related with changes in cell wall composition and, particularly, in the loss of pectic substances as the production of pectic enzymes increases on ripening. This has been observed in a wide variety of temperate (Whitaker *et al.,* 1997) and tropical fruits (Joseph and Aworh, 1991; Aina and Oladunjoye, 1993). Softening of fruits is brought about by the removal of the methyl ester groups from pectin (Rexova-Benkova and Markovic, 1976) in the cell wall by pectin methyl esterase, allowing the action of polygalacturonase over the resulting polymer producing a reduction in the intercellular adhesiveness and tissue rigidity (Sakai *et al.,* 1993). Rahman *et al.* (1995) reported that the concentration of polygalacturonase and pectin esterase in jackfruit (*Artocarpus heterophyllus* L.) was 12-fold and 40-fold higher respectively in mature fruit of the soft form compared to mature firm fruit. However, pectin modification is important in textural changes (Ahrens and Huber, 1990). An increase in the activities of the enzymes polygalacturonase and pectin methyl esterase has been shown in guava (*Psidium guajava* L.) (Jain *et al.,* 2003). They also reported that among the cell wall polygalacturonase activities increased significantly from 85 units at MG (mature green) stage to 162 units/g fruit weight at OR (overripe) stage. These results showed that polygalacturonase plays a major role in fruit ripening. Crisosto *et al.* (1993) in an experiment on postharvest performance evaluation of plum (*Prunus salicina*) reported that fruit firmness decreased significantly over the 9-day experiment period. Ribeiro *et al.* (2007) reported that pulp firmness in mango fruit decreases with increasing ripening period as the pectin content decreases and the soluble solids content increases.

2.7.2 pH

pH is an important parameter in color changes of ripening fruits. In full-grown fruit, cells are compromised mainly of the vacuole, with the cytoplasm being reduced to a

thin layer compressed between the tonoplast and cell wall (Kays, 1999). The vacuole accumulates organic acids, sugars and phenolic compounds including anthocyanin pigments. The accumulation of organic acids results in a buffered solution. This buffering capacity an increase in pH and a reduction in titratable acidity (TA) in the juice of strawberry was measured by Holcroft and Kader (1999). They reported an increase in pH over the 10-day storage period and a decrease in titratable acidity (TA) in the juice of strawberry. Since pH has a profound effect on anthocyanin stability and color expression, particularly in an aqueous solution, changes in pH could result in significant losses in color. Minor changes in pH can have a significant consequence on the colour expression of anthocyanin since the acidity of the solution affects the ratio between the various forms of the pigments (Holcroft and Kader, 1999). Changes in color and the stability of anthocyanins have been reported over pH range 2.0–8.7 in tamarillo fruit (*Solanum betaceum* Cav.) (Hurtado *et al.*, 2009). The color variation in the aqueous solutions of anthocyanin crude extracts was within the pH range 2.0–6.2, that is to say under and above the most common pH values in foods (Hurtado *et al.*, 2009).

Some studies have suggested that pH and mineral composition, may affect the catalytic activity of cell wall enzymes (Chun and Huber, 1998; Almeida and Huber, 1999). The acidification of the apoplast over the pH range can provide a mechanism for the regulation of the catalytic activity of cell wall enzymes (Pinheiro and Almeida, 2008). Pinheiro and Almeida (2008) reported that pH affected pectin dissolution and pericarp softening. In a study on the physical and chemical changes during ripening of blackberry fruits, it was observed that the acidity was inversely related to pH (Tosun *et al.*, 2008). The ripened sample which had a low acid content had a correspondingly high pH.

2.7.3 Total Soluble Solid Content (TSS)

Soluble solids include the soluble sugars sucrose, glucose and fructose as well as acids. Total soluble solids (TSS) or sugar content is considered to be an important parameter of quality attribute for many fresh fruits (Lu, 2004). TSS is also an important parameter to consider in determining the time of harvesting. Increase in soluble solids could be attributed to the decomposition of the cell wall which causes release of water-soluble components (Rees *et al.,* 1981). Reaves (1959) reported that the increase in total soluble solids is probably due to the increase in water-soluble galacturonic acids from the degradation of pectic substances by polygalacturonase (PG). Sharaf and El-Saadany (1987) indicated that the increase in soluble solids content in guava could be attributed to the conversion of starch to sugars. Increase in total soluble solids (TSS) and decrease in acidity are some indicators of sweetness of fruits such as mango (Lakshminarayana, 1980). Various researchers (Medlicott *et al.,* 1990; Perkins-Veazie *et al.,* 1996) reported increase in total soluble solids (TSS) for mango and blackberries in during ripening. Holland *et al.* (1999) also showed that the total soluble solids content increased during maturity of Fortune mandarins fruit, whereas the acidity decreased. Islam *et al.* (2001) reported that the percentage TSS of banana increased during ripening. They explained that increase in TSS of fruits might be attributed due to increase in soluble sugars, soluble pectin and soluble organic acids. In sweet orange total soluble solids declined during storage up to 45 days and then increased at the interval of 75 days (Ladaniya *et al.,* 2003).

Javanmardi and Kubota (2006) showed that during maturation and ripening of tomato fruits were changes in total soluble solid such as the ratio of glucose to fructose and organic acids during storage. Ribeiro *et al.*, (2007) reported that the total soluble solids content increases in mango during ripening. It has also been reported that during ripening there was a slight and insignificant increase in the soluble solids content in blackberry at the green and

red ripening stages. However at the ripened stage, the change in soluble solids was significantly different ($p < 0.01$) (Tosun *et al.*, 2008).

2.7.4 Titratable Acid (TA) Content

Juice acidity is an important parameter in defining quality. Total acidity which is also loosely referred to as titratable acidity is a measure of the total acid in the fruit. It is related to pH but the concepts are not identical. While pH measures acid strength or proton concentration, TA measures the amount of acids present and these are generally weak acids such as malic acid in apple, citric acid in citrus, oxalic acid in rhubarb, tartaric acid in wine and lactic acid in sour milk, which do not contribute much protons in solutions and thus although being acidic, do not change the pH drastically.

Fruit taste is a balance between acids, sugars and volatiles present. Holland *et al.*, (1999) showed that the total soluble solids content increased during maturity of Fortune mandarins fruit, whereas the acidity decreased probably due to increasing water content and size of the fruit. He also suggested it could be due to the use of the acids as respiratory substrates. Clementine fruit that has less than 0.8% acidity is more and more considered of low quality as the sugars triumph over the acids and thus the fruit has an insipid taste and is also more prone to post harvest decay organisms. Ong *et al.* (2006) working on jackfruit, observed that the titrable acidity throughout ripening process was in the range of 0.3-0.9%. However, Tosun *et al.* (2008) showed that titratable acidity increased during development in blackberry, but decreased in ripe fruits. The change in total titrable acids during storage was probably mainly due to the metabolic activities of living tissues during which depletion of organic acids takes place.

In addition Chahidi *et al.*, (2008), reported that juice titratable acidity of citrus fruit ranged between 0.8 and 1.5% (in during 3 months storage), which had the greatest value

(3.2%) at the first date of harvest, and declined with time as fruit over matures. Acidity is also perceived in degrees of sourness and decreases as the grapes become ripe. Tartaric acid is the primary acid, but others such as malic and citric can be found as well. As the harvest date draws near, TA in the fruit drops (due to the respiration of malic acid). It is important to pick the fruits with enough TA or an adjustment will need to be made.

2.7.5 Total Sugar Content

One of the most important biochemical changes during ripening in fruits is the increase in sugar concentration and this decisive factor has also been often used in quality determination. Mono and disaccharides with a sweet flavor are commonly called sugars and are present in a significant amount in fruits and their derivatives, glucose, fructose and sucrose being the main sugars which can be found in fruits and fruit juices (Rambla *et al.*, 1997).

The sweetness and energy content of fruits depend to a large degree on its sugar to acid ratio. Therefore, an increase in the content of the simple sugars usually brings about a sweeter fruit especially if this is accompanied by a decrease in the organic acid and phenolics content to minimize acidity and astringency (Nair and Tung, 1980). In most fruit, there are high percentages of fructose, glucose and sucrose during ripening and there may be an absence of very minute amounts of free sugar during the immature stages. There is an increase in free sugar content (fructose, glucose and sucrose) of fruit with increasing ripeness (Jordan *et al.*, 2000; Ong *et al.*, 2006). Jordan *et al.* (2000) has shown that starch lost during ripening was accounted for by the increase in the glucose and fructose sugar pools in kiwifruit. Chan *et al.* (1979) reported that sucrose made up less than 18% of the total sugar content in mature green *Carica papaya* (110 days after anthesis). However, it increased rapidly to make up 80% of the sugars, 25 days later (fully riped stage).

Bashir and Abu-Goukh (2003), reported a remarkable increase in total sugars in guava was attributed to an increase in the activity of enzymes responsible for starch hydrolysis and the decline in the rate of sugar breakdown by respiration. Adao and Gloria (2005) also reported that in banana the green fruit had high starch and low soluble sugars levels. Starch levels decreased significantly throughout ripening whilst fructose and glucose levels increased.

Kulkarni and Aradhya (2005) working on pomegranate arils showed an increase in concentration of TSS, total sugars and reducing sugars during fruit development. The lowest TSS (13%), total sugar (12.6%) and reducing sugar (12.2%) contents were recorded in 40 day-old fruits. A significant increase in all of the above three constituents was recorded after the 80 th day of fruit development and the highest TSS (15.3%), total sugar (16.6%) and reducing sugar (15.7%) contents were recorded in 140 day-old fruits.

The changes in sugar content with fruit development in oriental melon showed that glucose and fructose were the major sugar of these fruits from 10 to 30 days after pollination. However a very small quantity of sucrose was detected. The changes in sugar content gradually increased from 10 to 30 days after pollination, and doubled 35 days after pollination. The change in sucrose content of fruits at 30 days after pollination was 0.2- 0.6 mg g^{-1}. However the sucrose content at 35 days after pollination rapidly increased up to 40.7- 45.1 mg g^{-1}. The sucrose content quickly increased in full ripens fruit (Shin *et al.,* 2007).While respiration represents the glycolytic carbon flux, the rate of sugar accumulation represents gluconeogenic carbon flux. During the ripening process, carbon is simultaneously shunted in both directions. Rapid fluctuation of fructose 2, 6-bisphosphate in pea and banana tissue may represent one means of fine control of the glycolytic pathway. Fructose 2, 6-bisphosphate stabilizes the enzyme ATP-phosphofructokinase

which favors glycolysis and inhibits fructose bisphosphatase which favors gluconeogenesis (Beaudry *et al.*, 1989).

2.8 The use of selected horticultural techniques improve to fruit growth, development and quality

Over the last few centuries many methods to improve crop productivity and yield were developed. These include plant growth regulators, tree management, orchard floor management, breeding and insect pest and disease management. The use of plant growth regulators and other horticultural techniques has become an important component of agrotechnical procedures for most of the world's cultivated plants and especially for fruit plants (Monselise, 1979). Plant growth regulation chemicals have been used extensively in fruit research and numerous commercial crop applications over the years. Many researchers have used them to influence fruit quality factors such as peel quality, colour, fruit size, juice acidity and to improve TSS, total sugar, phytochemicals and antioxidant activity in different fruit species throughout the world (Berhow and Vandercook, 1992). However, in recent times some of these strategies have become increasingly viewed as inappropriate due to its possible effects on the environment and human health. The use of plant growth regulators, hydrogen peroxide and girdling on improving fruit growth and quality are reviewed.

2.9 The effect of gibberellic acid on growth and development

Gibberellins (GAs) are a group of diterpenoid acids that function as plant growth regulators influencing a range of developmental processes in higher plants including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, and leaf and fruit senescence (Hori, 1898) (Fig. 2.9). The discovery of gibberellin can be traced

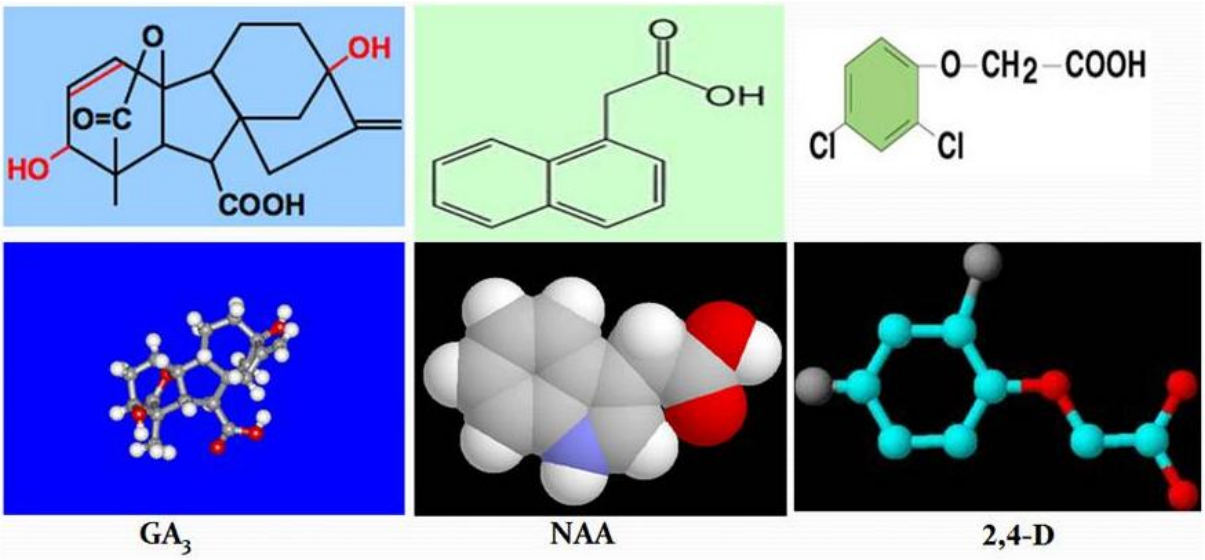


Fig. 2.9. Growth regulators gibberellic acid (GA_3) and synthetic auxins (NAA and 2, 4-D).

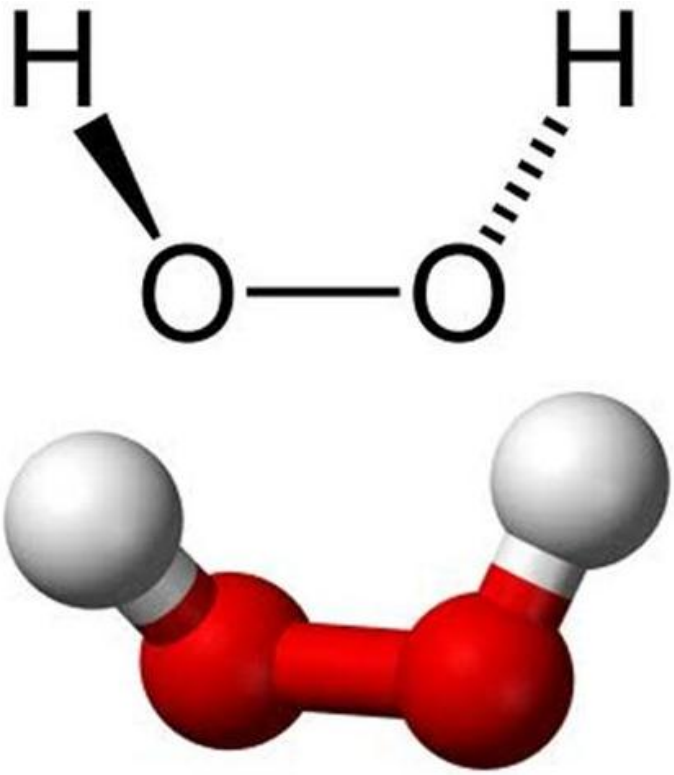


Fig. 2.10. Growth promoting chemical hydrogen peroxide (H_2O_2)

The cause of this exaggerated growth was due to the production of *Gibberellia fujikuroi.* Improper crystals containing a mixture of active ingredients were isolated in the late 1930s (Phinney, 1983). Gibberellic acid was identified, crystallized and synthesized in the 1950s (Brain and Hemming, 1955)

The use of growth regulators has become an important component of agrotechnical procedures for many the cultivated plants, especially for fruit plants (Monselise, 1979). GA_3 alone or combined with mepiquat chloride increased the leaf area and chlorophyll content in grape (Lim *et al.* 2004). Ilias *et al.* (2007) reported that chlorophyll fluorescence are linearly correlated with the functionality of PSII, where F0 and Fm are the chlorophyll fluorescence yields corresponding to open and closed PSII reaction centre respectively. Application of gibberellic acid increased the maximum quantum yield of primary photochemistry (Fv/Fm) and the ratio of Fv/F0 in *Capsicum annum* (Georgia *et al.* 2010). Gibberellic acid is used widely in horticulture for improving fruit set and to control apple russeting (Taylor and Knight, 1986) cracking in pomegranate fruits (Sepahi, 1986) and litchi (Sharma and Dhillon, 1986) and to inhibit flowering of *Prunus* species (Coneva and Cline, 2006; Lenahan *et al.* 2006).

Gibberellic acids (GAs) have been shown to increase fruit set and growth in apples, pears (Weaver, 1972) and seedless grapes (Zabadal and Dittmer, 2000). The GAs also play an important role in litchi fruit development (Huang, 2005). Xiang *et al.* (1994) reported that endogenous GA_3 was dominant in stage I (growth of pericarp and seed coat) and stage II (growth of cotyledon and initial aril) on litchi ontogeny. A spray of GA_3 at 50 mg/l 5 weeks after flower blooming reduced fruit drop in ‘Huaizhi’ (Ji *et al.,* 1992), and a spray of GA_3 at 50–100 mg/l at full bloom also enhanced fruit retention and fruit size in ‘Early seedless’ and ‘Calcuttia’ litchi in India (Singh and Lal, 1980). Stern and Gazit, (2003)

studied on the effects of GA_3 on fruit growth in 'Yu Her Pau' litchi over 2 years in Taiwan and reported that hypothesize that sprays of GA_3 during stage I of fruit growth would increase fruit and aril weight. Singh and Lal (1980) observed that spraying GA_3 once at full bloom in some Indian litchi cultivars at concentration of 50 and100 mg/l, were effective in improving fruit size. However, the proportionate increase in fruit weight ranged from 21.9 to 27.4% in the 'Yu Her Pau' litchies, while it ranged from 7.7 to 26.6% in the Indian cultivars. Seed produced GAs has been reported to assist fruit growth in tree crops (Westwood, 1993). Jer-Chia and Tzons-Shayam (2006) found that GA_3 sprays also enhanced the growth of pericarp and aril, in the absence of a normal seeds in 'Yu Her Pau'. It has been shown, in many fruits, that gibberellins influence fruit development, especially at the younger stage of fruit development (Kondo and Mizuno, 1989). Gibberellic acid (GA_3) sprays have been used in many cherry growing regions to reduce the risk of crop loss by making the fruit more resistant to cracking. Sprays of GA_3 have been widely adopted in commercial orchards because they have consistently been shown to increase fruit size and firmness of fruits such as cherry (Facteau 1986; Özaki *et al.,* 2006). Trees drop their fruits when the concentration of auxins decreases and the concentration of abscisic acid (ABA) increases as the endogenous hormones and their balance play a modulating role in the mobilization of nutrients to the developing organs (Marinho *et al.* 2005). Almedia *et al.* (2004) and Davies and Zalman (2006) who reported that the application of 2, 4-D and GA_3 significantly reduced the preharvest fruit drop in citrus species. Saraswathi *et al.* (2003) also observed that growth regulators such as 2, 4-D and GA_3 and their combinations significantly influenced the fruit weight in mandarin. Thomas and Lovatt (2004) and Davies and Zalman (2006) reported that preharvest application of growth regulators, 2, 4-D and GA_3 significantly increased the total number of fruits at the

time of harvest and fruit weight per plant by reducing the preharvest fruit drop. Thus in citrus fruits, excessive fruit drop can be controlled by the exogenous application of plant growth regulators, namely the auxins and gibberillins.

Application of gibberellic acid (GA_3) before or at full developmental stage increases the color of fruits (Curry and Williams, 1983). Foliar application of different levels of GA_3 (5, 50, 100 and 500 mg/L) to young fruitlets just after fruit set have been reported to clearly increase the fruit weight, peel thickness, juice content with improved taste of grapefruit (Berhow, 2000). The 'Baldy' mandarin fruit weight, diameter, volume, juice percentage, TSS, TA, TSS: TA ratio and ascorbic acid in juice were found to be affected by mid November spray treatments of GA_3 and $CaCl_2$ (El-Hammady *et al*., 2000).

Fruit size increase in many fruit species results from cell division, cell expansion or a coordinated series of cell division and expansion (Zhang *et al.,* 2006). They also reported that mesocarp cell size accounts for most variability in fruit size within a genotype and was significantly influenced by the environment, indicating that cultural practices that maximize mesocarp cell size should be used to achieve a cultivar's fruit size potential (Olmstead *et al.,* 2007). It has been suggested that application of gibberellins (GA_3) during the stages II–III transition to increased fruit size and delay maturation (Facteau *et al.,* 1985; Clayton *et al.,* 2006).

Polar GA_3 are known to inhibit flower bud initiation in many temperate fruit crops (Sedgley and Griffin, 1989). Luckwill (1970) hypothesized that gibberellins produced in seeds inhibit flower formation since they coincide with embryo growth in developing fruit lets in pome fruits. In many polycarpic plants (pome, mangoes and apricot), the application of gibberellin (GA_3) during flower induction interrupts the floral process and partially reduces the intensity of flowering (Turnbull *et al.,* 1996; Southwick *et al.,* 1997; Tromp,

2000). It depends on the date of treatment, concentration applied, and the total amount of active material applied per tree (González-Rossiaetal *et al.,* 2007). Yang *et al.* (2007) reported that GA_3 applied at an early stage of flower induction significantly reduced flowering rates in loquat trees, but the authors did not determine the appropriate concentration and date of treatment, thus more studies are needed to make the best use of GA_3. The application of GA_3, together with auxin treatment, can reduce the occurrence of puffy fruit (Yamasaki *et al.,* 1961). Talon *et al*. (1990) reported that fruit development is triggered by hormones and the endogenous gibberellin status of the developing citrus ovaries is the limiting factor for the initiation of fruit development

2.10 GA_3 on fruit quality

It has been reported that treatment with gibberellic acid (GA_3) influences sweet cherry fruit quality and can reduce negative the effects of rain and premature picking (Looney, 1996). The use of GA_3 increased fruit firmness at harvest, while decreasing decreased the rate of fruit softening and delayed fruit maturity for the late-maturing genotypes, but had no significant effect on early-maturing fruits (Choi *et al.,* 2002). GA_3 increased fruit firmness; soluble solids and fruit weight (Basak *et al.*, 1998) and delayed the time of ripening (Demirsoy and Bilgener, 2000; Facteau *et al.,* 1985). GA_3 decreased the activities of polygalacturonase (PG) and pectin methylesterase (PME) in strawberry (Andrews and Shulin, 1995). Fruits treated with GA_3 maintain their higher firmness during storage (Clayton *et al.,* 2003).The fruit colour is the most reliable indicator of sweet cherry fruit maturity (Proebsting and Mills, 1981; Drake and Fellman, 1987), therefore the visual estimation of the cherry colour was essential in defining the time of harvest. Demirsoy and

Bilgener (2000) reported that GA_3 influences cuticular thickness, and dimensions of the epidermal cells but this effect differed according to the cultivars in sweet cherry.

The application of GA_3 sprays on cultivars of cherries (Merton Premier, Bing, and Dawson) indicated that GA_3 delayed harvest, increased fruit firmness, weight, and soluble solids, but had no influence on fruit cracking (Horvitz *et al.,* 2003). Dawood (1986) reported that application of 15 mg/L GA_3 at 6 and 3 weeks before harvest increased fruit weight and the length: width ratio of cherry cultivars Stella and Merton Glory. It has been reported that sprays of GA_3 increase fruit soluble solids in sweet cherry (Dawood 1986; Clayton *et al.,* 2006)

Atawia and El-Desouky (1997) and Matthew and Davis (2002) reported that the application of growth regulators at flowering and preharvest significantly increased the juice percentage in various citrus species. The application of growth regulators, auxin and gibberellins significantly increase the total soluble contents of the fruit in citrus species (Atawia and El-Desouky, 1997; Huang and Huang, 2005). Xiao *et al.* (2005) reported that the application of 2, 4-D, GA_3 and NAA significantly reduced acidity percentage in citrus fruits. They also observed that preharvest application of growth regulators increased vitamin-C contents of the citrus fruits. Ingle *et al.* (2001) and Wang *et al*. (2004) found that application of 2, 4-D, GA_3 and other growth regulators increased the sugar contents in various mandarin and sweet orange cultivars. Javachandran *et al.* (2005) while testing three plant growth regulators observed that GA_3 at 100 ppm gave higher moisture contents (82 - 90%), ascorbic acid (225.9 mg/100 g) and total sugars (7.3%).

Taylor and Grotewold (2005) stated that flavonoids play a vital role in the physiology of plants by producing the red and purple anthocyanin pigments. GAs has also been

reported to promote synthesis of flavonoids, as studies had shown that an increase in anthocyanin synthesis by GA_3 promoted levels of flavonoid-specific mRNAs (Weiss *et al.*, 1990). Current study suggests that flavonoid levels of plants were significantly affected by PGRs. GA_3 significantly promoted these secondary metabolites, which shows the significance of these PGRs in the biosynthesis of flavonoids (Klessig and Malamy, 1994). In addition to their anti-oxidant properties, flavonoids have anti-proliferative, anti-tumor and pro-apoptotic activities and being used as medicinal plant, the relationship of PGRs on total flavonoids contents may be of great economic value.

It has been suggested that GAs promote synthesis of flavonoids and had shown that an increase in anthocyanin synthesis by GA_3 promoted levels of flavonoid-specific mRNAs **(**Weiss *et al.*, 1990). Various phytohormones (like GA, Kn and SA) can increase the plant growth, biomass and polyphenolic compounds in strawberry, maize and apple (Sharma and Singh 2009; Pan *et al.,* 2008). Taylor and Grotewold (2005) reported that flavonoids play a vital role in the physiology of plants by producing the red and purple anthocyanin pigments. They also suggested that flavonoid levels of plants were significantly affected by PGRs. The sprays of PGRs significantly affect in titrable acidity content and increased formation of organic acids in plant tissues most likely to accelerate activities within the Krebs cycle (Graham and Ballesteros, 2006). Thakur *et al.* (1996) reported that application of GA_3 and 2, 4-D decrease the acidity of tomato fruits was reduced; however, the ascorbic acid content significantly increased with higher doses of 2, 4- D and Para-chlorophenoxy acetic acid.

Kwack *et al.* (1997) and Chung *et al. (1998)* observed that PGRs can regulate the of anthocyanin synthesis. Hee Ock *et al.* (1997) reported that gibberellins and cytokinins promote phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) synthesis

and in turn increased anthocyanin contents, proposed that, there was a close physiological relationship between PAL and anthocyanin formation. Similar effects of GA_3 on anthocyanin formation and elevated the level of PAL and TAL activities were observed in *Fragaria ananossa* fruits (Montero *et al., 1998).* Deikman and Hammer (1995) also reported that the application of cytokinins increased anthocyanin pigments and PAL synthesis in *Arabidopsis thaliana* plants. It also has reported that, the mixture of GA and BA increased the anthocyanin content in strawberry fruits (Thakur *et al.*, 1991*).* Zhou and Tan (1997) stated that the sprays of GA_3 increase total phenolic content in pineapple fruits. They also suggested that the increased accumulation of phenolic compounds after GA application may depend on the increase of PAL and TAL activities.

2.11 Naphthalene acetic acid (NAA) and 2, 4-Dichloroacetic acid (2, 4-D) on growth and development

Auxin is a class of plant growth regulators which contained some morphogen-like characteristics (Fig. 2.9). Auxins regulate many growth and behavioral processes in the plant's life cycle and are essential for plant body development. Firstly, auxin was discovered by Charles Darwin in the 1880s when he was trying to find the reason why plants bend towards light. He observed that the tip of coleoptiles sensed light and there was a diffusible substance produced there that caused the shaded side to grow more rapidly than the illuminated side (Jacobs, 1979). From lower plants auxin (IAA) was identified in the 1930s and finally auxin isolated from higher plants in the early 1940s (Thimann, 1977). Augusti *et al.* (1995) reported that synthetic auxins (NAA and 2, 4-D) can influence cell enlargement, bud formation, root initiation, control the growth of stems, roots, and fruits, and convert stems into flowers. They also found that synthetic auxin reduced the bud and fruit dropping, increase fruit size, weight and yield as well as quality of the fruits.

It is well documented that spraying Naphthalene Acetic Acid (NAA) increased the chlorophyll content in the leaves (Grewal *et al.,* 1986). Gutam *et al.* (2009) also reported that foliar application of NAA increased the chlorophyll content in Bell pepper. The technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiological studies. By measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained. Synthetic auxins (NAA and 2, 4-D) stimulated the synthesis of chlorophyll. It was reported that auxin increased the yield of chlorophyll fluorescence. Studies have shown that synthetic auxin increased the stomatal conductance in soybean plants (Nahar and Takeshi, 2002).

Synthetic auxins have been reported to effective in enhancing fruit growth, when applied during the second stage of fruit development (Westwood, 1993). These auxins are known for their ability to increase cell enlargement (Westwood, 1993; Arteca, 1996; Davis, 2004), thus enhancing fruit growth in certain species such as citrus (Agusti *et al.,* 1995), peach (Agusti *et al.,* 1999; Flaishman, 2006), litchi (Stern *et al.,* 2000), apricot (Agusti *et al.,* 1994) and loquat (Agusti *et al.,* 2003). In all the species studied, synthetic auxins had the potential in increasing fruit size without inducing thinning. In citrus, peach and litchi, it was found that application of the synthetic auxin 3, 5, 6-trichloro-2-pyridyloxyacetic acid (3, 5, 6-TPA), at concentrations between 10 and 20 mg/L, were recorded the highest fruit size, whereas in apricot and loquat, 2, 4-dichlorophenoxypropionic acid (2, 4-D P) at 25–50 mg/L, had the optimum effect. The application of NAA 15 days after full bloom has been reported to be effective in thinning loquat fruit, increasing fruit diameter by 10%, and yield by 20% per tree compared with non-thinned trees, although total yield and fruit size of treated trees were not significantly higher than those of hand-thinned trees (Agusti *et al.,* 2000).

Pre-harvest fruit drop is a wide spread problem in many fruit species, guava trees also suffer badly from this menace. El-Shewy (1976) observed that 50 mg/L NAA and 50 mg/L GA_3 at full bloom and three months after the first spray were most effective treatments in reducing pre harvest fruit drop as well as fruit seed contents in guava. More recently Dutta and Banik (2007) applied GA_3 and NAA before flowering, followed by three weeks after fruit setting and observed that foliar application of NAA significantly increased fruit length, diameter and fruit weight and ultimately crop yield of guava. Similar results were also reported by Maurya and Singh (1981). Improved fruit yield and quality can thus be obtained by reducing heavy fruit drop. Lima and Davies (1984) successfully reduced summer drop in with 20 ppm 2, 4-D or in combination with 20 ppm GA applied to nine weeks after mid bloom. It had been shown that naphthalene acetic acid increased final fruit size through its thinning effect, so reducing competition among developing fruits (Agusti *et al.,* 2000). The highest fruit drop control was exhibited by 2, 4-D resulting in high yield and quality. It is believed that during part of the period of abscission, both this process and hence fruit set and fruitlet growth rate are related to carbohydrate and other metabolites availability.

Fruit size is important economic parameter, not only because it is a component of productive yield, but also determines the acceptance by the consumer. The application of synthetic auxin at the beginning of pit hardening caused a significant improvement in fruit size and total yield in Bing'cherry (Stern *et al.,* 2007). Guardiola (1992) stated that juice percentage is an extremely important parameter for its industrial processing, being also related to size, which, in turn, although determined by the genetic characteristics of each cultivar, can be affected by cultural practices such as application of plant growth regulators.

PGRs application can re-enforce hormone balance in the peel, reducing or retarding this precocious fall and the losses at harvest. It has been reported that the spraying of auxins prevented the dropping of fruit by maintaining the cells at the zone of abscission, preventing the synthesis of hydrolytic enzymes, such as cellulase, which decomposed the cell walls (Monselise and Goren, 1978). The application of 2, 4-D promote size and to control fruit and leaf drop (Hield *et al.*, 1964). El-Otmani, (1992) reported that the combined application of GA_3 and 2, 4-D reduces the precocious drop of fruit through the action of auxin and retards the softening and senescence of the peel, by the longer harvest time, and more economical storing in areas where stocking capacity is limited and the cost is high.

Barros (1992) reported that the application of GA_3 alone or with 2, 4-D can increase fruit diameter significantly. Similar observation were recorded by Coggins and Henning (1988), who reported that the application of GA_3 at 40 mg/L on 'Valencia' oranges caused increase of fruit size as measured for length and diameter. Aranguren *et al.* (1988) also found that length and diameter of 'Valencia' oranges were increased significantly with pre-harvest application of 2, 4-D in concentrations of 20 and 25 mg/L. It has also been suggested that 2, 4-D at 10, 20, or 30 mg/L can reduce the rate of premature fall of 'Kinow' mandarins (Patil *et al.*, 1989).

Gorquet *et al.* (2005) reported that the application of plant growth substances induced the tomato fruit growth. It is well documented in the literature that, after anthesis auxin stimulated the fruit set and development (Sjut and Bangerth, 1981). They also stated that GA_3 and auxin application provokes different histologic development of tissue ovaries.

Plant growth regulators (PGR's) are known to have a great influence on fruit drop and fruit retention in fruit trees. An imbalance of auxins, cytokinins and gibberellins for

example may lead to the formation of abscission layer at the stem point and eventually fruit drop (Chen *et al.,* 2006). The compound 2, 4-D is regarded as one of the most effective ones in preventing fruit drop in citrus (Coggins and Lovatt, 2004). Other auxin-type PGR's, that can also be utilized to prevent fruit drop include 2, 4, 5 trichlorophenoxy propionic acid (2, 4, 5-TPA) and naphthalene acetic acid (NAA) predominantly, as well as other PGR's such as gibberellic acid (GA) (Michael *et al.,* 1999). Besides reducing fruit drop, NAA may enhance background colour development and fruit softening, under circumstantial environmental conditions (Yuan and Carbaugh, 2007).

2, 4-D is a synthetic auxin type growth regulator that brings about a growth response in plants at a low concentration but at higher concentration it becomes a herbicide (Lee, 2003). Agusti *et al.* (2006) also described similar observations that, 2, 4-D at 15 mg/L treatment reduced abscission by 50-75% and that had no effect on the external and internal characteristics of the fruit. The amount of rainfall before the fruit harvesting have also positive correlation with fruit drop in oranges (Davies and Albrigo, 1994)

Photosynthesis of plant is the primary process which forms the basis for yield determination. The total chlorophyll content in the plants regulates the photosynthetic capacity of the cotton and influences the rate of photosynthesis, drymatter production and the yield (Krasichkova *et al*., 1989). The application of NAA increased the net photosynthetic rate due to increase in stomatal aperture which facilitates more CO_2 conductance (Guinn and Brummett, 1993). They also stated that NAA increased transpiration and stomatal conductance in treated plants.

2.12 Auxin (NAA and 2, 4-D) on fruit quality

Similar to the above reports on the effects of gibberellic acid on fruit quality, auxins have also been credited with improving fruit yield and quality. Growth regulators treatments

have a positive effect on TSS, total sugars and reducing sugars. These are a good signs and the treatments might be selected for the improvement of fruit quality of different varieties. The application of synthetic auxins at the beginning of cell enlargement also increases final fruit size (Agustí *et al.,* 1994). In accordance with the above, this effect might operate via increases in photosynthesis and carbohydrate availability (Mehouachi *et al.,* 1995, 2000); in fact, the increase in fruit size due to auxin treatment is associated with an increase in absolute juice content and also in pulp and rind content, i.e. dry matter (El-Otmani *et al.,* 1993).

Dubay *et al.* (2002) reported that NAA sprayed at 250 mg/L resulted in higher guava yield and quality (cv. *Allahabad Sufeda*). Yaday (2002) also reported that NAA at 60 mg/L gave a higher yield, TSS, total sugars and vitamin-C (ascorbic acid) contents in guava fruits. Plant growth regulation chemicals have been used extensively both in basic citrus research and numerous commercial crop applications. These have also been used to influence fruit quality indicators like peel quality, colour, fruit size, juice acidity and to improve TSS in different citrus species in the world (Berhow and Vandercook, 1992).

However it has also been reported that spray applications of 2, 4-D, GA or their combination did not affect the juice content of Washington Navel (Lima and Davies, 1984). Many scientists reported no effect of growth regulators on fruit quality parameters like TSS, sugars, acidity, TSS/ Acidity ratio etc (Lima and Davies, 1984). Besides, Saleem *et al.* (2008) reported that application of growth regulators had significant effects on acidity, vitamin-C and TSS/acidity ratio in blood red ornage. NAA has also been shown to promote maturation (softening and anthocyanin formation) in mesocarp discs of peach fruit (Ohmiya, 2000), as well as 2, 4-D P advanced fruit maturity in apricots (Agusti *et al.,* 1994) and 3,5,6-TPA in peaches and nectarines (Agusti *et al.,* 1999). Ravi *et al.* (2005)

reported that foliar application of plant growth regulators had favorable effect on physico-chemical characteristics of guava fruit (cv. Sardar). Iqbal *et al.* (2009) observed that NAA at 45 ppm controlled fruit drop and increased yield, TSS and vitamin-C while acidity decreased. They also reported that fruit quality improved with lower NAA concentrations and deteriorated at higher rates.

Plant phenolics constitute one of the major groups of compounds that act as primary antioxidants or free radical terminators (Ramandthan and Das, 1992). The antimutagenic and anticarcinogenic effects of phenolics have also been demonstrated (Newmark, 1987; Deschner *et al.,* 1991), as have their protective roles against cancer, cardiovascular diseases, and cataracts (Hollman *et al.,* 1996). Phenolics possess both antibacterial (Tomas-Lorente *et al.,* 1992) and antifungal effects (Weidenberger *et al.,* 1990). Flavonoids, some of the most important natural phenolics, are members of a highly diverse and widespread group of compounds (Agrawal, 1989) that also possess a broad spectrum of chemical and biological activities, including radical-scavenging properties. The phenolic phytochemicals present in the roots of *E. angustifolia* can be used as markers to assess its quality in a given product. Synthetic auxin (IBA) at a 2 mg/L in tissue culture medium increases the total phenols in tomato plants (Chun *et al.,* 2006). But a higher dose synthetic auxin had negative effects on total phenols and biomass production. Elisa *et al.* (2007) reported that auxin increased the total polyphenolic content as well as nutritional content in the berries. Some other results suggested that pre-sowing seed with synthetic auxin (NAA, IAA and IBA) have profound effects on improving the quality of tomato.

2.13 Effects of girdling on growth and development

Girdling is an old practice that has been used to improve crop productivity. The removal of a small strip of bark around a branch or trunk will obstruct basipetal phloem

transport and make available more photosynthetic metabolites to the growing regions above the strip (Casanova *et al.,* 2009). Although removing the strip of bark wounds the tree, it heals within several weeks. It has been well documented that the ringing of trees can bring about an increase in the size and sugar content of fruits and cause them to mature a few days to a week earlier (Turkey, 1978; Arakawa *et al.,* 1997; Hossain *et al.,* 2006). Arakawa *et al.* (1997) reported that girdling in apple significantly increased flowering the following spring. It has been suggested that girdling can change the fruit quality, via increased SSC and reduced acid concentration, by blocking the translocation of sucrose from leaves to the roots. Hossain *et al.* (2006) reported that girdling, as a form of partial ringing, reduced shoot growth of four-year-old peach trees but promoted fruit quality development. From an agronomic point of view, that practices such as girdling can improve carbohydrate availability to fruits and as a consequent lead to an increase fruit-set and yield (Goren *et al*., 2003; Rivas *et al*., 2004). Guardiola (1997) reported that girdling branches, a technique which increases carbohydrate availability to fruit lets as it is believed to remove competition with the roots, reduces fruitlet abscission but had no effect on flower abscission in apple. The increase in carbohydrate supply caused by girdling correlated with a transient reduction in fruitlet abscission in ponkon mandarin (Mataa *et al*., 1998). Branch girdling, which interrupts the phloem pathway and hence disrupts the transport of carbohydrates in or out of the branch, has been utilized experimentally to control dropping as well as increase the fruit setting of apple (Priestly, 1976)

Girdling is still widely used worldwide in the cultivation of crops such as citrus, grape, peach and other fruit trees, mainly to increase flowering, fruit set and fruit size (Tuzcu *et al.*,1994). The best-known effects of girdling are presumably brought about by accumulation of assimilates above the girdle (Tuzcu *et al.,* 1994). However it has also

been reported that the cytokinin and gibberellin content of shoots is also modified by girdling (Barut and Eris, 1994). They also observed that on the upper part of girdling leaf N content, C/N ratio and carbohydrate were increased. Hossain *et al.* (2007) reported a new modified girdling techniques as a form of partial ringing and observed that the peach plant can survive 98% girdling with improved fruit size, although the peach plants with 100% girdling do not survive.

The best-known effects of girdling are presumably brought about by accumulation of assimilates above the girdle (Chun *et al.,* 2003). As a results of girdling leaf N content, C/N ratio and carbohydrate were improved in the upper part girdle. Therefore, flowering and fruit set were increased (Shao *et al.,* 1998). Rivas *et al.* (2008) reported that girdling few weeks before flowering reduced fruitlet abscission, increased leaf chlorophyll content and chlorophyll fluorescence. Furthermore, it increased quantum yield and carbohydrate concentration in various flowering and vegetative shoots in citrus. Mostafa and Saleh (2006) reported that girdling plus potassium spray increased the total number of fruits and yield weight per tree. It has been well documented that the ringing of trees can bring about an increase in the size and sugar content of fruits and cause them to mature a few days to a week earlier (Onguso *et al.,* 2004; Hossain *et al.,* 2007; Hossain and Boyce, 2009).

Allan *et al.* (1993) reported that girdling resulted in a greater number of fruits, larger and of desirable marketable size (>90 g) than the control, in the low chill peach cultivar Florida prince. One of the possible causes for this enlargement is the increase in sucrose levels a few days after girdling which would enhance its availability for cell division and growth of fruitlets (Iglesias *et al.,* 2006). Girdled treated fruit grew at a faster rate and were larger than untreated control fruit. As mentioned above many workers have suggested these due to the accumulation of carbohydrates above the girdle (Di Vaio *et al.,* 2001).

However the precise biochemical changes resulting from girdling have been studied only in selected crop and a detailed interpretation of the physiological effects of girdling is still lacking (Beruter and Feusi, 1997).

2.14 Effects of girdling on leaf and fruit quality

It has been reported that in some cases girdling may induce excessive accumulation of carbohydrates originating a feedback inhibition of photosynthesis by reducing photosynthetic capacity (Rivas et al., 2007) and CO_2 assimilation rate (Iglesias et al., 2003). Girdling has been demonstrated to induce oxidative stress in citrus leaves, triggering enzymatic and non-enzymatic antioxidative responses (Rivas et al., 2008). Rivas et al. (2011) stated that girdling change the pattern and ratio of leaf pigments, being these alterations more acute in young than in mature leaf. They also reported that girdling increased abscisic acid in young leaves and decreased it in mature leaves. Matsui *et al.* (1979) reported that anthocyanin and sugar accumulations increased proportionally with increasing leaves per cluster of girdled shoots of grapes. These changes were accompanied by a significant increase in leaf carotenoids, carotenoids: chlorophylls ratio, xanthophylls and xanthophylls cycle pool size and its de-epoxidation state in vegetative and multi flowered young leafy shoots.

As mentioned above, it has been reported that girdling can increase the fruit size and sugar content of fruits (Turkey, 1978). Recently it was reported that trunk girdling stimulated fruit color development and fruit softening after harvest of Japanese persimmons (Shinji *et al.,* 2006). These can be related to the observation that the application of sugars in vitro culture, especially sucrose, improves the color of skin disks of wax apple (Liaw *et al.,* 1999).

As mentioned earlier, girdling has been shown to alter the partitioning of photosynthates, mineral nutrients and plant growth regulators in the tree (Mataa *et al*., 1998). Thus girdling can be effective at increasing yield (Rivas *et al*., 2004). Iwahori *et al.* (1976) reported fruit color and soluble solids enhancement from phloem ringing compared to control in ponkon mandarin. Girdling improved the rind/peel color of fruits (Yamanishi *et al.*, 1995). Mostafa and Saleh (2006) reported that girdling practices increased the yield. Juice percentage of fruit a little bit reduce by girdling treatments in Japanese persimmon (Hasegawa *et al.*, 2008).

Girdling increased total phenolic content and L-phenylalanine ammonia lyase activity in peach fruit (Kubota *et al.*, 1993). Casanova *et al.* (2009) reported that scoring (one type of girdling) significantly increased the total sugar content in grape. It has been reported that girdling increased the levels of total anthocyanin and individual anthocyanin in the berry skin of crimson seedless grapes (Harsimranjit *et al.*, 2008). Rivas *et al.* (2008) reported that girdling treatment increased the leaf soluble sugar content and all antioxidant enzyme activities. They also found close relation between leaf soluble sugar and antioxidant enzyme.

2.15 Influence of H_2O_2 on fruit growth and development

Hydrogen peroxide is the two electron reduction product of O_2 (Fig. 2.10). It is potentially reactive oxygen, but not a free radical (Halliwell *et al.*, 2000). Hydrogen peroxide is well-known as an antiseptic because of its cytotoxic effects on many bacterial strains and it also used as a disinfectant at wound sites (Halliwel and Gutteridge, 1999). It has been reported that H_2O_2 as one of the major and the most stable reactive oxygene species (ROS) that regulates basic acclimatory, defense and developmental processes in plants (Slesak *et al.*, 2007). Many researchers have been shown that hydrogen peroxide

had a significant role in plant biochemistry and physiology, and various functions of H_2O_2 in plants have been described in many review papers (Hung *et al.*, 2005).

Hydrogen peroxide is a stable partially reduced oxygen form; its rapid turnover is characteristically mediated by enzyme action and plays a dual role in plants. Dat *et al.* (2000) stated that at low concentrations, it acts as a messenger molecule involved in adaptive signaling, triggering tolerance against various abiotic stresses. Hydrogen peroxide at high concentrations orchestrates programmed cell death (Alvarez *et al.*, 1998). Hydrogen peroxide is used for many purposes including cleaning, bleaching, sterilizing, rocket fuel, animal feed treatment and in addition many miraculous claims about its health benefits have been made. Rugine *et al.* (2001) reported various use of hydrogen peroxide in hydroponics, soilless gardening and root initiation in cuttings

It has been reported that accelerated leaf carbon exchange rates have been associated with fruit and seed development in cucumber (Barrett and Amling, 1978). Huber *et al.* (1983) suggesting the pivotal role of leaf photosynthesis that occurs concurrently with fruit growth. During and after photosynthesis fruit sugars are exported from the source leaves to other plant parts. An approach to improve fruit sweetness should be to enhance the sugar biosynthesis during photosynthesis. The sugar in question in sucrose. Sucrose is synthesized from triple phosphates make in Calvin cycle and exported from the chloroplasts. It is converted fructose 6-phospates which is the combine with UDP-glucose to form sucrose phosphate, catalyzed by sucrose phosphate synthase. It has been shown that sucrose phosphate synthase (SPS) activity in rice seedlings can be induced at the transcriptional level by treatment with hydrogen peroxide (Uchida *et al.*, 2002), the nonspecific signaling molecule in various abiotic stresses (Prasad *et al.*, 1994). Furthermore, adaptations to abiotic stresses involve complex signal transduction pathways,

and together with its components may lead to increased soluble sugar contents (Carvajal *et al.,* 2000). The plants treated with 20 mM hydrogen peroxide appeared healthier than that of the control, and the dry weight of shoot and fruits increased approximately 1.6-fold when treated with 20mM hydrogen peroxide; although, at higher concentrations (50 mM) shoot weight declined (Ozaki *et al.* 2009). They observed that hydrogen peroxide treatment increased leaf photosynthetic activity considerably and suggested that a low level increase in endogenous hydrogen peroxide acts as the signal transduction for soluble sugar synthesis, thereby enhancing the soluble sugar content in leaves and fruits. Ozaki *et al.* (2009) also suggested reactive oxygen species such as hydrogen peroxide could be the key factor to activate the Calvin cycle and sugar metabolism. Another view suggests that hydrogen peroxide treatment contributes to absorption of nutrients through roots, and that may be the cause for the activation of Calvin cycle and sugar metabolism (Aonuma, 1993). It has been suggested that, the increased photosynthates formed as a result of the exposure of plants to hydrogen peroxide, are either converted into starch or exported from chloroplast to cytosol for soluble sugar synthesis resulting in an enhanced level of soluble sugar and starch in melon leaves as well as an increased dry weight of shoots and fruits (Ozaki *et al.,* 2009). In many fruits, reactive oxygen species including H_2O_2 are reported to be associated with fruit development, ripening and senescence (Woods *et al.*, 2005)

2.16 Influence of H_2O_2 on fruit quality

One of the important qualities of fruits is their sweetness, which is closely related to the soluble sugar content. Treatment of melon plants with hydrogen peroxide increased the fructose, glucose, and sucrose content in leaves (Ozaki *et al.,* 2009). They reported that the highest amount of fructose, glucose and sucrose increased by 256, 294 and 288%, respectively, relative to untreated control plants with 20 mM hydrogen peroxide treatment

(Ozaki *et al.,* 2009). They also reported that the dissolved solid content (TSS) in melon fruits increased from 11% in control to 17% in fruits treated with 20 mM hydrogen peroxide solution. Chun- yanl *et al.* (2007) reported that spraying of hydrogen peroxide on plants increased the antioxidant level in *Brassica campestris.*

Uchida et al. (2002) reported that the activity of sucrose phosphate synthase (SPS) is a key enzyme for the formation of sucrose from triose phosphates during and after photosynthesis, can be induced by transcriptional level by treatment with hydrogen peroxide. Hydrogen peroxide has been reported to play an important role in regulating the ripening or senescence course in fruits and vegetables (Hodges and Orney, 2000). Carvajal et al. (2000) reported that abiotic stresses involve complex signal transduction pathways, which, along with its components, may lead to increased soluble sugar contents. It has been reported that potassium uptake and translocation is high when H_2O_2 accumulation is less in plants (Shin and Schachtman, 2004). H_2O_2 may also responsible for activating gene expression of PAL, CHS and stylbene synthase enzymes, which are related with the synthesis and accumulation of secondary metabolites phenols and flavonoids (Nyathi and Baker, 2006). They also reported that hydrogen peroxide treatment increased the antioxidant status of fruits. The antioxidant status of *Melilotus officinalis* depends on its flavonoid and phenolic compounds (Pourmorad *et al.,* 2006). Liu *et al.* (2004) also reported that exogenous application of H_2O_2 increased the activity of antioxidant enzymes in cucumber leaves.

Ukuku (2004) reported that application of hydrogen peroxide significantly improved colour development, general appearance and shelf life of cantaloup and honey dew melons. It has been reported that reported that exogenous application of hydrogen peroxide increased phenylalanine ammonia lyase activity and the anthocyanin content in rice leaves

(Kou *et al.*, 2008). Hydrogen peroxide and other active oxygen species elevated the level of carotenoid and other pigments in *Haemato coccus pluvialis* without *de novo* protein synthesis.

2.17 Plant growth regulators on color development and anthocyanin formation

Color is an important factor determining the appearance of fruits and subsequently its quality as well, as consumers are greatly influenced by the appearance of fruits. The disappearance of the green color in fruits and vegetables, as they ripen and mature, is usually the result of chlorophyll degradation and the concomitant unmasking or synthesis of other pigments such as carotenoids and anthocyanins which gives the fruits a yellow to red color (Shewfelt, 1993; Shewfelt and Prussia, 1993). In many fruits, external features like fruit color, and peel texture are the important parameters to estimate the quality of the fruit. Fruit peel color is an important aspect of both natural and processed fruits particularly for commercial reason. In fruits like bananas there is no net synthesis of carotenoids but the loss of chlorophyll unmasks the yellow color present. Several factors and events come into play to bring about these changes, such as chlorophyllase, oxidative systems inherent in the plant cells, hormones, light and pH.

The relationship between color and degree of maturation has been widely studied and reported for fruits such as peaches, and nectarines (Mitchell, 1987; Luchsinger and Walsh, 1993), tomatoes (Choi *et al.*, 1995). Ong *et al.* (2006) reported that the hue value (pure spectrum colors) increased with increasing carotenoid content in ripe jackfruit pulp.

PGRs have been used as both pre and post-harvest treatments to hasten or slow the ripening process, color development, and maturation of specific fruit tissues to improve the quality of the fruits. Fruit quality reflects numerous external and internal attributes, on the basis of which, minimum standards of palatability and commercial acceptability have been

established over the years (Davies and Albrigo, 1994). Additionally color or pigments in fruits and vegetables reflect the presence of certained biological active phytochemicals and antioxidant that reportedly can promote good health. It has been suggested that synthetic auxin enhanced the fruit color development of loquat fruits. Saleem *et al.* (2008) reported that application of plant growth regulators specially (GA_3 and 2, 4-D) increase the peel and pulp color of blood red orange. They also suggested that growth regulators increase the taste and appearance of the blood red orange.

Anthocyanins contribute greatly to the antioxidant properties of certain colorful foods, such as grapes and cranberries (Wang *et al.,* 1997). As pigments, they are almost exclusively responsible for the red, blue and purple colours in fruits. Cyanidin is the most common anthocyanidin, and the 3-glucoside is the most active antioxidant anthocyanin (Wang *et al.,* 1997). Glycosylation and hydroxylation of the anthocyanidin backbone affects antioxidant activity (Wang *et al.,* 1997). It has been estimated that Americans ingest as much as 180–215 mg anthocyanins per day (Kuhnau, 1976). Anthocyanin formation is affected by a number of factors including, light temperature, growth regulators, and developmental stages (Jones, 1984). McGlasson *et al.* (1978) reported that plant growth regulators have a significant effect on anthocyanin biosynthesis of fruit. Application of GA_3 on strawberry plant increased the anthocyanin content significantly (Roussos *et al.,* 2009).

2.18 The effects of plant growth regulators on phenylalanine ammonia-lyase (PAL) enzyme activity

One of the most important enzymes in plant metabolism is phenylalanine ammonia-lyase, usually abbreviated as PAL. In plant metabolism one of the most central metabolic pathways is the Shikimic Acid Pathway which leads to the synthesis of the aromatic amino

acids, namely, tyrosine, tryptophan and phenylalanine. In some plants it accounts for more than 50% of its metabolism because from its many important plant compounds, secondary metabolites are synthesized. PAL (EC number 4.3.1.24) catalyzes the following chemical reaction: L-phenylalanine → trans-cinnamate + NH_3

This enzyme has one substrate, L-phenylalanine, and two products, Transcinnamate and ammonia (NH_3). PAL enzyme belongs to the family of lyases, specifically ammonia lyases, which cleave carbon-nitrogen bonds. The systematic name of this enzyme class is L-phenylalanine ammonia-lyase (trans-cinnamate-forming). Other names in common use include tyrase, phenylalanine deaminase, tyrosine ammonia-lyase, L-tyrosine ammonia-lyase, phenylalanine ammonium-lyase, PAL, and L-phenylalanine ammonia-lyase. This enzyme participates in five metabolic pathways: tyrosine metabolism, phenylalanine metabolism, nitrogen metabolism, phenylpropanoid, and alkaloid biosynthesis (Fig. 2.11).

Phenolic compounds such as flavonols, anthocyanins and phenolic acids, including benzoic, cinnamic and coumarin represent the largest group of plant secondary metabolites that embrace a variety of structural classes, for example as precursors of lignin and they originate from trans-cinnamic acid, which is produced by the action of PAL, the key regulatory enzyme in the first stage of the phenylpropanoids pathway.

PAL links the primary metabolism to the secondary metabolism by catalyzing the deamination of the primary metabolite L-phenylalanine to produce trans-cinnamic acid, thus, leading to the formation of a wide range of secondary metabolites with the

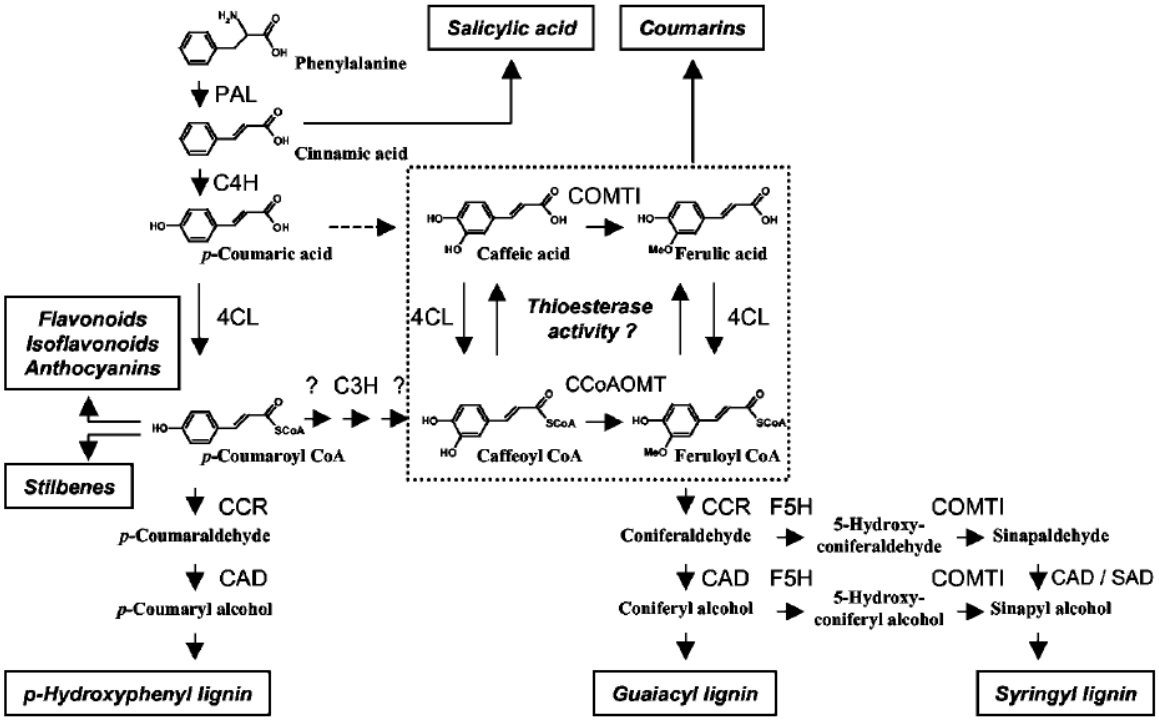


Fig. 2.11. Outline of the phenylpropanoid biosynthetic pathway. *4CL*, 4-hydroxycinnamoyl-CoA ligase; *C3H*, *p*-coumarate 3-hydroxylase; *C4H*, cinnamate 4-hydroxylase; *CAD*, cinnamyl-alcohol dehydrogenase; *CCoAOMT*, caffeoyl-CoA *O*-methyltransferase; *CCR*, cinnamoyl-CoA reductase; *COMTI*, caffeic/5-hydroxyferulic acid *O*-methyltransferase I; *F5H*, ferulate 5-hydroxylase;*PAL*, phenylalanine ammonia-lyase; *SAD*, sinapyl-alcohol dehydrogenase. (Hoffmann *et al*., 2003).

phenylpropane structure, e.g. lignin, which impregnate xylem cell walls during differentiation and suberins which are integral constituents of the cell wall matrix in endodermal and phellogen tissues, and a wide variety of natural products such as anthocyanins, absorbent of ultraviolet (UV), phytoalexins, phenolic compounds and flavonoids (Singleton and Esau, 1969; Lister *et al.,* 1996).

The development of the red pigmentation with maturity in some fruits is dependent on an increase anthocyanin during the maturing period (Wang *et al.,* 2000). Anthocyanin synthesis is a process that involves many steps from the primary precursor (phenylalanine) to the end products, glycosides of cyaniding (Wang *et al.,* 2000). As mentioned above PAL is the first enzyme to catalyze the elimination of NH_3 from L-phenylalanine to give trans-cinnamate. PAL activity has been reported to positively correlate with anthocyanin synthesis in grapes (Kataoka *et al.,* 1983), strawberries (Given *et al.,* 1988) and apples (Faragher and Chalmers, 1977; Arakawa *et al.,* 1986).

Ripening is an important natural process in fruits, which involve changes in color, flavor, and texture, and thereby making them most acceptable for edible purposes. A large number of physiological, biochemical, and structural changes occur during the ripening of fruits which include the degradation of starch and other storage polysaccharides, the production of sugars, the synthesis of pigments and volatile compounds, and the partial solubilization of cell wall (Tucker, 1993). Two groups of compounds are known to be responsible for all the colors found in plants and these are flavonoids and the carotenoids. The flavonoids are hydrophilic phenolic compounds, unlike the carotenoids which are lipophilic, accumulation in the vacuole of the plant cell and are responsible for a wide variety of colors from red to orange, yellow, blue and purple. The main flavonoids group responsible for the colors is anthocyanins.

Phenylalanine ammonia-lyase (PAL) is one of the key enzymes in controlling anthocyanin biosynthesis from phenylalanine (Tucker, 1993). Li *et al.* (2001) reported that PAL activity high in young fruits, decreased to a white maturity stage and then increased until the fully ripe stage in strawberry fruits. Yueming *et al.* (2003) reported that PAL activity increase versus time in strawberry fruits. McGlasson *et al.* (1978) suggested that these substances have an important incidence on the biosynthesis of the anthocyanins. The synthesis of these phenylpropanoid compounds begin with the conversion of the amino acid phenylalanine to cinnamic acid, coumaric acid and coumaryl CoA, catalyzed by the enzyme phenylalanine ammonia lyase or PAL. PAL has become one of the most extensively studied enzymes in plant secondary metabolism because from it is derived the lignins, chalcones, flavonones and tannins. It has been reported that exogenous treatments of GA_3 improve weight, size and color of strawberry fruits, and affect PAL (phenylalanine ammonia lyase) and TAL (tyrosine ammonia-lyase) activities (Teresa *et al.*, 1998). They also stated that the anthocyanin content and PAL activity are enhanced by the exogenous treatment of GA_3 in the range of 30 µg /liter.

2.19 The effects of gibberellic acid on antioxidant content, phenols and flavonoids of treated plants

2.19.1 Antioxidant assays

Several assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables and their products and foods for clinical studies including the 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS assay) (Miller and Rice-Evans, 1997; Leong and Shui, 2002), and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH assay) (Brand-Williams *et al.*, 1995; Gil *et al.*, 2002), ferric reducing antioxidant power (FRAP) (Benzie and Szeto, 1999; Jimenez-Escrig *et al.*, 2001; Guo *et al.*, 2003), and the oxygen

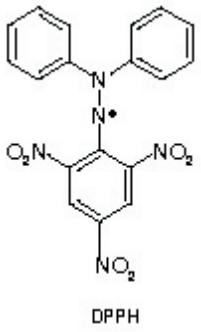
radical absorption capacity (ORAC) (Cao *et al.,* 1993; Ou *et al.,* 2001). The ORAC assay is said to be more relevant because it utilizes a biologically relevant radical source (Penza *et al.,* 2007). These techniques have shown different results among crop species and across laboratories.

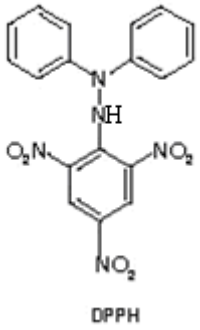
2.19.2 The DPPH assay

The radical-scavenging activity of antioxidants may be influenced by the radical system and other testing conditions. Two or more radical systems are needed to better study a selected antioxidant for its radical scavenging properties. Free radical scavenging is one mechanism by which antioxidants inhibit lipid oxidation. Antioxidant capacity of polyphenols in foods, vegetable, fruits and plant extract is usually tested with 1, 1-diphenyl-2-picrylhydrazyl hydrazyl (α, α-diphenyl-β-picrylhydrazyl). DPPH is a stable free radical in a methanolic solution, and has been used to estimate the radical-scavenging capacities of antioxidants and to evaluate the kinetics and thermodynamic properties of radical-antioxidant reactions (Yu *et al.,* 2002) (Fig. 2.12). Scavenging of DPPH by antioxidant is due to their hydrogen-donating ability (Singh and Bhat, 2003). The DPPH method is performed in a polar medium such as methanol at ambient temperature without any additional oxygen (Brand-Williams *et al.,* 1995). The radical is stable because the spare electron is delocalized over the whole molecule. This delocalization is responsible for its deep violet color. Because of its odd electron, the radical is paramagnetic. However, it can accept an electron or hydrogen radical to become stable and diamagnetic (Fig's 2.11). This yields the reduced form of with the loss of the deep violet color, occasionally giving a residual pale yellow color due to picryl group (Molyneux, 2004).

The decreasing absorbance of the stable radical is monitored at a characteristic wavelength after the single occupied orbital is filled up with an electron provided by the

antioxidant (Krings and Berger, 2001). The maximum absorption of DPPH occurs at 515-520 nm, which disappears as the unpaired electron becomes stabilized, or rather upon reduction by an antiradical compound.





Free radical Non radical

Fig. 2.12. Diphenylpicrylhydrazine (free radical) and Diphenylpicrylhydrazine (non radical).

This is a de coloration assay produced by the addition of the antioxidant to a DPPH solution in ethanol or methanol. Absorbance measurement is not affected by the color of the extracts in the reaction medium. DPPH will oxidize ascorbic acid, tocopherols (Vitamin E), glutathione, polyhydroxy aromatic compounds and aromatic amines (Blois, 1958). Besides studying the sample extracts, a standard/positive control such as ascorbic acid or α-tocopherol should be included. Representing the DPPH radical by $Z^{\bullet}$ (where $Z^{\bullet}$ is as a free radical) and the donor molecule by ascorbic acid (Vitamin C), as an antioxidant, the primary and second reactions are shown in Figure 2.13. The DPPH molecule has two adjacent sites for hydrogen abstraction which are internally connected, as is the case with ascorbic acid (Vitamin C). This leads to a 2:1 stoichiometry, that is, two molecules of DPPH are reduced by one molecule of ascorbic acid. Sanchez-Moreno *et al.,* (1999) proposed that the reaction be monitored until it has reached a plateau and the reaction kinetics plotted, followed by determination of percentage inhibition at steady state from these graphs.

The values of percentage inhibition should then be transferred onto another graph showing the percentage inhibition as a function of antioxidant concentration.

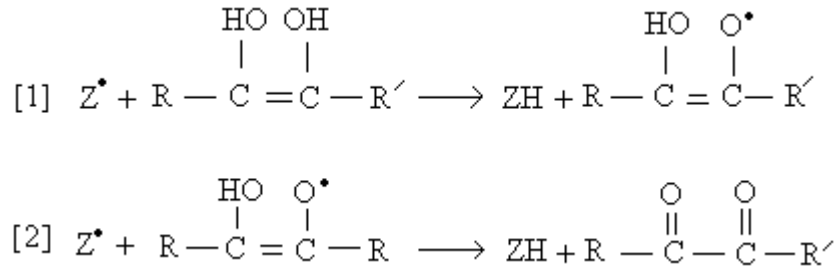


Fig. 2.13. The neutralizing reactions free radical by ascorbic acid.

Finally, Molyneux (2004) stated that usage of the EC_{50} value has a drawback because the higher the antioxidant activity, the lower the value of EC_{50} (substrate concentration to produce 50% reduction of the DPPH).

A experiment had shown that the DPPH scavenging activity of the unripe guava as measured by the AEAC value, is primarily due to its higher total phenol content (TPC) relative to ascorbic acid content (AAC) (Lim *et al.*, 2006). A decrease in AEAC during ripening suggests that the antioxidant activities of guava fruit declined during fruit ripening. They also compared antioxidant properties of guava with other tropical fruits and observed that both varieties of guava fruit contain relatively high quantity of antioxidants as shown by the high amount of TPC and AAC recorded. In the case of AAC, guava (*jambu batu*) contained as much as ten times the quantity of the antioxidant as that of other fruits such as banana; dragon fruit, star fruit and sugar apple have a comparable. On the whole, the results suggest that guava is a healthy fruit to consume from the antioxidant viewpoint, and is better than temperate fruits such as oranges and apples.

For antioxidant activities, this can be primary or secondary. Primary antioxidant properties are generally measured by the DPPH assay (expressed as AEAC and IC_{50}) and FRAP. The DPPH assay measures the ability of the fruit extract to donate hydrogen to the DPPH radical resulting in bleaching of the DPPH solution. The greater the bleaching action, the higher the antioxidant activity (AEAC value), and this is reflected in a lower IC_{50} value. Kondo *et al.,* (2005) reported that DPPH-radical scavenging activity in the skin of guavas, mangoes, and papayas had lower IC_{50} values than those in the flesh throughout development. However, banana skin had a higher DPPH IC_{50} value compared to the flesh, and DPPH IC_{50} of the skin and flesh in rose apples (*Syzygium jambos* Alston) showed no significant difference except at 56 days after full bloom (harvest).

Kulkarni and Aradhya (2005) working on pomegranate arils showed a rapid decrease in antioxidant activity (by 13%) during 20 to 60 days of fruit development, which immediately replenished to its peak activity with 10.6% increase on the 80^{th} day. The lowest antioxidant activity (61.6%) was recorded in 60 day-old fruits, probably due to a reduced concentration of total phenolics and ascorbic acid in the arils 73.9% and 80.1%, respectively. It has been reported that results of the DPPH assay showed that red chili spur pepper had the highest antioxidant capacity, followed by bird chili (red and green) holy basil (red and white), green chili spur pepper, garlic and pumpkin (Wangcharoen and Morasuk, 2007).

2.19.3 The ABTS assay

The ABTS assay is also designed to measure the overall antioxidant capacity within a given sample. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS (2, 2 -azino-di-[3-ethylbenzthiazoline sulphonate). A method for the screening of antioxidant activity is reported as a decolorization assay

applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, and carotenoids. The pre-formed radical monocation of 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) is generated by oxidation of ABTS with potassium per sulfate and is reduced in the presence of such hydrogen-donating antioxidants (Fig. 2.14). The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity (Re *et al.,* 1999).

In an experiment carried out on guava fruits, it was reported that correlations among antioxidant activity measured in methanol extract (AOAM) on the ABTS, DPPH, FRAP, and ORAC assays, were positively high and ranged between 0.68 and 0.97 (Thaipong et al., 2006). The highest correlation was between ABTS and FRAP (0.97) and the lowest correlation was between DPPH and ORAC (0.68). ABTS and DPPH assays are based on the reduction of ABTS and DPPH free radicals by the samples, but values from DPPH assay might be lower than those from the ABTS assay.

It has been shown that some compounds which have ABTS scavenging activity may not show DPPH scavenging activity (Zhang *et al.,* 2008). Wangcharoen and Morasuk (2007) reported that in the ABTS assay, the plants with the highest antioxidant capacity were bird chili (red and green) and red holy basil, followed by red chili spur pepper and white holy basil, green chili spur pepper, garlic, and pumpkin.

2.19.4 Antioxidant activity in fruits

Antioxidants are substances that are capable of neutralizing the damaging harmful molecules called free radicals that are chemically active atoms or molecular fragments that have a role in both plant and animal ageing process, chronic diseases as cancer, heart disease, stroke, alzheimer's disease, and atherosclerosis. Free radicals containing oxygen,

known as reactive oxygen species (ROS), are the most biologically significant free radicals. ROS are partially reduced forms of oxygen such as singlet oxygen, superoxide anions (O_2^-), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) (Abassi *et al.,* 1998).

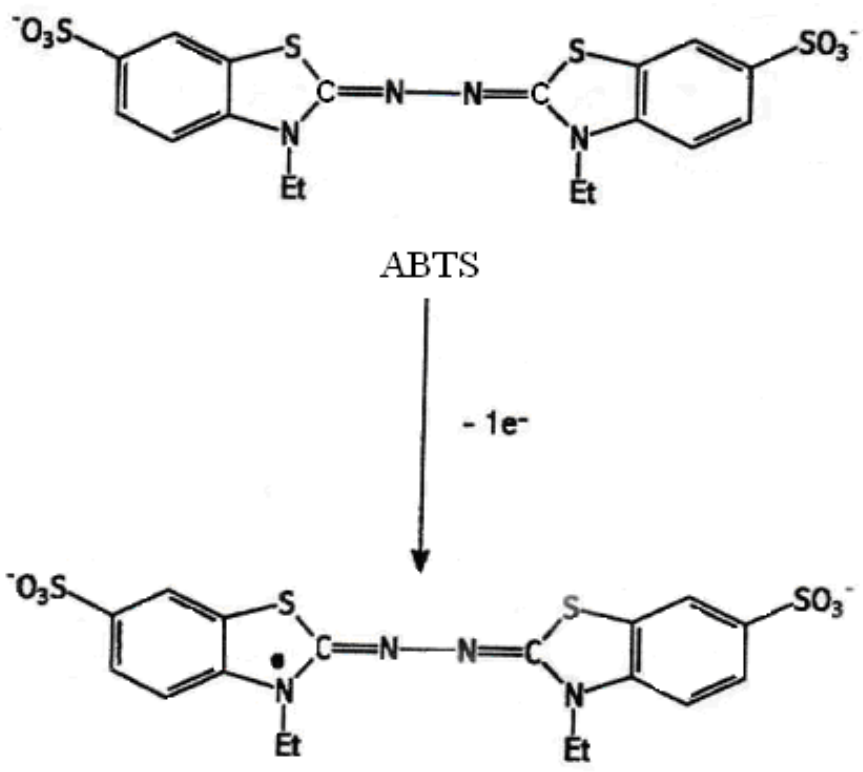


Fig. 2.14. Oxidation of ABTS to $ABTS^{\bullet+}$ radical (Akerstrom *et al.,* 2007; Rojo *et al.,* 2009).

Reactive oxygen species (ROS), are capable of causing damage to DNA, have been related with carcinogenesis, coronary heart disease, and many other health problems related to advancing age (Uchida, 2000).

An antioxidant protects the cell from the harmful effects of free radicals and when an antioxidant reacts with a free radical, it yields an electron, is oxidized, and becomes a weak, non-toxic free radical that is stable and unable to propagate the reaction (Gorinstein *et al.,* 2007). Natural antioxidant defense system include enzymes like superoxide

dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and monodehydroascorbate reductase (MDHAR) and water soluble compounds such as ascorbic acid, glutathione, phenolic compounds and flavonoids and lipid soluble compounds like carotenoids and tocopherols, etc., help in scavenging of ROS (Lelandais *et al.,* 1994).

There is an increasing awareness among consumers of the importance of natural antioxidants, especially from fresh fruit and vegetables. Epidemiological studies have shown that frequent consumption of natural antioxidants is related with a lesser risk of cardiovascular disease and cancer (Renaud *et al.,* 1998; Temple, 2000). Natural antioxidants in fruits and vegetables are classified in three major groups: vitamins [vitamin E (tocopherol), vitamin C (Ascorbic acid) and ß-carotene], phenolics, and carotenoids. Hydrophilic antioxidants are Ascorbic acid and phenolics, while carotenoids and tocopherols are known as lipophilic antioxidants (Halliwell and Gutteridge, 1995). In addition, there are also several antioxidant enzymes, including catalase, superoxide dismutase (SOD), and glutathione peroxidase, that counteract many types of free radicals in the body

Guava (*Psidium guajava* L.), also known locally as *jambu batu*, is rich in ascorbic acid (vitamin C), at levels far higher than most imported and local fruits. The fruit, in particular the pink flesh cultivar, has a fair amount of vitamin A (β-carotene) and also has a high quantity of antioxidants such as phenols (Lim *et al.,* 2006). Other fruits such as banana (Mokbel and Hashinaga, 2005), pomegranate (Kulkarni and Aradhya, 2005), black caraway, carrot, cranberry (Yu *et al.,* 2005), tomato (Javanmardi and Kubota, 2006), apple (Maffei *et al.,* 2007), blood orange (Kelebek *et al.,* 2008), and Chinese bayberry fruit (Zhang *et al.,* 2008), are also considered to be good sources of natural antioxidants.

Several quantitative studies investigating the phenolic content and antioxidant potential of edible fruits have been carried out and widely reported, since the role that these factors play in health and disease chemoprevention are important for human well being. It has led to an upsurge of interest in phytochemicals as potential new sources of natural antioxidants. From the literature, previous phytochemical studies of the leaves of *S. samarangense* have shown the presence of ellagitannins (Lee *et al.,* 1992), flavanones (Liu *et al.,* 2005), flavonol glycosides (Ross *et al.,* 2005), proanthocyanidins (Hosseinian and Mazza, 2009), anthocyanidins (Molan *et al.,* 2009), triterpenoids, chalcones (Srivastava *et al.,* 1995), and volatile terpenoids (Wong and Lai, 1996).

2.19.5 Phenolic content

The phenolic content in food, fruit, vegetable and beverages has been correlated with a reduced incidence of several diseases (Martha-Estrella *et al.,* 2008). There is considerable epidemiological evidence indicating a relationship between fruit and vegetable rich diets and a decreased risk of certain forms of cancer. The role of polyphenolic compounds from higher plants as antioxidants, antimutagenic, anti-inflammatory and antimicrobial agents is widely recognized (Hatano *et al.,* 2002). The health impact of antioxidants in foods and the hazardous effects of synthetic preservatives have led to active research in the field of natural antioxidants.

Phenolic compounds represent the largest group of plant secondary metabolites that embrace a variety of structural classes (e.g. as precursors of lignin) and biological functions. Phenolic compounds include a great diversity of compounds derived from the aromatic amino acids phenylalanine and tyrosine and they are widely distributed in fruits,

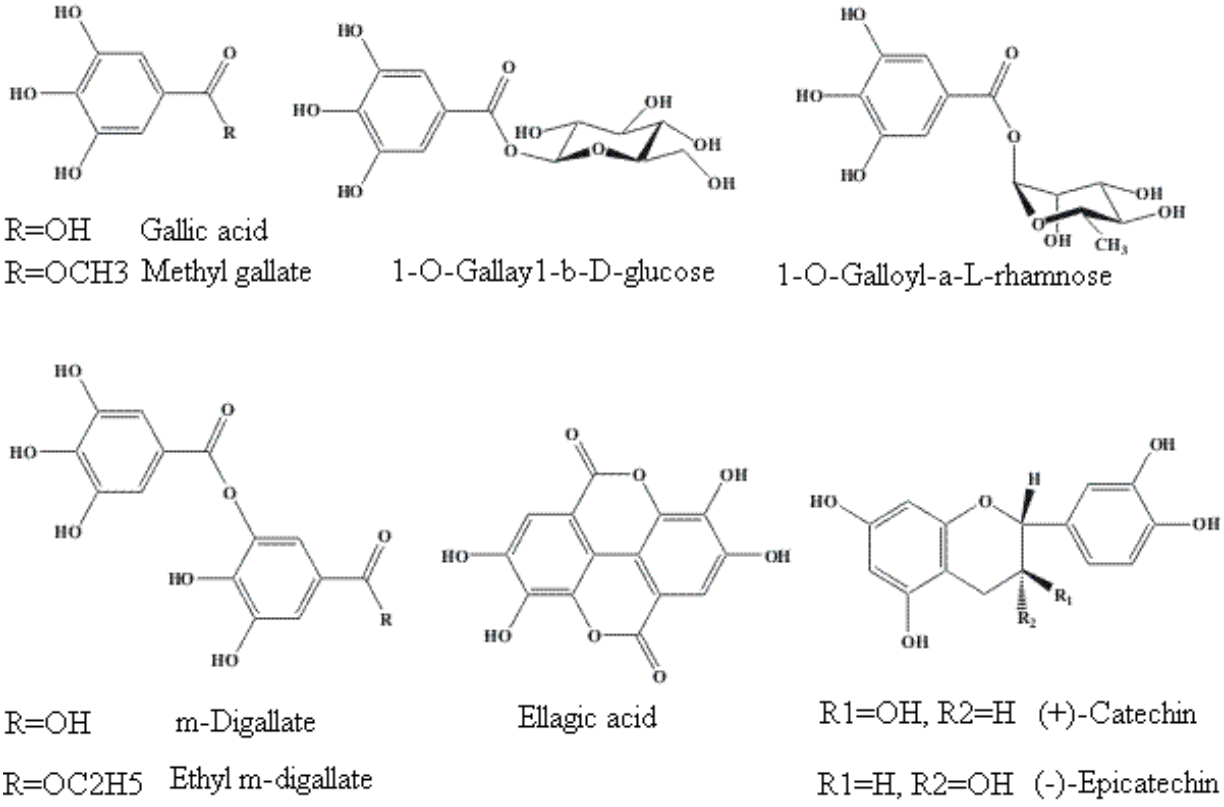


Fig. 2.15. Phenolic compounds (Rice-Evans *et al.*, 1996; Tomas-Barberan *et al.*, 2000)

vegetable, seeds, medicinal plants and herbs. The general characteristic of the compounds within this group is to have aromatic rings with variable degrees of hydroxylation (Shahidi and Naczk, 1995) (Fig. 2.15). The phenol subunits are classified in two groups which include simple phenols and polyphenols. The simple phenols contain phenolic acids or phenols with a carboxyl group underlying the specificity of their function. Polyphenols have at least two phenol rings such as flavonoids (Marinova *et al.*, 2005).

In plants, they are important for pigmentation. Polyphenolic anthocyanins are responsible for the blue, red, orange, purple and violet colour of plants and plant products. Phenolics are a source of natural antioxidants and are important in food and biological systems because they are preferentially oxidized, thus sparing nutrients, cells and tissues (Imeh and Khokhar, 2002). The antioxidant activity of polyphenols is higher than mono phenols. Polyphenols scavenge superoxide and hydroxy radicals, inhibit lipid peroxidation, prevent oxidative damage to important biological membranes and reduce lipid peroxyl radicals (Mayo *et al.*, 2003).

The beneficial properties of berry fruits on human health have been associated in part with the presence of relatively high levels of phenolic compounds (Seeram *et al.*, 2006). Cocoa bean and its products, cocoa liquor, cocoa powder, and dark chocolate, are food sources rich in phenolic compounds. Cocoa beans have a high phenolic content of about 12–18% (dry weight) in unfermented beans (Kim and Keeney, 1984). It is reported that 60% of the total phenolics in raw cocoa beans are flavanol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer) (Dreosti, 2000). These compounds have been reported to be potential candidates to combat free radicals, which are harmful to our body and food systems (Adamson *et al.*, 1999).

In a study on Bulgarian fruits and vegetables has shown that the highest total phenolic content (TPC) is in blueberries (670.9 mg gallic acid equivalents (GAE)/100g), dogwood berries (432.0 mg GAE /100g) and sour cherry (429.5 mg GAE / 100g). The lowest TPC were observed in peaches (50.9 mg GAE / 100g) **(**Marinova *et al.,* 2005**).** For vegetables the highest phenolic content were in green peppers (246.7 mg GAE / 100g) and red peppers (173.2 mg GAE/ 100g) and lowest was in leek (stem) (27.7 mg GAE /100g) **(**Marinova *et al.,* 2005**).**

Righetto *et al.* (2005) in an experiment on acerola (*Malpighia emarginata* DC**)** reported that the total phenol contents decreased during ripening, from 3.8 mg of catechin/g for immature acerola juice to 1.4 mg of catechin/g in mature acerola juice. Ninfali *et al.* (2005) working on vegetables, showed that the phenolic content matched the concentration of the two phenolic subgroups, namely, flavonoids and flavanols. They also showed that the diversification and the combination into salads of different vegetables provides an opportunity to introduce a variety of phenolics with the possibility of markedly increasing the total antioxidant capacity of the vegetable portion.

Kondo *et al.* (2005) also reported that the total phenolic content of guava, mango, and rose apple skin were higher than in the flesh and at the immature stage and decreased as the fruit ripened. In contrast, total phenolics of banana pulp were higher than in the skin, while those of papaya skin and flesh showed no significant differences. The composition of polyphenolics varied among fruit, and except for bananas, the skin contained a greater range of polyphenolics than the flesh. In an experiment carried out by Kulkarni and Aradhya (2005) pomegranate arils showed a rapid and significant ($P \leq 0.05$) depletion (by 54.5%) in total phenolics during the initial stage of fruit development from 20 to 40 days. At the later stages, the decrease was gradual but significant up to 140 days. The highest

phenolic content (506 mg/ 100 g arils) was recorded in 20 day-old fruits. There was nearly a 73.9% reduction in total phenolics from 20 to 140 days of fruit development.

It was reported that the total phenolic content (TPC) in guava was high compared to other fruit crops (Thaipong *et al.,* 2006). The ranges of TPC (mg/100 g) in other fruits were 14–102 in nectarines, 21–111 in peaches and 42–109 in plums (Gil *et al.,* 2002), 142.9 in star fruit, 47.9 in pineapple, 56.0 in mango, 57.6 in papaya, 28.8 in litchi (Luximon-Ramma *et al.,* 2003). In a study on some Thai culinary plants it was shown that the red bird chili had the highest amount of phenolic compounds (3.48 mg GAE / g) and the pumpkin had the lowest amount of phenolic compounds (Wangcharoen and Morasuk, 2007).

2.19.6 Flavonoid content

Flavonoids are widely distributed in plants fulfilling many functions including producing yellow or red/blue pigmentation in flowers. They also play a role in protecting the plants from microbe and insect attacks. They are also referred to as bioflavonoids and are secondary metabolites, meaning they are organic compounds that have no direct involvement with the growth or development of plants. There are different classes of flavonoids (Le Marchand, 2002) viz. a) flavones and flavanols; b) flavanones, flavanols; c) isoflavones; d) proanthocyanidins; and e) anthocyanidins (Fig. 2.16). Over 4000 different flavonoids occurring in plants have been described (Hollman, 2001).

Flavones include rutin, luteolin and apigenin, while the most abundant flavonols are quercetin and kampferol (Manach *et al.,* 2004). Onions are rich in these compounds. Blueberries also have high levels, especially in the peel, because synthesis is stimulated by exposure to light. Celery is a good source of flavones. Flavones are also present in citrus, but they are associated mainly with the fruit peel. Isoflavones are phytoestrogens present in

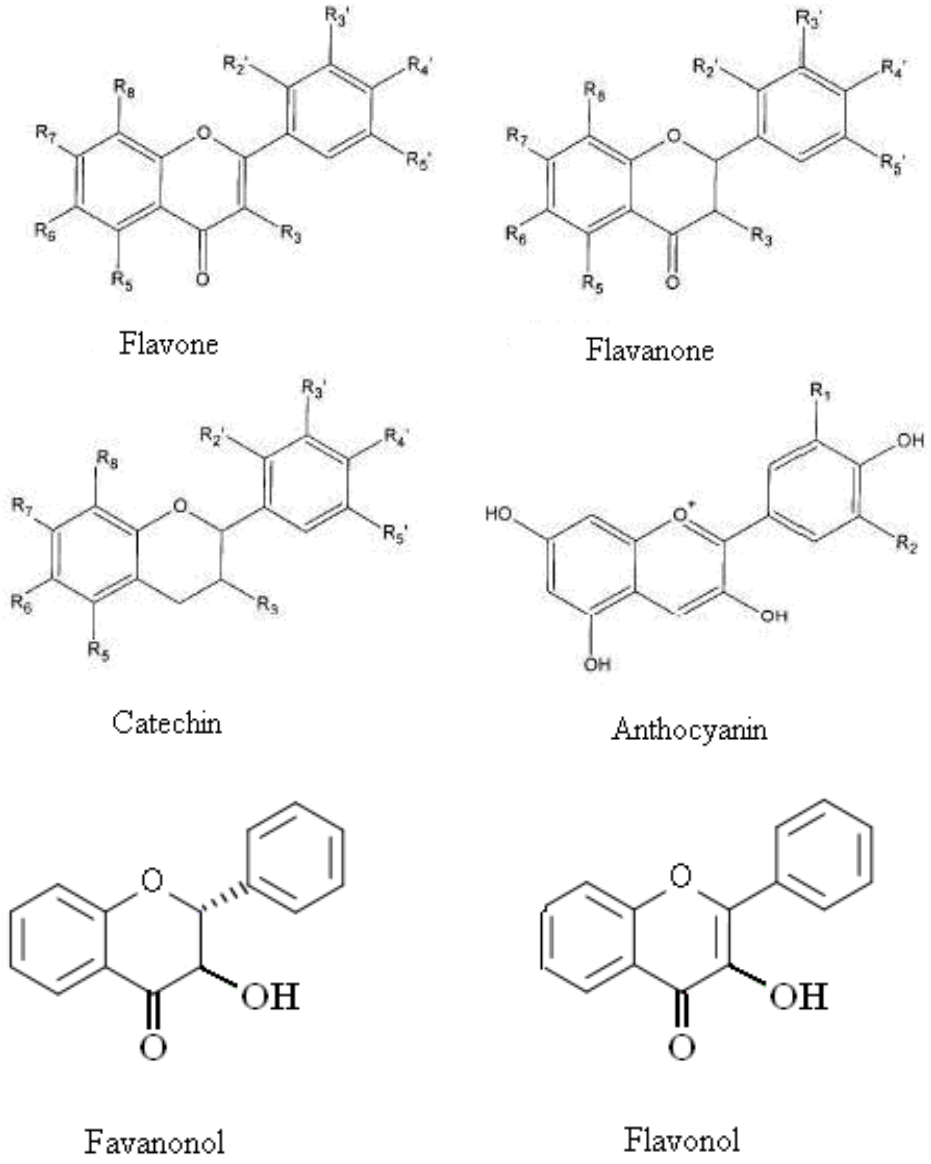


Fig. 2.16: Some of flavonoids classes: Flavone, Flavanone, Catechin, Anthocyanin, Flavanonol, Flavonol (Santos *et al.*, 2002; Jakus *et al.*, 2004)

legumes. Soybean products are a good source of these compounds (Manach *et al.,* 2004). The three most commonly found isoflavones are genistein, glycitein and daidzein. Proanthocyanidins are oligomeric flavonoids (usually dimers or oligomers of the flavanols catechin and epicatechin). They are common in the peel and seeds of grapes (Gu *et al.,* 2004). Other sources of these compounds include apple, almond and blueberry. Anthocyanidins are pigments giving several fruits their characteristic red or purple colors, although in some conditions they can be uncolored. Besides being pigments, anthocyanidins have great relevance due to their contribution to the antioxidant capacity of fruits and vegetables.

Fruits and vegetables are high in flavonoid content. Flavonoids impart color and taste to flowers and fruits, and it is estimated that humans consume between a few hundred milligrams and one gram of flavonoids every day (Hollman and Katan, 1999; Pietta, 2000). Fruit phenolic compounds include mainly flavonoids (e.g. flavonols, flavones, isoflavone, anthocyanins, flavanones, chalcones), phenolic acids, quinones, and tannins (Sakakibara *et al.,* 2002). In an experiment on dates it was reported that the total flavonoid content (TFC) varied considerably from 1.62 to 81.79 mg in terms of catechin equivalents/100 g of sample (Biglari *et al.,* 2008). Zhang *et al.,* (2008) reported that the antioxidant capacity also increased with the increase in fruit maturity at harvest in Chinese bayberry. These changes were well correlated with the increases in the contents of total phenolics, flavonoids and anthocyanins.

In other fruits the biosynthesis of different anthocyanin types and other flavonoids may continue after harvest and during air storage even at low storage temperature in dark conditions as was found in blueberry (Kalt and McDonald, 1996), pomegranates and strawberry (Holcroft and Kader, 1999). Tomas-Barberan *et al.,* (2000) observed an

increase in anthocyanin concentration in nectarine, cherry, grape and strawberry fruits during air storage whereas flavonoids and hydroxyl cinnamic acid derivatives remained constant except for resveratrol in grapes and ellagic acid in strawberry, which increased.

2.20 Growth regulators effects on photosynthesis

Hedden and Phillips (2000) stated that gibberellins (GAs) are plant growth regulators that regulate the growth and development of higher plants and only a few of them are believed to possess biological activity. It has been reported that GAs can promote stem elongation, seed germination, leaf expansion, flowering, and fruit development, and the mediation of environmental signals such as day length, stimulate both cell elongation and cell division (Hedden and Proebsting, 1999; Kende and Zeevaart, 1997).

Photosynthesis is the most important metabolic event and process to understand in order to maximize plant productivity on earth (Dean, 1994). Thimann (1980) reported that gibberellins and cytokinins can delay the loss of chlorophyll whereas ethylene and abscisic acid enhance the rate of chlorophyll loss. Application GA_3 and white light significantly delayed the loss of chlorophyll content in leaves of alstroemines cut flower (Jordi *et al.,* 1993). They also stated that the chlorophyll loss affect the photosynthetic activity. Genty *et al.* (1989) reported that gas exchange measurements provide information on the net CO_2 uptake and stomatal control whereas fluorescence signals give a measure of the electron transport rate through photosystem II.

Nagel and Lambers (2002) stated that very little information is available how GAs affect photosynthesis, which supplies carbon skeletons and energy for growth. The few available reports reveal contradictory results showing that application of GA_3 either stimulates (Yuan and Xu, 2001; Ashraf *et al.,* 2002) or reduces the photosynthetic rate (Dijkstra *et al.,* 1990). Ashraf *et al.* (2002) reported that GA_3 treatment of salt-stressed

wheat plants increased photosynthetic capacity and greater dry matter production. Similar, a stimulatory effect of GA_3 on photosynthesis was also observed in broad bean and soybean (Yuan and Xu, 2001). Cramer *et al.* (1995) reported that no significant differences in photosynthetic rates were found between a GA-deficient tomato mutant and wild type plants. Nagel and Lambers (2002) stated that the reason for these contradictory results were may be for different ways of measuring and calculating photosynthesis as well as the different experimental systems used such as; application time and methods of GAs or GA inhibitors, use of mutants.

Lenz (1979) reported that the photosynthetic capacity of the leaves of fruiting and non-fruiting plants is regulated by current demand for assimilates and regulatory mechanisms such as hormonal influence, and assimilate concentration. Fruit has relatively high concentration of phytohormones (Luckwill, 1975). Wareing (1968) suggested that hormones deriving from the fruit regulate photosynthesis by directly activating ribulose diphosphate carboxylase. It has been reported that GAs, auxin and cytokinin can stimulate the rate of photosynthesis and elevated the activity of ribulose diphosphate carboxylase in leaves (Huber and Sankhla, 1973). The application of IAA increased photosynthesis of chloroplast through enhancing photophosphorylation (Tamas *et al.,* 1972).

Photosynthesis and transpiration are the exchange processes, inside and outside, in which CO_2 and water molecules are transported, respectively, and the main channels are the stomata. Mesophyll cells water, CO_2 exchange, and water use efficiency controls the stomatal movement (Liang *et al.,* 1999). CO_2 exchange is influenced by stomatal and mesophyll cell factors at the same time; therefore, stomatal limiting theory (Farquhar and Sharkey, 1982)

It has been shown that applications of GA_3 can alter the partitioning of photosynthates in grapevines and in other plant species. Long-term treatment of GA_3 (often for several days) increased photosynthetic CO_2 uptake rate in leaves of bean (Marcelle *et al.*, 1974), pea, rice, and tomato (Hayashi, 1961). GA_3 application could lead to the changes in plastid development and chloroplast structure (Marcelle *et al.*, 1974).

RubisCoase is a key enzyme controlling photosynthetic carbon fixation of plants. It is assumed that the quantity of activated RubisCoase is closely related to the rate of photosynthetic carbon assimilation. There was a good correlation between the light-saturated leaf photosynthetic rate and the activity of RubisCoase (Björkman, 1981). Furthermore, the relation between the initial slope (CE) of response curve of Pn to Ci and RubisCoase quantity has been demonstrated in spinach leaves (Bjorkman, 1982). It was also reported that the increase in photosynthetic rate after GA_3 short term treatment can be attributed to the enhancement of Rubisco content and activity.

GA_3 treatment increased the carboxylation efficiency of soybean and the RubisCoase activity in broad bean leaves (Yuan and Xu, 2001). They also reported that short-term application of GA_3 increased the ratio of Rubisco LS and total soluble proteins, or the relative content of Rubisco LS, in soybean leaves and broad bean protoplasts. Yuan and Xu (2001) reported that GA_3 treatment had a stimulatory effect of on Pn, therefore, may be attributed to the increase in Rubisco content. They also stated that the increase in leaf net photosynthetic rate caused by GA_3 short-term treatment is mainly due to the increases in the content and activity of RuBPCase but not due to increased stomatal conductance and GA_3 stimulates the synthesis of Rubisco protein at translation rather than transcription level.

Hydrogen peroxide treatment contributes to absorption of nutrients through roots, and that may cause the activation of Calvin cycle and sugar metabolism (Aonuma, 1993; Koga, 1999). Ozaki e*t al.* (2009) reported that hydrogen peroxide treatments increased the photosynthesis and the increased photosynthates are either converted into starch or exported from chloroplast to cytosol for soluble sugar synthesis resulting in an enhanced level of soluble sugar and starch in melon leaves as well as increased dry weight of shoots and fruits. Shin *et al.* (1998) observed that increased photosynthetic activity in melon leaves during CO_2 enrichment condition enhanced the yield and soluble sugar content in fruits. They also reported that the increased invertase activity in leaves of hydrogen peroxide-treated plants suggests for the enhanced sucrose synthesis and vice versa due to increased photosynthetic pro-ducts. Treatment of melon plants with an optimum concentration of hydrogen peroxide did not decrease the plant growth and fruit yield, albeit the increased in the soluble sugar content in leaves and fruits of the melon plants, thus improving the fruit quality (Ozaki *et al.,* 2009).

2.21 The **effects of gibberellic acid on protein, sucrose phosphate synthase activity and rbcA gene of treated plants**

Total Protein Content

Proteins are vital parts of organisms and contribute in every process within cells. Many proteins are enzymes that catalyze biochemical reactions that are essential to metabolism. About 4,000 reactions are known to be catalyzed by enzymes. A group of enzymes such as the polyphenol oxidases (PPO), that catalyzes the oxidation of polyphenolic compounds by molecular oxygen, are responsible for enzymatic browning reactions happening during harvesting, handling, processing and storage of many plants (Robb, 1984; Sheptovitsky and Brudvig, 1996). It has been reported that levels of PPO and

phenolics may change during fruit development and ripening which may influence the potential damage, in loquat fruit (Bru *et al.,* 2006). Ayaz *et al.* (2008) reported that the total phenolic content decreased, while PPO activity increased in fruits during ripening.

Other groups of enzymes are the antioxidant enzymes such as, superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and monodehydroascorbate reductase (MDHAR) that are part of the antioxidant defense system against the damaging effect of reactive oxygen species (ROS) (Mondal *et al.,* 2004). They also reported that antioxidant enzyme activities increased in during the ripening of tomato but not until the later stages of ripening. During plant senescence, Lysyl oxidase (LOX) activity increases, while SOD, CAT and POX activities fall, resulting in a concomitant decline in the ability to scavenge free radicals (Mondal *et al.,* 2004). They also stated that increases in lipid peroxidation products during later stages of fruit ripening could also be mediated through increased LOX activity. Vincent *et al.* (2004) reported that lipid peroxides increases during ripening in fruits. These changes are primarily due to enzymatic reactions and thus, energy demand especially for protein synthesis increases during ripening (Brady *et al.,* 1970).

It has been shown that the highest total protein (209 mg/100 g) was observed in 20 day-old pomegranate fruits followed by a rapid decrease (66.9 %) in total protein up to 80 days of fruit development (Kulkarni and Aradhya, 2005). The amount of protein varied significantly with the fruits; Myrtus berries (0.9 g protein/100g), blueberry (0.7g/100g), strawberry (0.6 g/100g), raspberry (1.2 g/100g), sweet cherry (1.1 g/100g), grape (0.7 g/100g), apple (0.3 g/100g) and orange (0.9 g/100g) (Gebhardt *et al.,* 2005). Bernardino-Nicanor *et al. (*2006) reported that guava seed content the larger proportion of protein.

Shakirova *et al.* (2003) reported that phytohormone regulates many physiological processes in plant. Gibberellins (GAs) are group of plant hormones that play an important role in regulating many physiological processes involved in the growth and development of plants (Hooley, 1994). Hormonal regulation could play a role in gene expression. The use of gibberellic acid (GA_3), together with auxin treatment, can reduce the occurrence of puffy fruit (Yamasaki *et al.,* 1961) and raise the setting ratio under high temperature conditions (Sasaki *et al.,* 2005). Iglesias *et al.* (2001) reported that GA_3 regulate the timing of chlorophyll disappearance by inhibiting or reducing chlorophyll biosynthesis.

Auxin and GA_3 mediated cell elongation is associated with changes in the expression of specific gene products (Chory *et al.* 1987; Shi *et al.* 1992; Hagen, 1995). Severa1 of these auxin and GA-responsive genes have been characterized, and the functions of the proteins have been proposed (Abel *et al.,* 1994). Given *et al.* (1988) reported that auxins delay ripening in strawberry fruit by altering the expression of many ripening-associated genes.

Gray *et al.* (1992) reported that investigations of fruit development using molecular genetics techniques have concentrated mainly on fruit ripening, although changes in polypeptide patterns and expression of severa1 genes have been reported during early fruit development in tomato, kiwifruit, strawberry, and pea (Reddy *et al.,* 1990; Perez-Amador *et al.,* 1995). The regulatory roles of auxin and GAs during early fruit development have focused on either auxin-or GA_3-regulated developmental processes (Reddy *et al.,* 1990; Granel1 *et al.,* 1992). Little is known on the relative roles of auxin and GA_3 or their interaction in the leaf during fruit development and ripening of wax apple fruits.

Ribulose 1, 5-bisphosphate carboxylase is the most abundant enzyme and functions in CO_2 fixation in the Calvin-Benson cycle (Andrews and Lorimer, 1987, Hartman 1992,

Spreitzer 1993). In plants, algae, and some autotrophic eubacteria, this enzyme is composed of octamers of each 50 to 55 kilodalton (kDa) large subunit (L, subunit A) and 12 to 18 kDa small subunit (*S, subunit B*), L8S8 (Akazawa, 1979). The enzyme requires activation for catalysis through carbamylation of a specific lysine residue and the subsequent chelation by Mg^{2+}. The activated enzyme catalyzes both the carboxylation and oxygenation of ribulose 1, 5-bisphosphate (RuBP). Several amino acid residues in the active site have been identified as essential for activity by means of chemical modification or site-directed mutagenesis (Andrews, and Lorimer, 1987). The only known exceptions are RubisCO from *Rhodospirillum rubrum* and form II RuBisCO from rhodopseudomonads, which are composed only of large subunits (Akazawa, 1979). Despite intensive work, the function of the small subunit is not well understood, although it is necessary for the activation and catalysis of the hetero-oligomeric enzyme (Akazawa, 1979, Andrews and Lorimer, 1987). The presence of two sets of genes coding RubisCO has been reported for the rhodopseudomonads (Tabita, 1988). Alcaligenes eutrophus (Klintworth *et al.*, 1985) and Nitrobacter hamburgensis (Harris *et al.*, 1985).

It has been reported that gibberellin treatment induced expression of several gene, namely; pyruvate dehydrogenase kinase1 (Jan *et al.,* 2006). Ogawa *et al.* (2003) reported that a recent study identified 138 up-regulated and 120 down-regulated gene**s** after a 12-h GA_4 treatment of imbibed *ga1-3* seeds, using the oligo-based microarrays representing about 8,200 Arabidopsis genes. Growth promoting chemicals cerium promotes the chlorophyll synthesis, the activities of two key enzymes in CO_2 assimilation, and the expression of *rbcL*, *rbcS*, and *rbcA*, thus leading to the enhancement of spinach growth under magnesium-deficient conditions (Yuguan *et al.*, 2009).

It has been stated that sucrose plays a pivotal role in plant growth and development because of its function in translocation and storage, and because of the increasing evidence that sucrose (or some metabolite derived from it) may play a non nutritive role as a regulator of cellular metabolism, possibly by acting at the level of gene expression (Jang and Sheen, 1994). It is the non reducing nature of the sucrose molecule that explains its wide distribution and utilization among higher plants. Sucrose-phosphate synthase (SPS) is the plant enzyme thought to play a major role in sucrose biosynthesis. Sucrose phosphate synthase (SPS) is a low-abundance protein (<0.1% of leaf soluble protein) and is also relatively unstable. The native SPS molecule is likely a dimer of 120-138-kDa subunits Sucrose synthesis can be catalyzed by two distinct enzymes in higher plants: sucrose-phosphate synthase (SPS) and sucrose synthase (SuSy) (Huber and Huber, 1996).

SPS activity has been reported to change rapidly according to light/dark transitions and involves a mechanism of light activation of the enzyme. Huber et al. (1989) characterized several species according to the extent of light modulation of the corresponding SPS enzyme. Stitt et al. (1988) reported that Sucrose phosphate synthase activity can be controlled by a number of factors, but also that its regulation is species specific, with possible variations in protein structure.

Sucrose phosphate synthase (SPS) plays an indispensable role in the regulation of photosynthetic sucrose formation. An enhanced SPS activity can be closely associated with an increase in sucrose accumulation in plant parts. It has been shown in several studies that photosynthetic sucrose formation directly or indirectly regulate SPS activity in leaves of higher plants (Cheikh *et al.,* 1992). They also reported that gibberellic acid was clearly identified as one of the possible signals in the regulation of SPS activity in soybean and spinach leaves. Uchida *et al.,* (2002) reported that H_2O_2 treatment increased the SPS

activity and sugar content of tomato and melon plants without any negative effects on plant growth or fruit productivity.

Judging from the literature discussed above, it is apparent that fruit growth, development, and ripening is a very complex process with various mechanisms involved. From a physiological and biochemical perspective, plant growth regulators, hydrogen peroxide and girdling plays a crucial role in fruit growth, development as well as ripening. Hence it can be said that plant growth regulators and others horticultural techniques had a enormous role in fruit growth, yield and quality development. The present study, examines few approaches discussed above, in order to understand growth, development and ripening of wax apple fruit.

CHAPTER 3

MATERIALS AND METHODS

3.1 a. Experimental site

Studies were carried out in an orchard located at Malaysian Agricultural Research and Development Institute (MARDI), Klang, and a commercial farm in Banting, Selangor, 2^0 30N, 112^0 30E and $1^0$28 N, 111^0 20E, at an elevation of about 45 meter above sea level (Fig. 3.1 and 3.2). The area under study had a hot and humid tropical climate. The soil in the both orchards was peat with a mean pH of 4.6 (Ismail *et al.,* 1994). This study was carried out for three years of fruit growth between December 2008 to May 2011, the first year at MARDI, Jalan Kebun, Klang, and the second and third years in Banting, Selangor. The 2^{nd} and 3^{rd} year plants were selected in Banting because the plant was cut down by the operating authority in MARDI, Klang. So in this thesis the experiment will display results from both experimental sites as mentioned above.

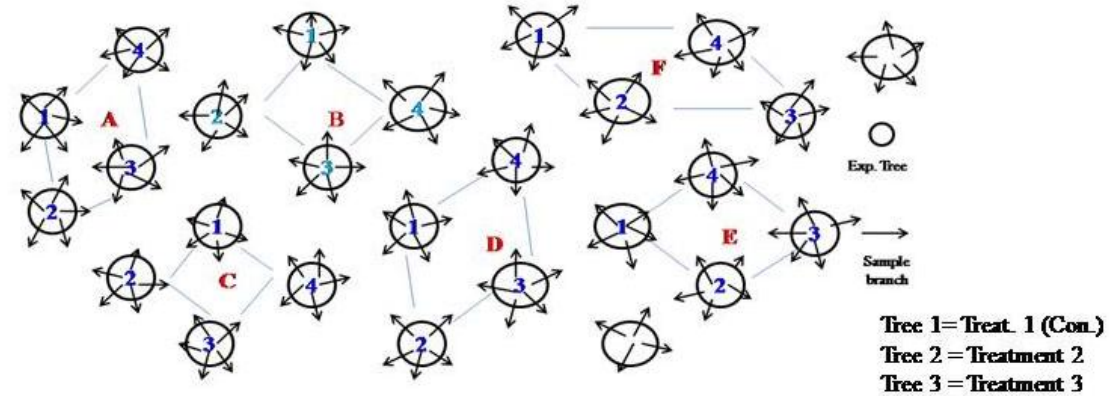
3.1 b. Experimental design: All the six experiments were arranged in a randomized complete block design (RCBD). Experiment 1, 2, 3, 5 and 6 have 4 treatments with 6 replications (Fig. 3.3a), while, experiment 4 has 6 treatments with 6 replications (Fig. 3.3b). A single tree was used as an experimental unit and from each tree five uniformed branches along with approximately same length, diameter and the same number of leaves were selected from a tree. An average value of 5 sample branches from a single tree for each treatment was used for statistical analysis.

3.2 Experiment

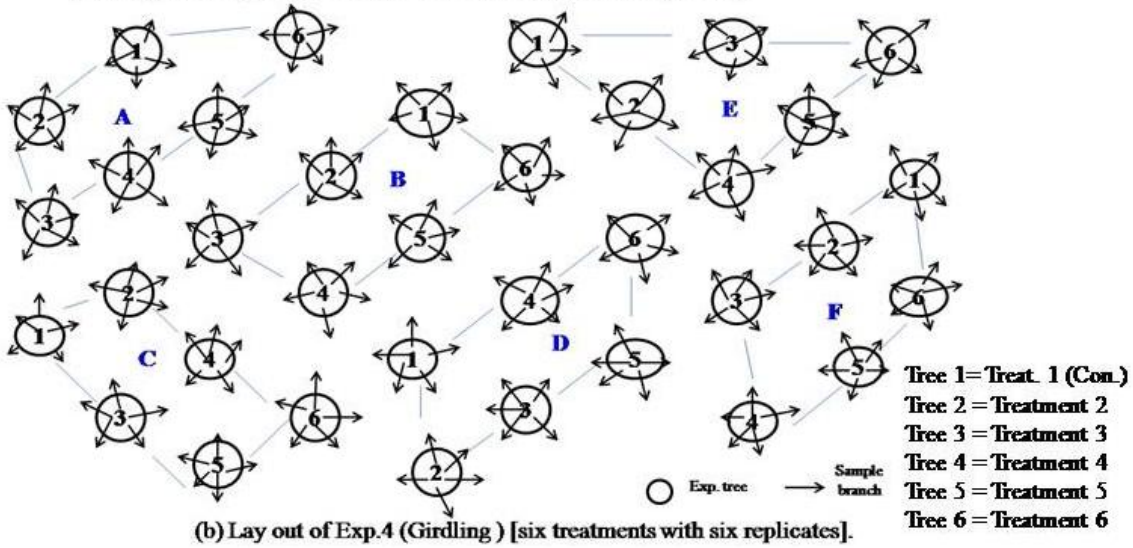
3.2.1 Experiment 1. The effect of GA_3 treatments on fruit growth, development and quality of wax apple.

Fig. 3.1. Google map showing experimental location in Klang and Banting (Shown in 'A' and 'B', respectively), Selangor, Malaysia.

Fig. 3.2. Experimental fields at (1) MARDI station, Jalan Kebun, Klang and (2a, b, c) Olak Lempit, Banting, Selangor.



(a) Layout of Exp.1 (GA_3), 2 (2,4-D), 3 (NAA), 5 (H_2O_2) and 6 (GA_3 on physiological process) [Each experiment consists of 4 treatments with six replicates].



(b) Lay out of Exp.4 (Girdling) [six treatments with six replicates].

Fig. 3.3: (a) Experiment 1, 2, 3, 5 and 6 and (b) Exp.4.

The effect of different GA_3 concentrations on the growth and development and quality of wax apple fruits were investigated.

(a) Plant materials

Twelve years old wax apple air *var. jambu madu* trees were selected for the study. The trees were planted at 4.40 m × 4.40 m and received the same horticultural management. The trees were irrigated every 15 days with fertilizers and insecticides applied according to the recommendations of the State Agricultural Department. A total of 24 trees were used GA_3 treatments.

Fertilizer dose: Before flowering, 2.5 kg N: P: K: MgO (12:12:17:2) fertilizer per tree were applied to the experimental trees. At the same time 15 kg (1bag) compost of sawdust and decompose cow dung was also applied per tree.

Insecticide: Insecticide Cyper-Ten (10.5 % w/w) was applied to the trees twice a month from bud development until the growth and development. The spray of insecticide was stopped at the time of ripening of fruits.

(b) Treatment application

The selected uniform branches were sprayed with 20, 50 and 100 mg/L GA_3 and water (control) once a week at the beginning of flower opening until fruit maturation (Fig. 3.4). For each treatment, 250 ml hormone solution was used for thirty branches and at the time of hormone application the branches were separated with a black cloth to avoid hormone spreading to other branches via air current.

(C) Measurements of physiological parameter

(i) Number of buds

The total number of buds was determined when bud size (diameter) was 0.8-1.0 mm from the tagging branches of experimental trees. As mentioned before, all the tagging branches were of the same length and diameter and uniform number of leaves.

(ii) Bud drop (%)

To determine the bud drop percentage from tagged branches on the experimental tree, the numbers of buds were counted and the bud drop percentage was calculated using the following formula:

$$= \frac{\text{Total number of buds at initial stage} - \text{Buds before blooming}}{\text{Total number of buds at initial stage}} \times 100$$

(iii) **Fruit set (%)**

For the determination of fruit set percentage on the tagged branches on the experimental tree, the number of flowers and total number of fruitlets were counted before and after anthesis. Fruit setting percentage was calculated using the following formula

$$\text{Fruit set (\%)} = \frac{\text{Total number of fruitlets}}{\text{Total number of flowers}} \times 100$$

(iv) Fruit drop (%)

Fruit dropping percentage was determined from tagged branches on the experimental tree by counting the number of fruitlets and total number of fruits immediately after anthesis and 35 days after anthesis. Fruit drop percentage was calculated using the following formula:

$$= \frac{\text{Total number of fruitlets} - \text{Number of fruits in 35 days after anthesis}}{\text{Total number of fruitlets}} \times 100$$

(v) Fruit growth

In the MARDI orchard and Banting farm six 1-year branches were selected on each tree. The development of 12 fruits (2 fruits per tree) per treatment was monitored weekly from the day of treatment until harvest, measuring the length and diameter of each fruit on the selected branches using a vernier caliper.

(vi) Fruit weight (average)

Average fruit weight was determined by weighing 30 fruits from five sample branches per tree on a digital UWE-ESP electric balance and the average weight was calculated.

(vii) Peel color (%)

The surface color of each tagged fruit was determined at three different parts of the fruit (proximal portion, distal portion and middle portion) using a standard color chart (Minolta, Osaka, Japan) and expressed as percentage of color cover.

(viii) Yield and fruit dry biomass

Yield per treatment was recorded by weighing and counting the total number of fruits per branch at the time of harvesting. Yield was expressed as kg/ treatment. Fruit and dry biomass of pulp was determined by weighing pulp mass at 0% moisture.

(ix) Leaf characteristics (Leaf length, wide, fresh weight and drymatter content)

Leaf size (length and wide) were measured before the harvesting of fruits with measuring scale. Fresh and Dry weight (0% moister) were measured with a electronic balance.

(d) Measurements of biochemical parameter

Fruits of different treatments were randomly harvested from the selected outside branches at fully ripening stage during the first and second seasons from the experimental trees at the orchard. Fruits maturation was closely monitored and was determined in the field using the standard color chart of USDA (1991) depending on external color of wax jambu fruits. Harvesting was carried out manually early in the morning with care to minimize mechanical injury. Fully ripened fruits were kept in the refrigerator at 4ºC and 80-90% RH for biochemical analysis.

(i) Determination of chlorophyll a, b and carotenoid content

The weighed samples of wax apple were homogenized in 80% acetone (10 ml for each 0.25g), in a mortar and pestle. The homogenate was centrifuged at 2500 rpm for 10 minutes and filtered with a Whatman No.1 filter paper. The absorbance of the filtrate were read at 663 nm, 645 nm, and 480 nm using 80% acetone as the blank for zeroing the machine. The formulas used in the calculation of chlorophyll *a,* chlorophyll *b* and total carotene levels as follows,

Chlorophyll a concentration in mM = 12.7 x A663 - 2.69 x A645

Chlorophyll b concentration in mM = 22.9 x A645 - 4.68 x A663

(Where A663 and A645 are the values for absorbance at wavelengths 663 nm and 645 nm, respectively)

Carotenoid concentration in mM = (A480 + (0.114 x A663) - (0.638 x A645)) ÷112.5

The basis for this protocol was taken from Hendry and Price (1993).

(ii) Protein determination by Bradford's method

For protein extraction, 2 g of leaf samples were cut into small pieces with a scissor and ground in mortar with 5 ml of phosphate buffer (pH 7.6) and transfer in a centrifuge tube. The homogenate was centrifuged at 8000 rpm for 20 minutes and the clear supernatants of different samples were put in separate tubes. The volume of all the samples in a tube were then made equal volume by adding phosphate buffer solution and the extraction was stored in a refrigerator at 4°c for further analysis. 30 µl of leaf sample was taken in a separate tubes and mix with 70 µl of distilled water.

After that 2.9 ml Coosmassic Brilliant Blue solution were add and mixed thoroughly. The total volume was 3 ml and all the tubes were kept for incubated about 5 minutes at room temperature. The absorbance at 595 nm was recorded against the reagent blank. Protein content in the extracted leaf samples were determined from the standard curve and the protein in mg/L was calculated (Bradford, 1976).

(iii) Juice content

The fruit juice of each harvested fruit was extracted and weighed and the average juice weight was calculated separately for each treatment. The fresh juice was collected and weighed with an electronic balance. The average juice percentage per fruit was obtained from the following formula

$$\text{Percentage juice} = \frac{\text{Juice weight per fruit}}{\text{Fruit weight}} \times 100$$

(iv) Fresh biomass and dry matter

After collection of fruit juice, fresh biomass was weighed separately for each treatment with an electronic balance. Leaf and fruit dry matter were determined at 0% moister level.

(v) Total soluble solids (TSS) and pH

A small fraction of the homogenous mixture of wax apple was centrifuged at 4000 ×g for 10 min and the clear supernatant was analyzed for TSS and pH. TSS was evaluated at 25°C with an Atago 8469 hand refractometer (Atago Co. LTD., Tokyo, Japan) and expressed as °Brix. The pH was determined using a Hanna pH meter (Hanna Instruments).

(vi) Titrable acidity and TSS/TA ratio

A 5 mL sample of the wax apple juice prepared by direct homogenization was titrated with 0.1 N NaOH using phenophthalein as indicator. The end point of the reaction was pH 8.0. Titratable acidity of the juice was defined as % citric acid. Sugar acid ratio of the wax apple juice was expressed as TSS/TA ratio.

(vii) Total ascorbic acid content assay

Total ascorbic acid content determination was carried out using the method modified by Hashimoto and Yamafuji (2001). Five g of fresh cut wax apple pulp was homogenized with cold 5 % metaphosphoric acid and then filtered through cheese cloth. A 0.8 mL sample of filtrate was reacted with 0.4 mL of 2% di-indophenol, 0.4 mL of 2% thiourea and 0.4 mL of 1% dinitrophenol hydrazine. The mixture was incubated at 37 °C for 3 hr and then 2 mL of 85% sulphuric acid was added. The solution was left at room temperature for 30 min. The absorbance at 540 nm was then recorded. Total ascorbic acid content was expressed as g ascorbic acid per 100 g fresh weight.

(viii) Measurement of the K^+ content in fruit juice

The K^+ content of the fruit juice was determined by using a Cardy Potassium meter. Immediately after harvesting the fruits, 6 fruits were taken for K^+ determination from each treatment. One gram of fruit pulp was homogenized in 5 ml distilled water in a mortar and pestle and centrifuged at 4000 rpm for 10 min. Then 3 to 5 drops of the supernatant liquid were dropped onto the calibrated sensor pad (Cardy Potassium Meter, Model-2400, USA), on a sampling paper placed on the sensor. The reading in ppm was taken from the display pad after it stabilized (30 to 43 s).

(ix) **Determination of total sugars**

a. Preparation of sample

For total soluble sugars, one gm of fruit pulp was homogenized in 4 mL of 0.5 M of sodium hydroxide and ground in a motar and pestle and then centrifuged at 3,500 rpm for 20 min at 4°C. The pellet was discarded and the supernatant neutralized with 0.5 M acetic acid. The resulting solution was made up to 40 ml and stored at 4°C until use. These extracts were then used for the determination of total soluble sugars according to the phenol-sulphuric method by Dubois, (1956).

b. Total Sugar assay

One milliliter of the fruit juice was placed in a test tube and 1 ml of phenol [5% w/v] was added followed by 5 ml of concentrated sulphuric acid. Then the mixture was shaken and incubated in a water bath at 25-30° C. The absorbance was then determined in a spectrophotometer at 490 nm. The sugar concentration was obtained by referring to a standard glucose graph. The assay for this standard glucose graph was carried out by adding phenol and sulphuric acid to a standard glucose solution with concentrations

ranging between 0-100 μg ml^{-1}. Total soluble sugars was expressed in g/100 g fruit fresh weigh.

(x) Glucose, fructose and inverted sugar

A small fraction of the homogenous mixture of wax apple fruit was centrifuged at 4000 ×g for 10 min and the clear supernatant was analyzed for determination of soluble sugars. Glucose, fructose and inverted sugar were evaluated at 25°C with Atago 8469 hand refractometer for glucose, fructose and inverted sugar (Atago Co. LTD., Tokyo, Japan) and expressed as percentage. Six samples per treatment were used for soluble sugars analysis.

(xi) The total phenolic content (TPC)

The total phenolic content of wax jambu fruits were determined by using the Folin-Ciocalteu assay of Singleton and Rossi, (1965). An aliquot (1ml) of the extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L) was added into a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank using H_2O was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to a volume of 25 ml with double distilled water and mixed. After incubation for 90 min at room temperature, the absorbance against reagent blank was determined at 750 nm with an UV-Vis Lambda 5 Spectrophotometer. Total phenolic content of the fruits was expressed as mg gallic acid equivalents (GAE)/100g fresh weight.

(xii) Total flavonoid content (TFC)

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen *et al.,* 1999). An aliquot (1 ml) of the extracts or a standard solution of catechin (20, 40, 60, 80 and 100 mg/L) was added to a double distilled water in 10 ml volumetric

flasks containing 4 ml of double distilled water. To the flask was added 0.3 ml 5% $NaNO_2$. After 5 min, 0.3 ml 10% $AlCl_3$ was added. One minute later, 2 ml 1M NaOH was added and the total volume was made up to 10 ml with double distilled water. The solution was mixed well and the absorbance was measured against a reagent blank at 510 nm. The total flavonoid content of fresh fruit was expressed as mg catechin equivalents (CE)/100 g fresh mass. Samples were analyzed in triplicates.

(xiii) Antioxidant assays

a. DPPH assay

Antioxidant capacity was determined by using the radical 2, 2-diphenyl-1-picryhydrazyl (DPPH) as described by Tadolini *et al.* (2000). The stock solution was prepared by mixing 75 mg DPPH in 1 L methanol overnight. In the assay, 0.75 mL of the extract, standard (0–0.1 mmol Trolox) and blank (methanol) was mixed with 1.5 ml DPPH solution. The absorbance at 517 nm was determined after 5 min. For each extract, a blank with 1.5 ml methanol, instead of the DPPH reagent, was included to correct for any sample absorbance at 517 nm.

b. TEAC assay

Evaluation of antioxidant capacity was done following the method of Millar and Rice-Evans, (1997).

c. Preparation of the ABTS solution

A 7 mM solution of 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) in milli-Q (ultra pure water) was prepared and ABTS was formed after addition of potassium per sulfate to the mixture in a final concentration of 2.45 mM. After a 12–16 h incubation

at room temperature, the stock solution was diluted with phosphate buffered saline (*PBS*) until an absorbance of 0.7 (±0.02) at 734 nm was reached (Re *et al.,* 1999).

Solutions of antioxidants were prepared in ethanol. To exclude the influence of light, the solutions were prepared in the dark. To a fixed concentration of an antioxidant, ABTS in a variable concentration was added. The approximate concentration of ABTS varied from 0 to 45 μM in several different incubations. After 4 min incubation at 37°C, the absorbance at 734 nm was determined. For each extract, a blank with 1 ml ethanol; instead of the ABTS reagent, was included to correct for any sample absorbance at 734 nm The concentration of ABTS was calculated, using a molar extinction coefficient of 1.5×10^4 M^{-1} (Re *et al.,* 1999).

Total antioxidant capacity (TAC) was expressed as Ascorbic acid equivalent to antioxidant capacity (AEAC) mg/100g sample. AEAC was calculated using the following formula:

$$AEAC = \frac{\Delta A}{\Delta A_{AA}} \times C_{AA} \times V \times \frac{100}{w}$$

ΔA= Change of absorbance after addition of fruit extract

C_{AA}= Concentration of AA (ascorbic acid) standard solution (mg/ml)

ΔA_{AA}= Change of absorbance obtained from a calibration curve when the same volume of AA standard solution as that of fruit extract was added

V= The volume of fruit (ml)

W= The weight of homogenate used for extraction.

(xiv) Total anthocyanin content

Total anthocyanin content of the extracts was measured by the pH-differential method described by Rodriguez-Saona *et al.* (2001). Samples were diluted with two different solutions: potassium chloride (0.025 M), pH 1.0; and sodium acetate (0.4 M), pH 4.5. The pH was adjusted with concentrated hydrochloric acid. Samples were diluted to give an absorbance of <1.2 at 530 nm. Diluted samples were held for 15 min before measuring the absorbance. The absorbance was measured at 530 nm and 700 nm with distilled water as a blank. The absorbance difference between the pH 1.0 and pH 4.5 samples was calculated as follows:

$$A= (A_{530nm}-A_{700nm})_{pH1.0}- (A_{530nm}-A_{730nm})_{pH\ 4.5}$$

The monomeric anthocyanin pigment concentration was calculated using the following equation:

Monomeric anthocyanin pigment (mg/L) = $(A\times MW\times DF\times 1000)/ (\varepsilon \times 1)$

MW= 449.2, ε = 26,900 and DF= Dilution factor

3.2.2 Experiment 2. Effects of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) on growth and development of wax apple

The effects of 2, 4-D on wax apple fruit growth and development of wax apple fruits were investigated under field conditions. Fruit growth, fruit drop, color development and yield were evaluated as well as some selected leaf physiological and chemical characteristics.

(a) Plant materials

Twenty four (24) trees were selected for 2, 4-D experiment. Plant spacing and all the horticultural management (Fertilization, irrigation and insecticide application) were similar to the previous experiment (3.2.1). Experiment consists of randomized complete block design (RCBD) with six replicates.

(b) Treatment application

Selected uniform branches were sprayed with 5, 10 and 20 mg/L 2, 4-D and water (control) once in a week at the beginning of flower opening until fruit maturation as above (Fig.3.4).

(C) Measurements fruit growth parameters

(i) Number of bud, bud drop, fruit set, fruit drop, average fruit weight and peel color

All the fruit growth and yield contributing characters **(**number of bud, bud drop, fruit set, fruit drop and average fruit weight) were determined as the same procedure described earlier (3.2.1 c).

(ii) Chlorophyll content (SPAD value)

The chlorophyll content in the leaves was recorded using a Minolta SPAD. The SPAD value of the leaves was determined at the fruit development stages 3 weeks after anthesis. Thirty readings were recorded per treatment.

(iii) Chlorophyll fluorescence

Leaves of selected uniformed branches were used for chlorophyll fluorescence determination between 12.00 to 2.00 pm on a sunny day (PAR 800-1500 µE m^{-2} s^{-1}) during the fruit developmental stage. Chlorophyll fluorescence was determined using a Plant

Efficiency Analyzer (Hansatech Instruments Ltd., England). A leaf clip was attached to the leaf and kept in the dark for at least 10 min minutes for dark adaptation to take place. Then the shutter plate was opened and light was applied on the leaf. The fluorescence signal was determined for 3 second and the quantum yield or photosynthetic yield determined as Fv/Fm. The maximum fluorescence (F_m) and minimum fluorescence (F_0) was read from the display pad of the Plant Efficiency Analyzer and the variable fluorescence (F_v) calculated as F_m-F_0. The quantum yield was determined according to the equation F_v/F_m.

(iv) Firmness of fruits

Flesh firmness was determined in the middle of the fresh fruit using TA-XT II, Texture analyser, Stable Microsystem England, equipped with P/2 probe (2 mm diameter). The result was expressed as the maximum force-penetrated (kg).

(v) Stomatal conductance

Stomatal conductance of treated leaves measured with a leaf porometer model SC-1 and was expressed as mmol $m^{-1}s^{-1}$. Measurements were carried out on a sunny at PAR (800-1500 µE m^{-2} s^{-1}) between 11.00 am and 2.00 pm during the fruit developmental period. A leaf clamber was attached to one of the leaf and kept in ambient temperature for 10 to 15 min to maintain sunlight adaption. A stomatal conductance was measured in 3 replicates from different spot of a single leaf.

(d) Measurements fruit biochemical parameters

Fruit quality parameters such as; total soluble solid (TSS), total sugar and anthocyanin content were determined as described earlier 3.2.1.d (iii, vii & xii).

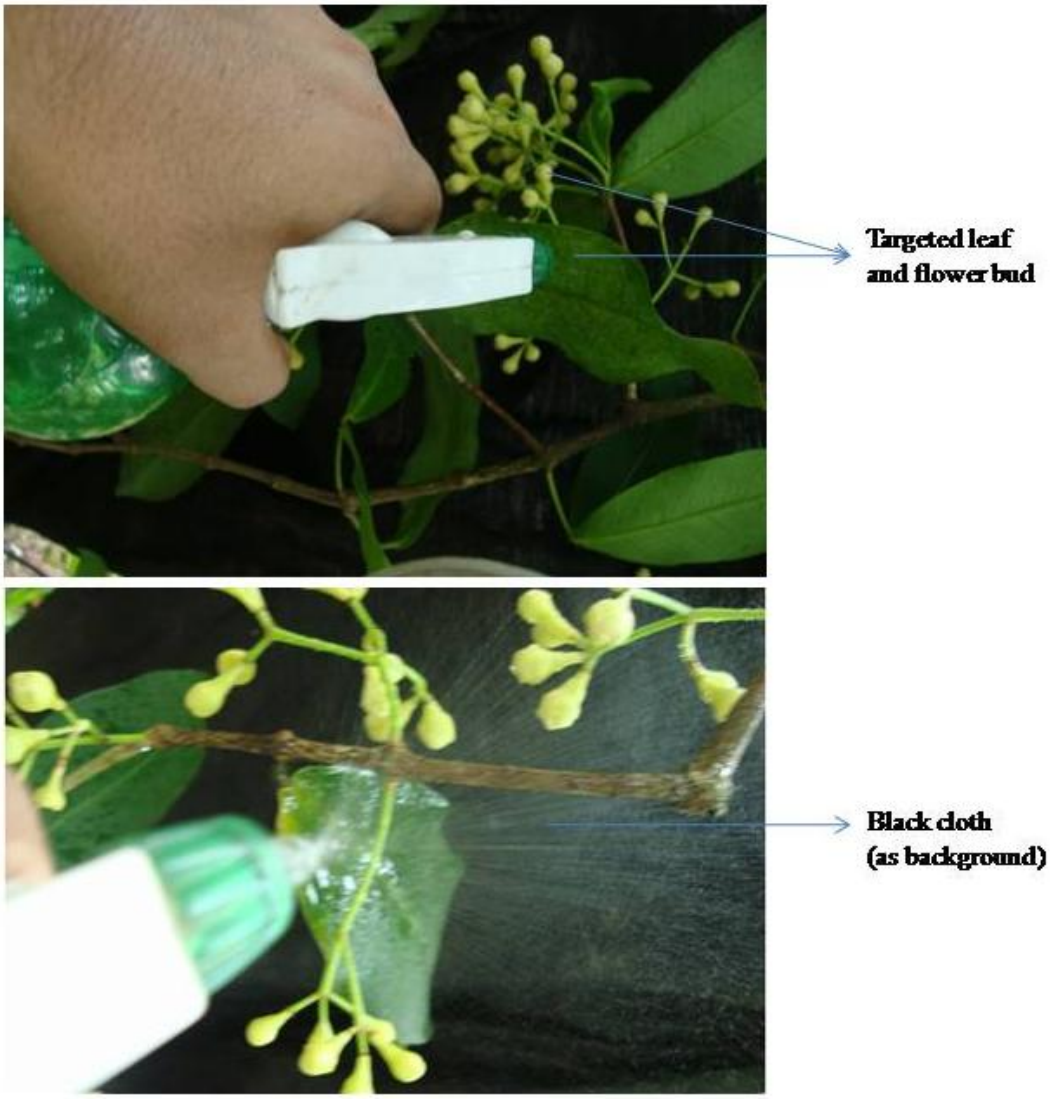


Fig. 3.4. Protected spray of growth regulators and hydrogen peroxide.

3.2.3 Experiment 3. Effects of naphthalene acetic acid (NAA) on growth, development and quality of wax apple

(a) Plant material: For NAA experiment, 24 trees were selected for each year, with the same horticultural management, irrigation and fertilization (3. 2. 1).

(b) Treatment application

For the application of NAA, the branches were sprayed with 5, 10 and 20 mg/L NAA and water (control) once in a week at the beginning of bud development until fruit maturation as same with GA_3 application (Fig. 3.4).

(c) Measurement of fruit growth and development characteristics

All the fruit growth and yield contributing characteristics (number of bud, fruit drop, fruit set, fruit drop, color development, yield, chlorophyll fluorescence and stomatal conductance) were measured using the methods described earlier (3.2.1 c).

(d) Determination of nutritional quality parameters

Fruit quality parameters (TSS, total sugar, juice K^+ content, phenol, flavonoid, anthocyanin and soluble protein content) were determined using the methods described earlier (3.2.1d)

(e) Phenylalanine ammonia lyase (PAL) enzyme assay

Enzyme extraction

The (1.0 g) fruit pulp paste was homogenized for 30 s in chilled 0.05 M potassium phosphate buffer (25 ml; pH 6.6) with 0.2 g of Triton-X using a polytron homogenizer. Polyvinyl polypyrrolidone (25 mg) was added and the suspension centrifuged at 4°C for 15

min at 25,900 g. The supernatant was stored on ice after filtering through glass wool and was used as a source of crude enzyme. L-phenylalanine ammonia lyase activity in the crude enzyme extracts were assayed by the method of Zucker (1965).

Assay

The assay mixture consisted of 0.06M sodium borate buffer (4.1 ml; pH) and crude enzyme (0.4ml) and the reaction was initiated by addition of 1 ml of a solution of L-phenylalanine (10mg/L; final concentration 11 mM). Tubes were incubated at 37°C for I hour. The reaction was stopped by addition of 35% (w/w) trifluoro acetic acid (TFA) (0.5 ml) and tubes were centrifuged for 5 min at 5000g to precipitate the denatured protein. The cinnamic acid was estimated by measuring the absorbance at 290 nm of the supernatant in 1 cm quartz cuvettes. Triplicate assays were performed for each extract, both with and without substrate in order to compensate for increases in absorbance even in the absence of added L-phenylalanine. L- phenylalanine ammonia lyase enzyme activity was expressed in nmol cinnamic acid yield of mg protein per min (nmol-cinnamic acid min^{-1} mg $protein^{-1}$). The molar extinction coefficient used for cinnamic acid was 19.207.

3.2.4 Experiment 4. Effect of different type of girdling on growth, development and quality of wax apple

The effect of different girdling techniques on the growth and development and quality of wax apple fruits were investigated.

(a) Plant material: For girdling experiment 36 trees were selected for each year, using the similar experimental tree selection and horticultural management (fertilization, irrigation and insecticide application) (3.2.1)

(b) Treatment application

Different girdling techniques namely, I-25% shaped, I-50% stress, 100% stress, V-shaped and C-shaped girdling were applied three weeks before flowering. To set I-25%, I-50% and 100% girdling treatment on the branches, 75%, 50% and 100% of the phloem tissue was removed, respectively (Fig. 3.5). For the C and V-shaped girdling techniques, the bark was carved a C and V-shape on the treatment branches. Girdling was performed using a girdling knife which simultaneously cuts and removes the bark strips. The width of the girdle was 5 mm. In all cases, the cut reached the cambium and was left bare without injury to the inner layer. Partial ringing (C and V) was done by removing a partial ring 4 cm long and leaving a connecting strip 5 mm of C and V shape on the main stem. In the case of complete bark girdling, the bark was removed in ring fashion around the stem, without any phloem connection left. All the girdling was carried out 3 weeks before flowering.

(c) Measurements of physiological characteristics

i. Inflorescence development

Inflorescence development of the tagged branches were monitored twice weekly. Observations were made at three-day interval. The number of days required for the full development of the inflorescence for each treatments branches and control were recorded.

ii. Fruit retention (%)

For the determination of fruit retention (%) from the tagged branches on the experimental tree, the number of fruits 7 days after anthesis and the number of fruits before harvesting was determined and calculated as follows:

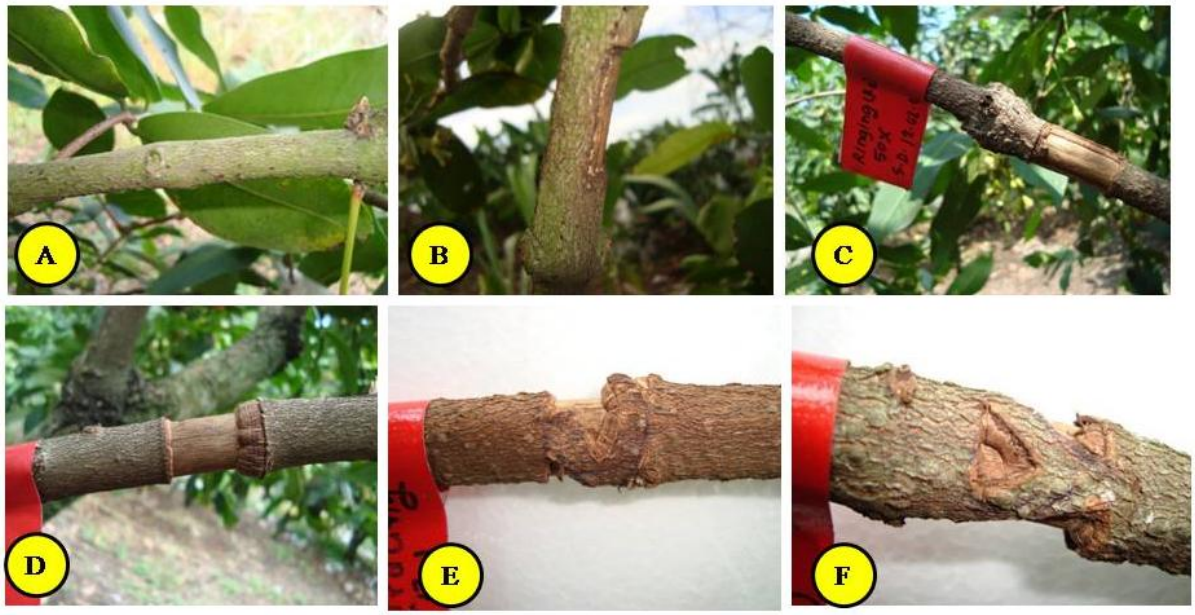


Fig. 3.5. Different girdling techniques were performed in this study. A: Control, B: I-25%, C: 50 %, D:100 %, E: C-shaped, and F: V- shaped girdling.

$$\text{Fruit retention (\%)} = \frac{\text{Number of fruits before harvest}}{\text{Number of fruits 7 days after anthesis}} \times 100\ \%$$

iii. Fruit growth and yield contributing characteristics

All the fruit growth and yield contributing characteristics (bud number, fruit set, fruit drop, fruit growth, peel color, fruit size, yield, chlorophyll fluorescence, photosynthetic yield, and dry matter of leaf and fruit were measured the methods described earlier (3.2.1. c)

(iv) Fruit size

Fruit size was determined by measuring the length and diameter of harvested fruits per treatment randomly with Vernier caliper.

(v) Determination of nutritional quality parameters

Fruit quality parameters (TSS, total sugar, juice K^+ content, phenol, flavonoid, anthocyanin and soluble protein content) were determined using the methods described earlier (3.2.1d)

3.2.5 Experiment 5.The influence of hydrogen peroxide on growth, biochemical and phytochemical properties of wax apple

In this group of experiments, the effects of hydrogen peroxide on physiological and biochemical properties of wax apple fruits were investigated under field conditions. The photosynthetic characteristics, fruit growth and development, color development, yield, biochemical, phytochemical and antioxidant properties were determined. Finally, correlation between physiological and biochemical parameter also carried out.

(a) Plant material: For hydrogen peroxide experiment 24 trees were selected for each year. Experimental tree selection and horticultural management (fertilization, irrigation and

insecticide application) were similar to the previous experiment (3.2.1). The experiments were arranged in randomized complete block design (RCBD) with six replicates.

(b) Treatment application

In this experiment the leaves, flowers and young small fruits of selected uniform branches were sprayed with 5, 20 or 50 mM H_2O_2 and water (the control) once each week from the beginning of flower opening through fruit development (Fig. 3.4). The hydrogen peroxide solution was freshly prepared for two hours before treatment application and a different sprayer were used for each treatment to avoid contamination. A total of eight spraying times were carried out; two times before anthesis and six times after anthesis and 450 ml H_2O_2 solution was used per treatment (thirty branches). It takes ten weeks from bud development to fruit ripening and all the fruits were harvested eight week after anthesis. All the harvested fruits were kept in a refrigerator at 4°C prior to biochemical analysis.

(C) Fruit growth and developmental characteristics

All the fruit and developmental characteristics (bud number, bud drop, fruit set, fruit drop, color development, fruit growth and yield) were measured as the methods described as earlier (3.2.1.c)

(d) Measurement of photosynthetical characters (net photosynthetic rate, transpiration and stomatal conductance)

To measure the activity level of photosynthetic carbon metabolism, the photosynthetic activity in terms of µmol CO_2 fixed m^{-2} s^{-1} was determined. Photosynthesis rate was measured using portable photosynthesis system (LI 6400, Li-COR, U. S. A) equipped with a 6400-02 LED light source. Measurements were made on leaves of treatment branches.

Net photosynthesis rate (*Pn*), stomatal conductance ($g_s w$), and transpiration (E) were measured at 1 day after treatment application. 24 leaves were selected for photosynthesis measurement. Photosynthesis of treated leaves were measured at 9.00 am to 1.00 pm (PAR at 800-1500 µE $m^{-2}s^{-1}$. Before measuring, the cuvette chamber conditions were set to provide photosynthetic photon flux density of 400, 800, 1200 and 2000 µmol m^{-2} s^{-1} and cuvette block temperature was maintained at 24°C, and concentration of the CO_2 was set at 350 µmol mol^{-1} with a flow rate of 500 mL s^{-1}. Humidity levels of the reference and sample chambers were set at 30 g kg^{-1}. Stomatal ratio was set at 0.5 and measurements made on a leaf of treated branch of wax jambu tree. The chamber was attached to a leaflet, the photosynthesis allowed to stabilize and the data recorded.

(e) Determination of fruit biochemical and phytochemical characteristics

Fruit biochemical and phytochemical characteristics (K^+ content, TSS, total sugar, total phenol, flavonoid, anthocyanin and carotenoid) were determined according to the methods described earlier (3.2.1.d)

(f) Phenylalanine ammonia lyase (PAL) activity

PAL enzyme extraction and assay was determined using the methods as mentioned earlier (see 3.2.3 e)

3.2.6 Experiment 6. Effect of GA_3 on selected physiological process of wax apple (*Syzygium samarangense*)

In an effort to understand the effects of the PGR, in particularly GA_3, which produced the most promising results, several physiological parameters were selected for

further study. These include PAL activity, anthocyanin formation, photosynthetic activity and SPS activity of wax apple.

(a) Plant material

To see the effects of GA_3 on selected physiological process of wax apple trees 24 trees were selected. All the horticultural managements (fertilization, irrigation and insecticide application) were the same as the previous experiment (3.2.1). Experiment six were carried out in one season in Banting, Selangor

(b) Treatment application

For the experiments involving the use of GA_3 hormones, the selected uniform branches were sprayed with 20, 50 and 100 mg/L of GA_3 and the control with water. The branches were sprayed once in a week. Two hundred fifty milliliter hormone solutions were used for each treatment. Spraying was performed at light intensity of 800-1500 $\mu E\ m^{-2}\ s^{-1}$ and a separate sprayer was used for each concentration and hormone used to avoid contamination.

(C) Measurement of physiological parameter

(i) Photosynthetical characteristics

Net photosynthesis, transpiration and stomatal conductance of treated plant measured as described earlier (3. 2. 5. d).

(ii) Sucrose phosphate synthase (SPS) activity

Enzyme extraction

Treated wax apple leaf samples (0.5 g) were homogenized in 2 mL of ice-cold 50 mM MOPS–KOH buffer (pH 7.3), 5 mM $MgCl_2$, 1mM ethylene di -amine tetra acetic acid (EDTA), 16 mM mercaptoethanol, 0.1% (v/v) TritonX-100, 10% (v/v) glycerol, 2 mM benzamidine, 1 mgL^{-1} leupeptin and 2 mM phenylmethyl-sulfonyl fluoride. The crude extract (0.8 mL) was desalted by centrifugal gel filtration using a 4 mL Sephadex-G25 (Pharmacia) column equilibrated with 50 mM MOPS–KOH (pH7.3), 5 mM $MgCl_2$, 1mM EDTA, 16 mM mercaptoethanol and 10% (v/v) glycerol. The desalted crude extract was used for enzyme analysis and protein content was determined by Bradford (1976) method using bovine serum albumin as standard. SPS activity was assayed under V_{max} condition as described by Huber *et al.* (1989).

Assay

For the V_{max} assay, 45 µl of desalted extract was added to 50 mM MOPS-NaOH, 15 mM $MgCl_2$, 2.5 mM DTT, 10 mM UDP-glucose, 10 mM fructose-6-P, and 40 mM glucose-6-P at pH 7.5 in a total volume of 70 µl. The V_{lim} assay consisted of the same mixture except that 10 mM Pi was added and the concentrations of UDP-glucose, fructose-6-P, and glucose-6-P were reduced to 2, 2 and 10 mM, respectively. The reactions were terminated after 10 min at 30°C with 70 µl of 1 n NaOH and boiled for 10 min to degrade un reacted fructose-6-P. After cooling, 0.25 ml of 0.1% (w: v) resorcinol in 95% ethanol and 0.75 ml of 30% (v: v) HCL were added and the tubes were incubated at 80°C for 8 min. After cooling for 5 min, the absorbance was read at 520 nm. Blanks were run in parallel using the complete assay reaction mix with enzyme denatured by boiling. Two separate extractions of leaf powder were each assayed three times. The sucrose phosphate synthase (SPS) activity were expressed in µ mol sucrose mg^{-1} protein h^{-1}.

(iii) Phenylalanine ammonia lyase (PAL) activity and anthocyanin content

PAL enzyme extraction and assay was determined as mentioned earlier (see 3.2.3 e). Anthocyanin content of treated fruits was determined as the method described earlier (3.2.1.d xii)

3.2.7 Statistical analysis

The experimental designs for all experiments were randomized complete block design (RCBD) with six replications. The data obtain from three seasons were pooled (except exp.3.2.5 hydrogen peroxide application) and analyzed using MSTAT-C statistical software. The one way ANOVA was applied to evaluate the significant difference of the parameter studied in the different treatments. Least significant difference (Fisher's protected LSD was calculated, following significant F-test ($p = 0.05$) except girdling treatments. Tukey's test (HSD) was used to compare treatments of girdling when ANOVA showed significant differences among mean.

CHAPTER 4

RESULTS

4.1. Effects of GA_3 on growth, development and biochemical properties of wax apple (*S. samarangense)* fruits.

The effects of GA_3 on physiological and biochemical properties of wax apple were observed and their results are reported below. The correlation between selected physiological and biochemical properties of wax apple fruits are herewith presented.

4.1.1 Number of buds and bud drop

As shown in Table 4.1, 50 mg/L GA_3-treated branches produced the highest number of buds amounting to about 60 buds per branch. Control branches showed the lowest number of buds, in the region of 49 buds per branch. The difference was statistically significant between the treatments and control. With regard to bud drop, our results showed that GA_3 treatments did not produced significant effect on reducing bud drop (Table 4.1).

4.1.2 **Fruit set and fruit drop**

Data in Table 4.1 shows that fruit setting was almost 2.6 times more in 50 mg/L GA_3 treated branches compared to control branches. All the GA_3 treated branches posted significantly higher fruit set values compared to the control which recorded about 27 % fruits set per branch. As can be seen in Table 4.1, control branches showed the highest number of fruit dropped (52%), with the least percentage of fruit drop observed (32%) in 20 mg/L treated branches followed by 50 and 100 mg/L GA_3 treatments.

Table 4.1: Effects of different treatments of GA_3 on number of buds, bud dropping, fruit setting and pre harvest fruit dropping and yield of wax apple fruits.

Treatment (mg/L)	Number of Buds	Bud drop (%)	Fruit set (%)	Fruit drop (%)	Yield (kg/treatment)	Average fruit weight (g)
Control	49^{b}	36^{a}	27^{c}	52^{a}	0.31^{d}	45^{b}
GA_3 20	55^{a}	29^{a}	35^{c}	32^{b}	0.40^{c}	49^{b}
GA_3 50	60^{a}	35^{a}	69^{a}	45^{b}	1.02^{a}	63^{a}
GA_3 100	59^{a}	35^{a}	49^{b}	48^{b}	0.79^{b}	52^{a}

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

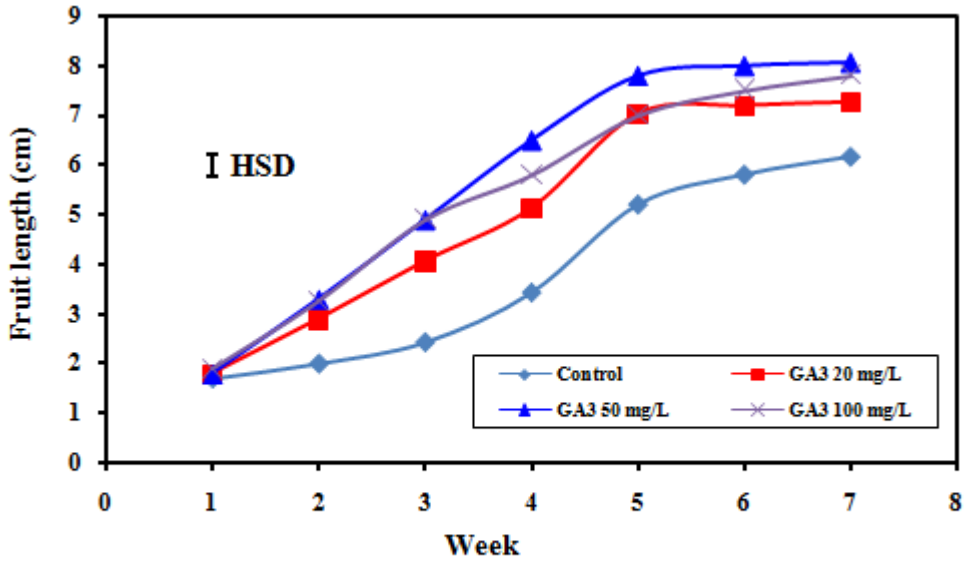


Fig. 4.1. Fruit growth (length) of wax apple as influenced by different treatments of GA_3 (n = 12).

All the GA_3 treated branches posted statistically significant higher fruit set values compared to the control.

4.1.3 **Fruit growth (length and diameter)**

The results showed that all the GA_3 treated branches exhibited higher fruit growth rate from the first week till the 7th week, with regard to fruit length and diameter compared to the control branch (Figs. 4.1, 4.2 and 4.3). At the 3rd week of observation, fruit length was 4.90 cm and 5.0 cm in the 50 and 100 mg/L GA_3 treatments whereas it was 2.43 cm in the control fruits. Fruit diameter was 3.26 and 3.06 cm in 50 and 100 mg/L GA_3 treated fruits, respectively, whereas it was 1.76 cm in control fruits. This growth trend was observed throughout the whole fruit developmental period until the harvesting period (Figs. 4.2 and 4.3.)

From the results, it can be seen that all the treated fruits grew at a faster rate and were larger than the untreated control fruits. At the 3rd and 7th week of observation, fruit growth (length and diameter) was found to be significant between the treatments and control. Finally, GA_3 treatments increased the fruit size and yield of wax apple (Fig. 4.5 a, b, c).

4.1.4 GA_3 on color development

Figure 4.4a shows that fruit color development was greatly enhanced by the GA_3 treatments used in this study, with the 50 and 100 mg/L GA_3 treated fruits exhibiting the greatest percentage color cover from day 14 till 28. Furthermore, it was observed that on day 14 after anthesis the red color of the fruits had already started to show in the treated branches compared to the control fruits, which only started coloring one week later. On the 28th day of observation, the 50 mg/L treated fruits showed more or less 95% red color whereas, control was only 35%.

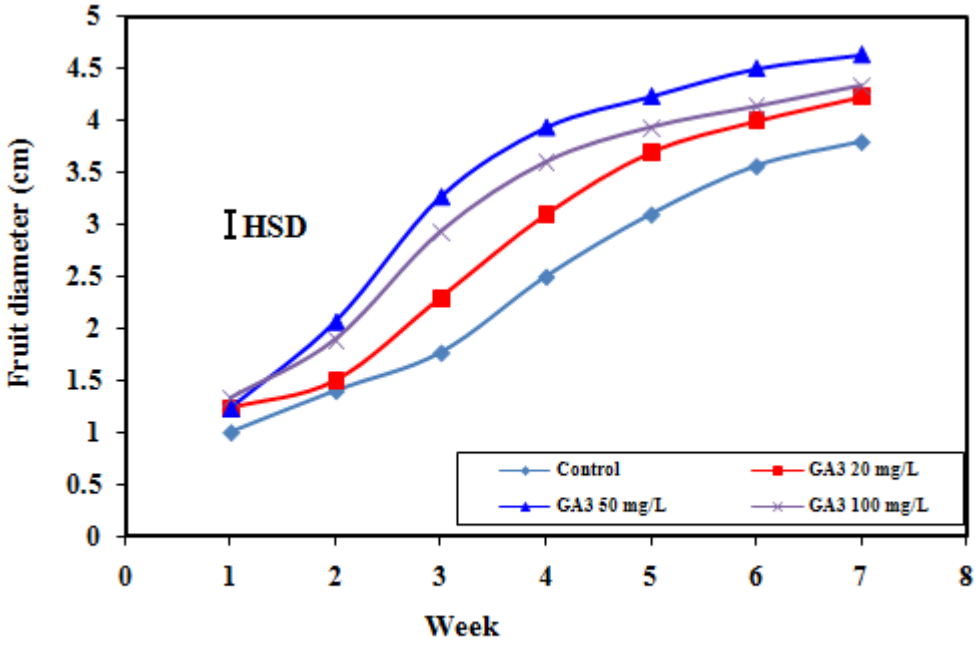


Fig. 4.2. Fruit growth (diameter) of wax apple as affected by different treatments of GA_3 (n = 12).

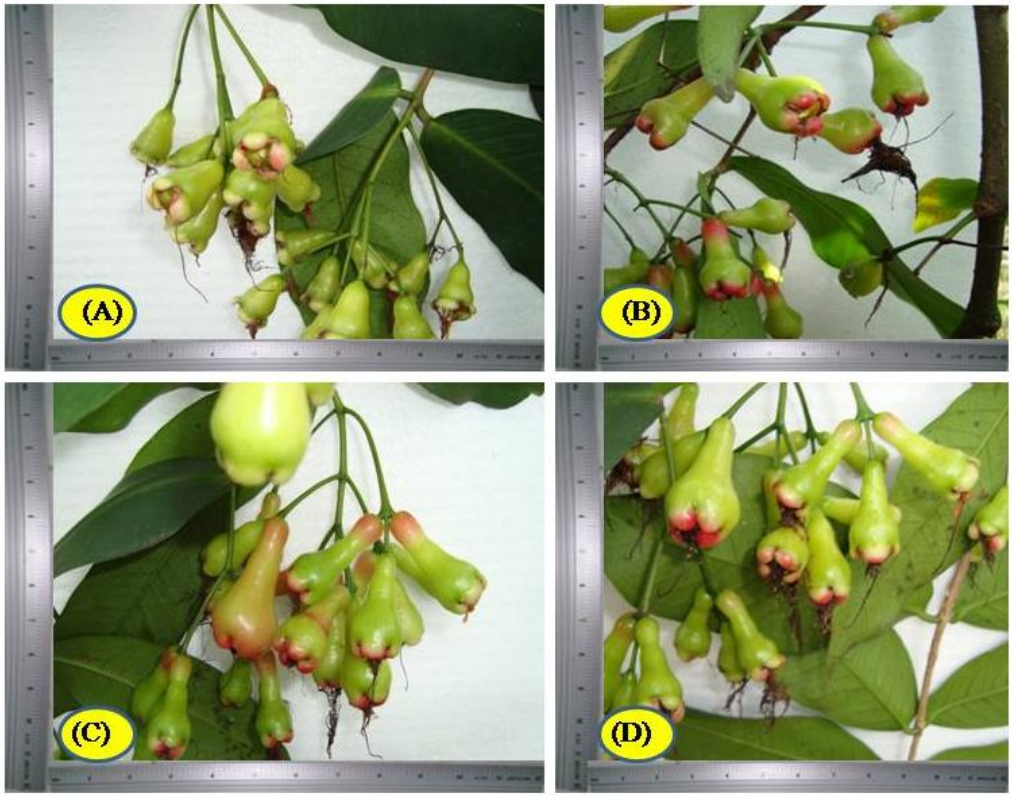


Fig. 4.3. Fruit growth of wax apple at one week after anthesis as affected by GA_3 treatments, (A): Control, (B): 20 mg/L, (C): 50 mg/L and (D): 100mg/L GA_3.

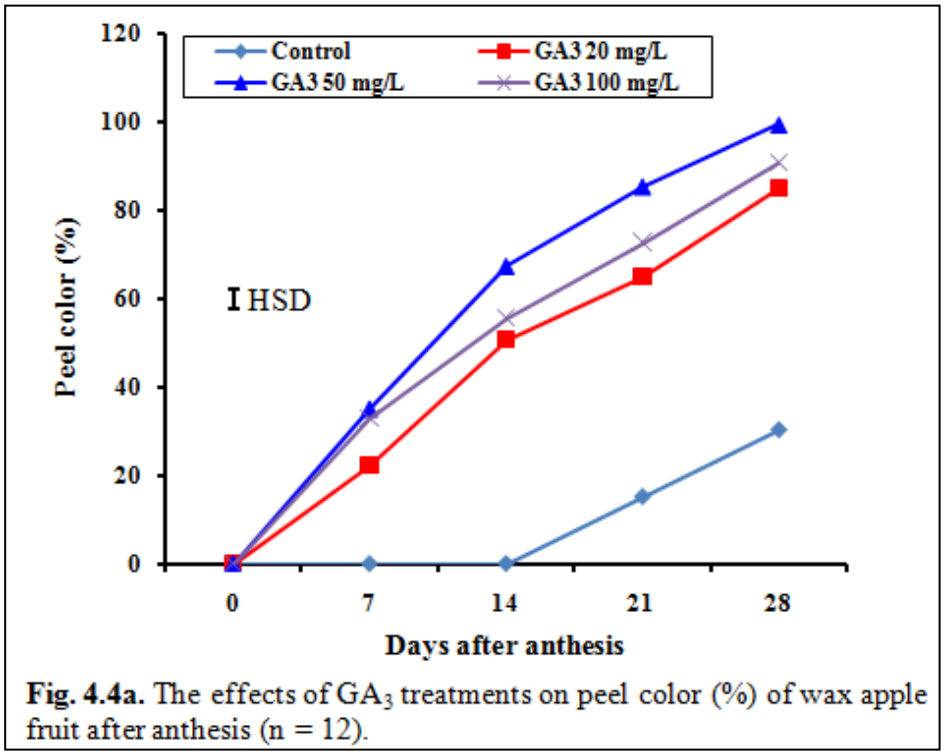


Fig. 4.4a. The effects of GA_3 treatments on peel color (%) of wax apple fruit after anthesis (n = 12).

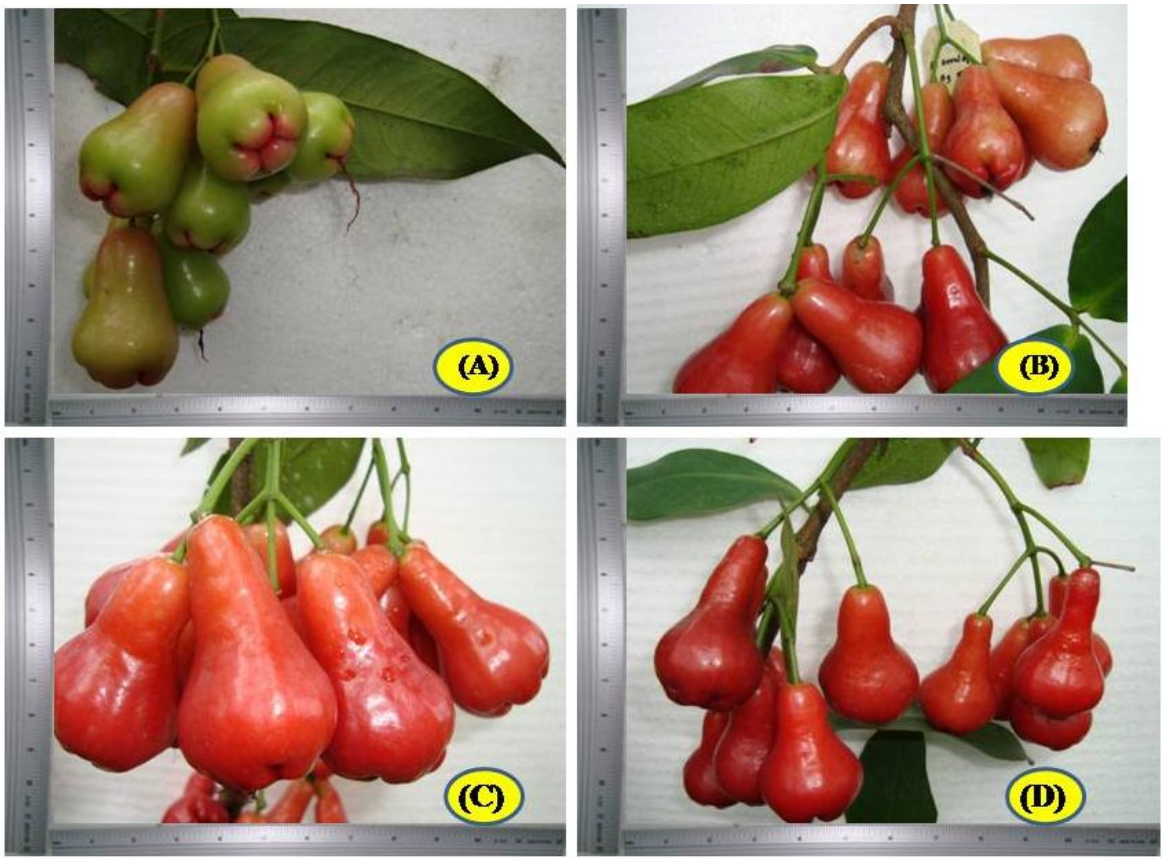


Fig.4.4b. Fruit size and color development of wax apple at three week after anthesis. (A): Control, (B): 20 mg/L, (C): 50 mg/L, and (D): 100 mg/L GA_3 treatments.

Fig. 4.4c. Color development of wax apple fruits at four week after anthesis. (A): Control, (B): 20 mg/L, (C): 50 mg/L GA3 and (D): 100 mg/L GA_3 treatments.

Fig. 4.5a. Fruit size of wax apple as affected by GA_3 treatments. (A): Control, (B): 20 mg/L, (C): 50 mg/L and (D): 100 mg/L treatments.

From the graph (Fig. 4.4 b) it can be seen that a significant difference ($p < 0.05$) was observed in peel color development between different GA_3 treatments and control.

4.1.5 **Yield and average fruit weight**

As shown earlier in Table 4.1, all the GA_3 treated branches in this study yielded higher fruit weight than the untreated control. The yield, on a fruit weight basis, was almost more than 3 times higher in the treated branches compared to the control (Table 4.1). From the results, it can be seen that 50 mg/L GA_3 treated branch produced the highest yield followed by the 100 and 20 mg/L treatments. The differences were was found to be statistically significant ($p < 0.05$) between the treatments and control (Fig. 4.5).

4.1.6 GA_3 on leaf size, weight and quantum yield

It is observed that GA_3 treatments increased the leaf size (length) significantly from the control treatment (Table 4.2). Results showed that the highest leaf length was in 100 mg/L GA_3 treatment followed by 50 and 20 mg/L GA_3 treatment, whereas the lowest leaf length was recorded in the control treatment. GA_3 treatments also had a significant effect on leaf fresh weight (Table 4.2). The highest leaf weight was recorded in GA_3 treatments, whereas control leave registered the lowest leaf weight.

From the Table 4.2, it could be seen that the highest quantum yield (0.88) was recorded in 50 mg/L GA_3 treatment, while, control leaves recorded the least of 0.82. The difference between 50 mg/L GA_3 treatment and other treatments and control was significant ($p < 0.05$) (Table 4.2).

Fig. 4.5b. Fruit size of wax apple as affected by GA_3 treatments. (A): Control, (B): 20 mg/L, (C): 50 mg/L and (D): 100 mg/L GA_3.

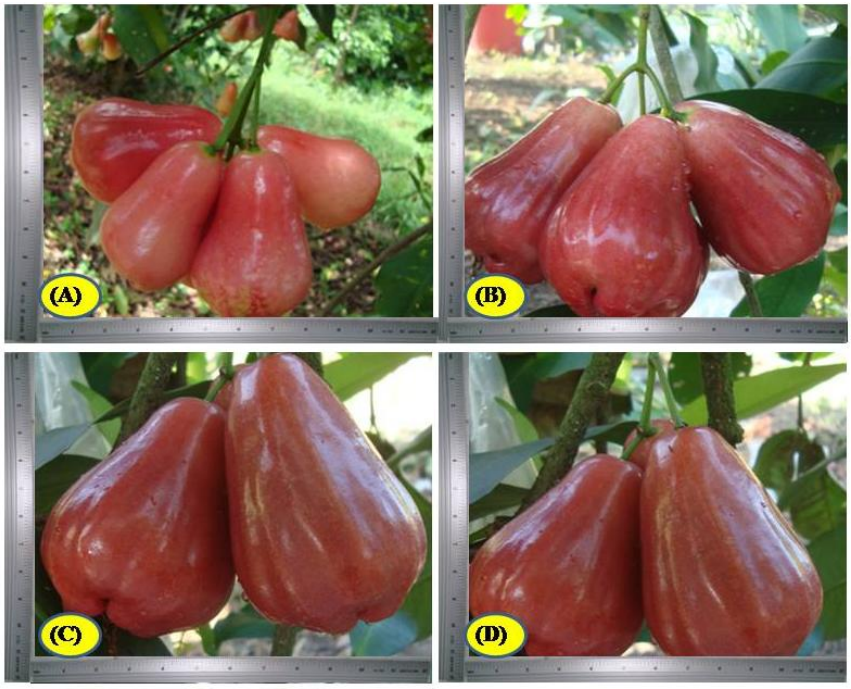


Fig. 4.5c. Fruit size of wax apple as affected by GA_3 treatments. (A): Control, (B): 20 mg/L, (C): 50 mg/L and (D): 100 mg/L GA_3.

4.1.7 **Chlorophyll content in leaf and fruit**

The chlorophyll values was found the highest in the 20 mg/L GA_3 treated branches, followed by control and 50 mg/L GA_3, whereas the GA_3 100 mg/L treated branches showed the lowest value (Table 4.2). In the case of total chlorophyll, this difference was statistically significant. GA_3 treatments also showed an significant effect on fruit chlorophyll content (Table 4.3). The highest chlorophyll content in fruit was observed in the control treatment followed by 20 and 100 mg/L GA_3 treatments, whilst, the lowest chlorophyll content was recorded in the 50 mg/L GA_3 treatment (Table 4.2).

4.1.8 GA_3 on soluble leaf protein

Protein content was determined in selective leaves of the treated branches. From the results, it can be seen that gibberellin treatment had a significant effect on leaves soluble protein content. It was discovered that 50 mg/ L GA_3 treated leaves produced the highest soluble protein content (7.54 mg/g) followed by 100 and 20 mg/L GA_3 with a value of fruits 7.36 and 7.21 mg/g FW, respectively, whereas, control produced the lowest amount of soluble protein content (6.76 mg/g FW) (Fig. 4.6).

4.1.9 Fruit juice

Fruit juice content, which is related to fruit size is an extremely important parameter in industrial processing of fruits. Fruit size in turn depends on genetic characteristics and cultural practices such as application of plant growth regulators. Table 4.3 shows that the highest amount of juice, 81 ml/100g, was observed in 50 mg/L GA_3 treated fruits, whereas, the lowest amount of 69 ml/100g of juice was found in the control treatment. These values were found statistically significant between the treatments and control. Fruit biomass was also significantly affected by different treatments and control.

Table 4.2 The effects of GA_3 on leaf size, weight and Chlorophyll fluorescence of wax apple

Treatment (mg/L)	leaf size (L) (cm)	Leaf weight (g)	Leaf chlo. *a* (mg /L)	Leaf chlo. *b* (mg /L)	Total chlo. *a*+*b* (mg/L)	Quantum yield Fv/Fm
Control	19.3[c]	3.10[b]	3.27[a]	2.28[a]	5.56[a]	0.82[b]
GA_3 20	21.3[b]	4.11[a]	3.72[a]	2.41[a]	6.14[a]	0.85[ab]
GA_3 50	21.7[b]	4.09[a]	3.28[a]	2.50[a]	5.46[a]	0.88[a]
$GA_3$100	22.3[a]	4.01[a]	2.84[b]	1.92[a]	4.77[b]	0.84[b]

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

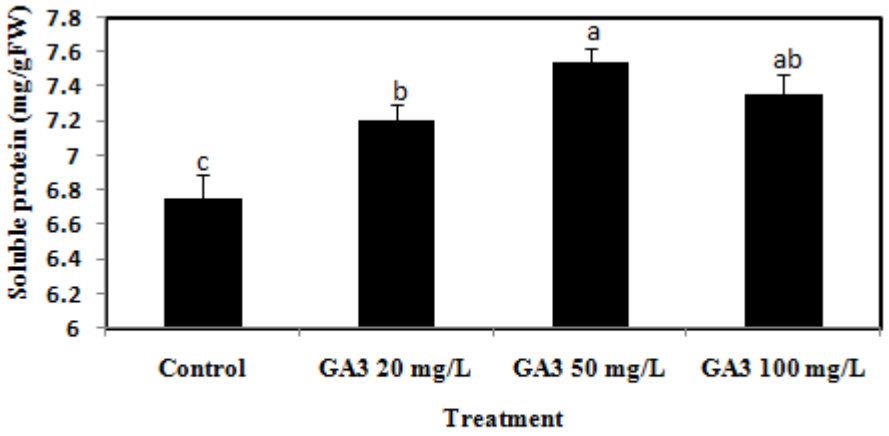


Fig. 4.6. The effect of GA_3 treatments on soluble protein (mg/g FW) of wax apple leaves. Values (n = 6) followed by the same letter were not significantly different at $p < 0.05$.

Results showed that, fruit biomass was 45%, 24% and 14% higher in 50, 100 and 20 mg/L GA_3 treated fruit, respectively, than the control.

4.1.10 pH of fruit juice

From the results presented in Table 4.3 it can be seen that the pH value of the fruit juice was significantly different between the treatments and control. Results showed that the highest pH value was observed in the 20 mg/L GA_3 treatment, while, the lowest pH value was recorded in the control treatment (Table 4.3).

4.1.11 K^+ content and total soluble solids (TSS)

Results showed that the K^+ content of the fruit juice was the highest in 20 mg/L treated fruits, whereas, the control fruits produced the lowest value. It was found significantly differences in treated and non-treated fruits (Table 4.3).

Total soluble solids (TSS) is considered an important fruit quality parameter. Significant variations between the fruits of the different GA_3 treatments were recorded with respect to TSS content in the fruit pulp. As can be seen in Table 4.4, the highest TSS value (11.88 °Brix) was observed in 50 mg/L GA_3 treated fruit, while, the lowest TSS (5.63) was recorded in control treatment (Table 4.4). The differences between the various GA_3 treatments were not significant but between the control and the GA_3 treatments it was significantly different.

4.1.12 Total soluble sugar

As pointed out in the literature review chapter, it is well known that plant growth regulators can play a role in increasing the sugar content in fruits.

Table 4.3: Effects of different treatments of GA_3 on leaf chlorophyll, fruit juice and pH of wax apple fruits.

Treatment (mg /L)	Juice (ml/100g)	Fruit biomass (fresh)	Chlorophyll fruit juice (mg /L)	pH	K^+ content (mg/kg)
Control	69^b	16.60^d	0.63^a	4.92^b	15.3^b
GA_3 20	80^a	18.90^c	0.44^b	5.29^a	31.3^a
GA_3 50	81^a	24.14^a	0.24^c	5.17^a	22.0^a
GA_3 100	80^a	20.60^b	0.26^c	5.15^a	28.0^a

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

Table 4.4: Effects of different treatments of GA_3 on TSS (% Brix), total sugar, flavonoids and total phenols, anthocyanin and carotenoids content in wax apple fruits.

Treatment (mg/L)	TSS (°Brix)	Total sugar (g /100g pulp)	Flavonoids (mg/100g)	Total phenols (mg GAE/100g)	Carotenoid (μg/g)	Anthocyanin (mg/L)
Control	5.63^b	3.32^b	21.5^c	311^c	5.97^c	2.43^c
GA_3 20	10.76^a	6.16^a	24.4^b	589^b	10.58^b	4.02^b
GA_3 50	11.88^a	6.57^a	36.9^a	535^b	11.32^a	5.60^a
GA_3 100	10.50^a	6.21^a	24.0^b	752^a	10.35^b	4.60^b

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

In this study, as shown in Table 4.4, fruits of different treated branches produced a significant difference in total sugar content. The highest sugar content of 6.57 g/100g was recorded in 50 mg/L GA_3 treated fruits, whereas, control fruits showed the lowest sugar content of 3.32 g/100g. Here again the differences between the various GA_3 treatments were not significant but between the control and the treated fruits it was significant.

4.1.13 Total flavonoids, total phenols, carotenoid and anthocyanin content

This experiment was carried out to correlate flavonoid with color development and antioxidant activity with the presence and content of phenols and flavonoids in the fruits. However as reported below, the relationship between these compounds and the two parameters was not a straightforward one. From the results it was observed that the treated fruits produced significant differences in flavonoid content than the control. As can seen from the Table 4.4, 50 mg/L GA_3 treated fruits recorded the highest (36.95 mg/100g) flavonoid content, followed by 20 and 100 mg/L GA_3 treatments, while, the control fruits produced the lowest flavonoid content value.

Furthermore the application of different concentrations of GA_3 had a significant effect on the total phenolic content of the wax apple fruits (Table 4.4). Fruits from 100 mg/L GA_3 treated branches exhibited the highest amount (752 mg GAE/100g) of phenolic content followed by the 20 and 50 mg/L treated fruits with values of 589 and 435 mg GAE/100g respectively. Control fruits showed significantly ($p < 0.05$) lower (311 mg GAE/100g) phenolic content. The application of various treatment of GA_3 had a significant effect on the carotenoid and anthocyanin content of wax apple fruits (Table 4.4). Results showed that carotenoid content of wax apple fruit increased by 90%, 77% and 73%, respectively, with 50, 20 and 100 mg/L GA_3 treatments than the control.

4.1.14 Glucose, fructose and sucrose content

In addition to the total soluble sugar content, we also studied the effect of the GA_3 treatment on the glucose, fructose and sucrose content. It was observed that the GA_3 treatments had a significant effect on glucose, fructose and sucrose content in the wax apple fruits. As can be seen in Table 4.5, the highest glucose content of 7.93 % was observed in 50 mg/L GA_3-treated fruits followed by 100 and 20 mg/L GA_3 treatments with a glucose content of 7.76 % and 7.05 %, respectively, whilst, the lowest glucose content was seen in the control fruits (5.6 %).

In the case of fructose, the 50 mg/L GA_3 treatment produced the highest amount of fructose, followed by 100 and 20 mg/L GA_3 treatments, whereas control treatment produced the lowest amount of fructose. Similarly sucrose was also the highest in the 50 mg/L GA_3 treatment followed by 100 and 20 mg/L GA_3, respectively, with the control recording the lowest value (Table 4.5). All these differences were statistically significant.

4.1.15 Titrable acidity and TSS and Acidity ratio

In these set of experiments, results showed that the highest titrable acidity was observed in control fruits (0.78 % citric acid) followed by the 20 and 50 mg/L GA_3 treatments with a acidity content of 0.74 and 0.71 (%) respectively, whereas, lowest titrable acidity (0.7%) were found in the 100 mg/L GA_3 treated-fruits. However these differences were not statistically significant (Table 4.5).The sugar acid ratio (TSS/TA) was significantly different between the different GA_3 treatments and control.

Table 4.5: Effects of different treatments of GA_3 on glucose, fructose, sucrose, TA, TSS/TA and vitamin-C content of wax apple fruits.

Treatment	Glucose	Fructose	inverted	Titrable acidity	TSS/acidity	Vitamin-C
(mg/L)	(%)	(%)	(%)	(% citric acid)	ratio	(mg/100g)
Control	5.61[c]	5.73[c]	6.25[c]	0.78[a]	8.67[d]	5.10[c]
GA_3 20	7.05[b]	7.08[b]	7.37[b]	0.73[b]	14.45[c]	5.96[b]
GA_3 50	7.93[a]	8.36[a]	8.12[a]	0.71[b]	16.65[a]	6.60[a]
GA_3 100	7.76[a]	8.18[a]	7.93[a]	0.70[c]	15.60[b]	5.90[b]

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

Results showed that the highest sugar acid ratio (16.65) was observed in 50 mg/L GA_3 treated fruits followed by 100 and 20 mg/L GA_3 treatments with a sugar acid ratios of 15.6 and 14.45 respectively, whereas, the control posted the lowest sugar acid ratio (8.67) (Table 4.5)

4.1.16 Ascorbic acid content

An increase in ascorbic acid content was observed in treated fruits. The different GA_3 treatments significantly affected the ascorbic acid content of fruits (Table 4.5). The highest ascorbic acid content (6.6 mg/100g) was found in the 50 mg/L GA_3 treatment followed by 20 and 100 mg/L GA_3 treatments with an ascorbic acid content of 5.96 and 5.9 mg/100g, respectively, whereas the control treatment yielded the lowest amount (5.1 mg/100g) of ascorbic acid content.

4.1.17 Color versus TSS content of fruit and total phenolics versus antioxidant activity

As shown in Figure 4.7, it was observed that fruit peel color had a strong correlation ($r = 0.97$) with flavonoid content of fruit. From the results with 50 mg/L GA_3 treatment, it was observed that fruit peel color correlated with its flavonoid content and increased simultaneously with peel color of fruits. Figure 4.8 shows the relationship between antioxidant capacity and the phenolic content of the *S. samarangense* fruits studied. A high correlation between the total phenolic content and DPPH measurements was observed ($r = 0.93$).

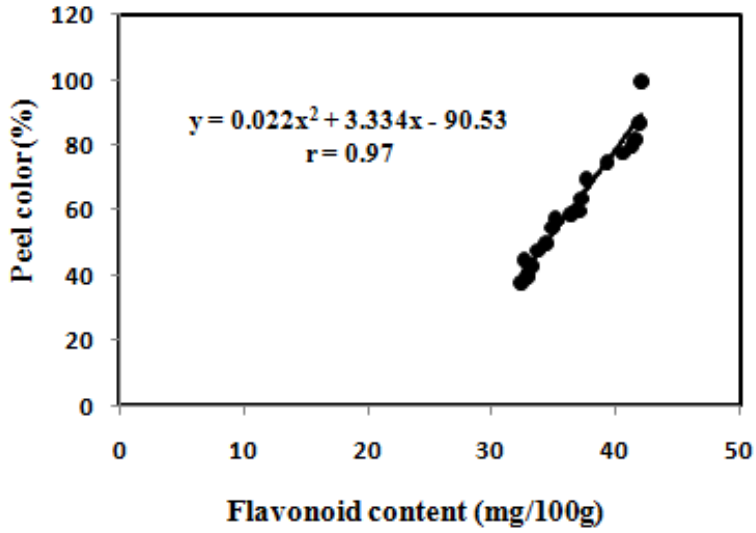


Fig. 4.7. Correlation between peel color and flavonoid content of wax apple fruits as affected by 50 mg/L GA_3 treatment.

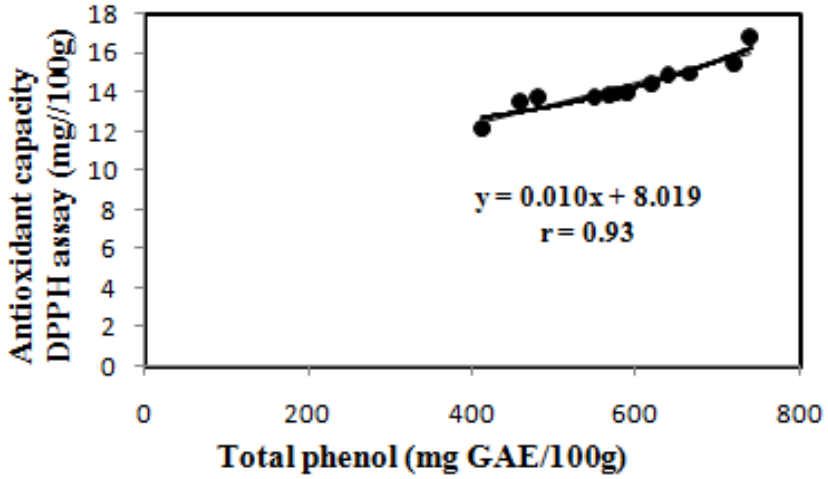


Fig. 4.8. Correlation between antioxidant capacity and total phenolic content of wax apple fruits as affected by 50 mg/l GA_3 treatment.

4.2 Effects of 2, 4-D on growth and development of wax apple fruits

4.2.1 Bud development, bud drop and fruit set

As shown in Table 4.6, there was no significant difference in bud number between the treatments and control. On the other hand, treatment of 2, 4-D produced a significant difference ($p < 0.05$) on bud drop in the wax apple fruits (Table 4.6). The highest bud drop (45%) were recorded in the 20 mg/L 2,4-D treatment followed by 10 mg/L 2, 4-D and control treatments with a value of 43 and 35%, respectively, whilst, the least (32%) bud drop observed in 5 mg/L 2, 4-D treatment.

With regard to fruit set, it was not found significant difference ($p < 0.05$) among the treatments and control.

4.2.2 Fruit drop and average fruit weight

Plant growth regulators (PGR's) are known to have a great influence on fruit drop and fruit retention in fruit trees. The use of auxins has been shown to prevent fruit drop by maintaining the cells at the zone of abscission, preventing the synthesis of hydrolytic enzymes such as cellulase, which decompose the cell wall. In this study, the application of 2, 4-D treatment had a significant ($p < 0.05$) effect on fruit drop in wax apple fruits (Table 4.6). Results revealed that control branches showed the highest (40%) fruit drop followed by 10 and 20 mg/L 2,4-D treatment with a fruit drop of 26 and 25%, respectively,

whereas, 5 mg/L 2, 4-D treatment showed the least percentage of fruit drop (18%). The application of 2, 4-D treatment also significantly ($p < 0.05$) increased the fruit weight as compared with control.

Table 4.6 Effects of different treatments of 2, 4-D on number of buds, bud drop, fruit set, pre harvest fruit drop and yield of wax apple

Treatment (mg/L)	Bud number	Bud drop (%)	Fruit set (%)	Fruit drop (%)	Av. fruit wt. (g)	DM (g/leaf)	Chlorophyll (SPAD)
Control	49[a]	35[b]	18[a]	40[a]	32[c]	1.12[b]	54[c]
2, 4-D 5	53[a]	32[b]	27[a]	18[c]	46[a]	1.44[a]	71[a]
2, 4-D 10	56[a]	43[a]	31[a]	26[b]	42[a]	1.66[a]	70[a]
2, 4-D 20	52[a]	45[a]	29[a]	25[b]	38[b]	1.41[a]	62[b]

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

As can be seen from the Table 4.6 average fruit weight was the highest (46 g) in 5 mg/L 2,4-D treated branches followed by the 10 and 20 mg/L 2,4-D treatments, whilst, the control treatment produced the lowest fruit weight (32 g).

4.2.3 Leaf drymatter and chlorophyll content

All the treated branches in this study exhibited higher leaf dry matter content in the treated branches compared to the untreated control (Table 4.6). The highest leaf drymatter (1.66 g) was recorded in the 10 mg/L 2, 4-D treated branches, whereas, the control treatment produced the least amount of drymatter (1.12 g). The difference between the treatments and control was statistically significant. Leaf chlorophyll content is another important factor that reflects plant health and productivity. The chlorophyll readings in leaves from all the 2, 4-D treatments were significantly higher than the control, up to 31 % higher, in the 5 mg/L 2, 4-D treated branch branches (Table 4.6).

4.2.4 Fruit growth (length and diameter)

The 2, 4-D-treated branches exhibited higher fruit growth rate from the first week till the 7^{th} week, with regard to fruit length and diameter (Figs. 4.9 and 4.10). At the 3^{rd} week of observation, fruit length was 3.5 cm and 3.2 cm in 10 and 5 mg/L 2, 4-D treatments, respectively, whereas, it was 2.6 cm in the control. At the 3^{rd} week of observation, fruit diameter, were 2.63 and 2.43 cm in 10 and 5 mg/L 2, 4-D treated fruits, respectively, whereas it was 2.13 cm in control fruits (Figs. 4.10, 4.11a, b and c). This growth trend was observed throughout the whole fruit developmental period until fruit maturation. All the treated fruits grew at a significantly ($p < 0.05$) faster rate and were significantly larger than the untreated control fruits (Figs. 4.11 b & c) from the 3^{rd} to the 7^{th} week of observation.

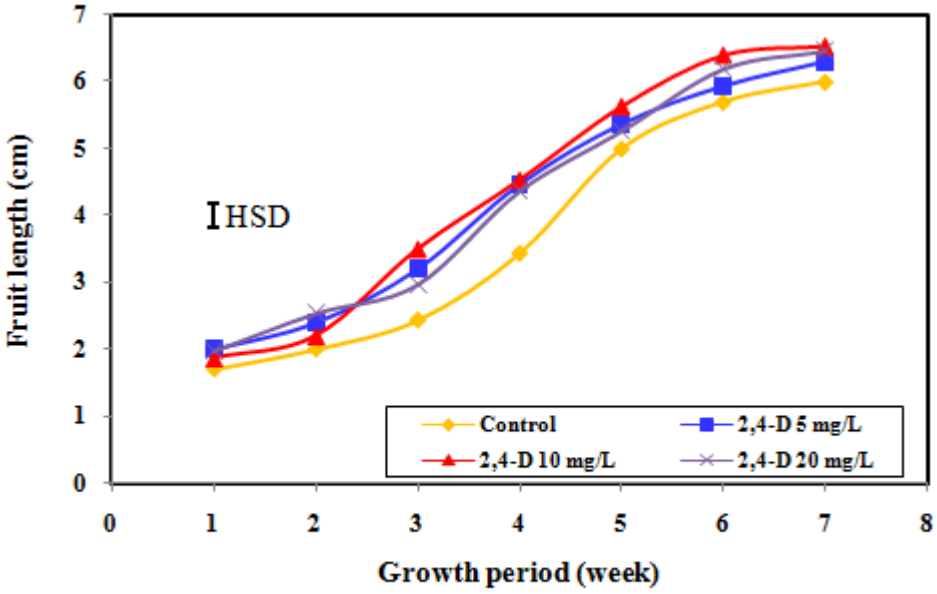


Fig. 4.9. Fruit growth (length) of wax apple as influenced by different treatments of 2,4-D (n = 12).

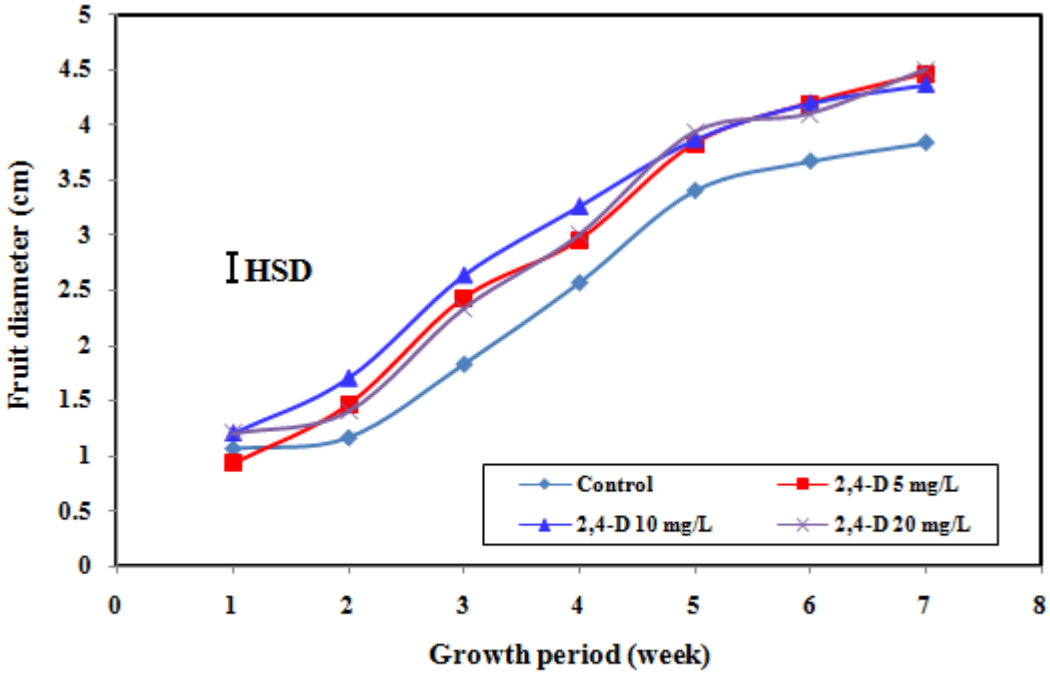


Fig. 4.10. The effects of 2,4-D treatments on fruit growth (diameter) of wax apple (n = 12).

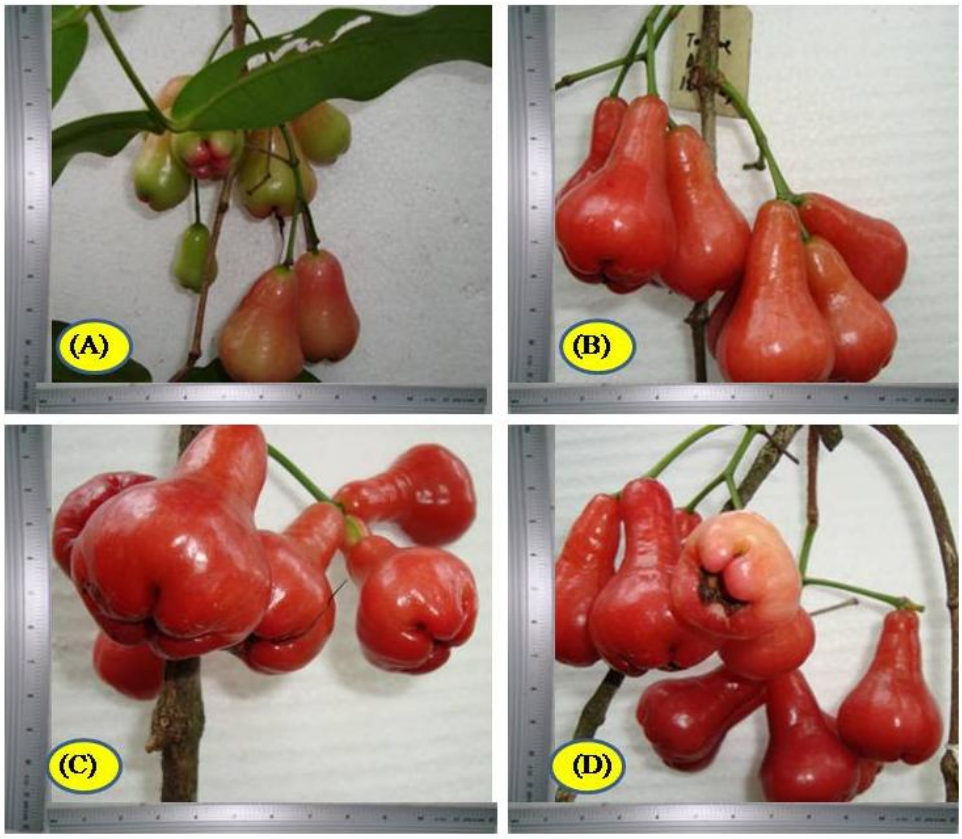


Fig. 4.11a. The effect of 2, 4-D treatments on fruit growth and color development of wax apple. (A): Control, (B): 5 mg/L, (C): 10 mg/L and (D): 20 mg/L 2, 4-D.

Fig. 4.11b. The effect of 2, 4-D treatments on fruit size of wax apple. (A): Control, (B): 5 mg/L, (C): 10 mg/L and (D): 20 mg/L 2, 4-D.

4.2.5 Chlorophyll fluorescence and photosynthetic yield

Chlorophyll fluorescence has recently become an important tool and a widely used technique available to plant physiologist and ecophysiologist looking at the various aspects of plant growth and development. Chlorophyll fluorescence provides information about the state of photosystem II and has been used shown to be correlated with photosynthetic yield.

Treatments of 2, 4-D had a significant effect on chlorophyll fluorescence (Fig.4.12). The highest maximum fluorescence (Fm) was observed in 5 mg/L treatment followed by 10 mg/L and 20 mg/L 2,4-D treatment respectively, whilst, control produced the lowest Fm value. The highest ground state fluorescence (F_0) was also observed in the 5 mg/L treatment. Relative variable fluorescence (Fv) was the highest in 5 mg/L 2,4-D treatment followed by 10 and 20 mg/L treatments respectively, whereas, control leaves exhibited least fluorescence (Fig.4.12).

The highest optimum quantum yield (Fv/Fm) (0.83) was recorded in 5 mg/L 2, 4-D treated leaves, whilst, the control showed the least quantum yield (0.71) (Fig.4.13). The difference between the treatments and control was found statistically significant.

4.2.6 Stomatal conductance and fruit yield

Stomatal conductance affects the photosynthetic rate by regulating CO_2 fixation in leaf mesophyll tissue and this in turn affects on accumulation of drymatter content. In this study, 2, 4-D had a significant effect on stomatal conductivity of treated leaves. Results showed that the highest stomatal conductivity (0.05 mol H_2O m^{-2} s^{-1}) was observed 5 mg/L 2, 4-D treatments followed by the 10 and 20 mg/L 2, 4-D treatment with a value of 0.047 and 0.040 mol H_2O m^{-2} s^{-1}, respectively, whereas, the control showed the least stomatal conductance (0.022 mol H_2O $m^{-2}s^{-1}$) (Fig.4.14).

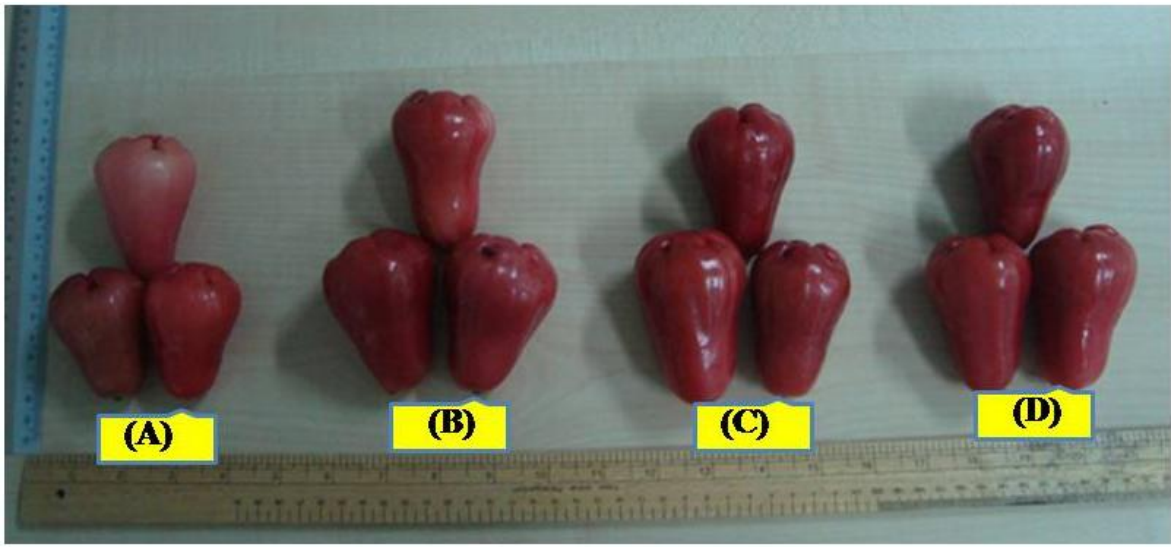


Fig. 4.11c. The effect of 2, 4-D treatments on fruit size of wax apple. (A): Control, (B): 5 mg/L, (C): 10 mg/L and (D): 20 mg/L 2, 4-D.

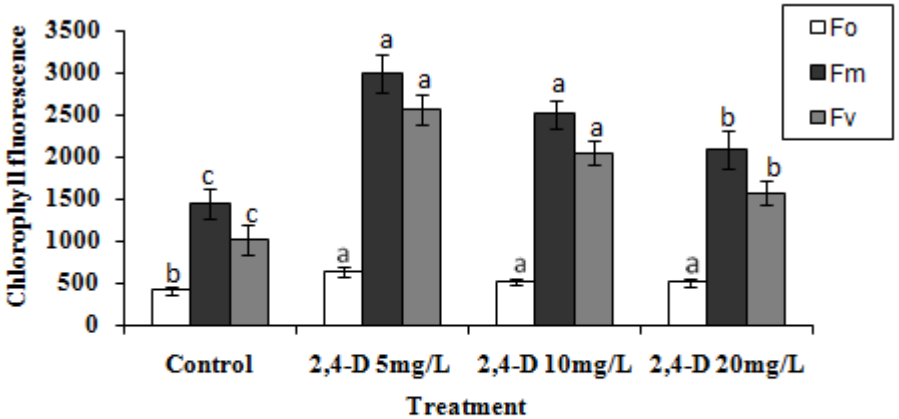


Fig. 4.12. Chlorophyll fluorescence intensity as affected by 2,4-D treatments. F_0= Lower fluorescence, Fm= Higher fluorescence and Fv= Relative variable fluorescence. Bars indicate ± S.E. and values (n = 30) followed by the same letter were not significantly different at $p < 0.05$.

With regard to fruit yield, results showed that all the treated branches exhibited a higher yield compared to control. The yield, on a fruit weight basis was almost 1.54 times higher in the fruits from the 5 mg/L treated branches compared to the control.

The 5 mg/L 2, 4-D treated branches produced the highest yield, followed by the 10 and 20 mg/L treatments, whilst, the control was the lowest. The results were found to be statistically significant between the treatments and control (Fig.4.14).

4.2.7 Total soluble solids (TSS) and peel color development

The TSS content of the fruit juice was found to be statistically significant ($p < 0.05$) between 2, 4-D treatments and the control. The highest TSS value (10.00 °Brix) was observed in 10 mg/L 2, 4-D treated fruit, while, the lowest TSS (5.63 °Brix) was recorded in the control fruit juice (Fig. 4.15). The application of 2, 4-D treatments also had a significant ($p < 0.05$) effect on peel color development of fruits. The highest peel color development was recorded in 10 mg/l 2, 4-D treated fruit followed by 5 and 20 mg/L treatments respectively, whilst control fruits showed the least color developed (Fig.4.15).

4.2.8 Color at 7th week, pulp firmness and anthocyanin content

The 2, 4-D treated fruits became completely red on the 7th week of observation, when, the control fruit was only 62% red (Table 4.7). The pulp firmness of wax apple fruits was significantly ($p < 0.05$) affected by 2, 4-D application, showing that pulp firmness increased with 2, 4-D application (Table 4.7). In relation to this 2, 4-D treatment had a significant ($p < 0.05$) effect on the anthocyanin content of wax apple fruits (Table 4.7).

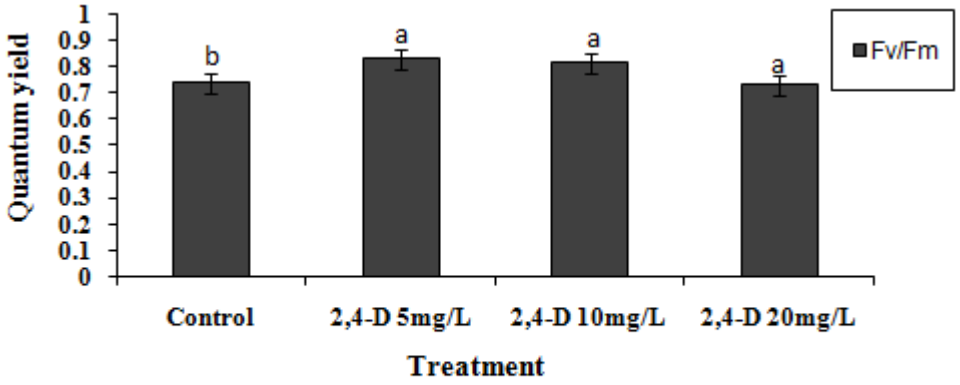


Fig. 4.13. Photosynthetic or quantum yield (Fv/Fm) as affected by treatments of 2,4-D. Bars indicate ± S.E. and values (n = 30) followed by the same letter were not significantly different at $p < 0.05$.

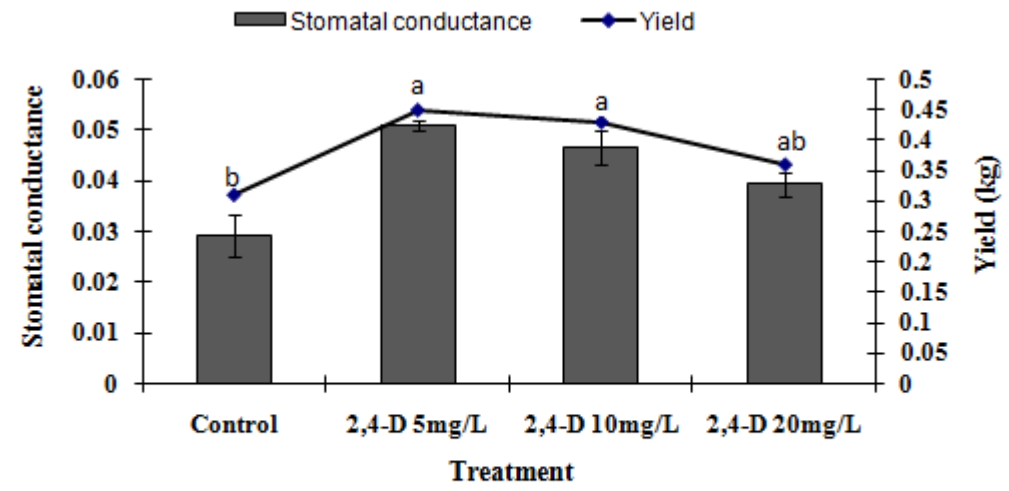


Fig. 4.14. Stomatal conductance (nmol m^{-2} S^{-1}) and yield (kg/treatment) as affected by 2,4-D treatments. Bars indicate ± S.E. and values (n = 30) followed by at least one same letter were not significantly different at $p < 0.05$ (LSD test).

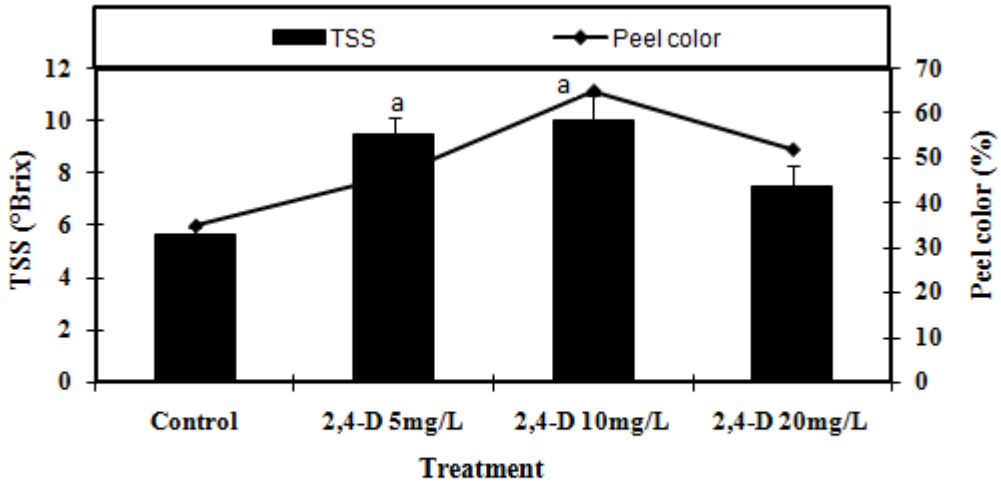


Fig. 4.15. TSS (°Brix) and peel color (%) as affected by 2,4-D treatments in wax apple fruits. Bars indicate ± S.E. and values (n = 12) followed by same letter were not significantly different at $p < 0.05$ (LSD test).

Table 4.7 Effects of different treatments of 2, 4-D on colour development, anthocyanin and total sugar content of wax apple fruit.

Treatment (mg/L)	Color at 7th week	Pulp firmness (N)	Anthocyanin (mg/L)	Total sugar (g/100g pulp) 1st year	2nd year	3rd year
Control	62[b]	6.5[c]	1.43[d]	2.54[b]	3.32[b]	3.95[b]
2, 4-D 5	95[a]	6.9[a]	2.00[b]	5.15[a]	5.83[a]	5.85[a]
2, 4-D 10	100[a]	7.0[a]	2.80[a]	5.20[a]	5.86[a]	5.90[a]
2, 4-D 20	100[a]	6.7[b]	1.80[c]	5.49[a]	5.81[a]	5.62[a]

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

The highest amount of anthocyanin (2.80 mg/L) was observed in 10 mg/L treated fruits followed by 5 and 20 mg/L 2, 4-D treatments with a value of 2.00 and 1.83 mg/L, respectively, whereas control fruits showed significantly ($p < 0.05$) least amount of anthocyanin (1.83 mg/L) (Table 4.7).

4.2.9 Total sugar content

With regard to total sugar content, treatments of 2, 4-D did not produced significant differences among themselves, whereas, between the treatments and control these were statistically significant ($p < 0.05$) in all three years (Table 4.7). In the first year, the total sugar content was 104% higher in 10 mg/L treated fruits compared with control.

4.3 **Effects of NAA on growth, development and quality of wax apple fruits.**

The effects of NAA on wax apple fruit growth, development and quality were also observed. Results of different quality parameters of wax apple are reported below

4.3.1 Total number of buds, fruit set, and fruit drop

5 mg/L NAA-treated branch produced 26% higher bud than the control treatment, followed by 10 and 20 mg/L NAA treatments, respectively. The differences were statistically significant at 5% level among the treatments and control (Table 4.8). Fruit set was almost 1.74 fold in 20 mg/L NAA-treated branches compared to those in the control (Table 4.8).The control branches produced the lowest fruit set. It was found statistically significant between the treatments and control. Regarding fruit drop, NAA treatments reduced the fruit premature fruit drop, although, their differences were not statistically significant (Table 4.8).

4.3.2 Leaf chlorophyll content

As shown in Table 4.8 the chlorophyll content, which can indirectly indicate the health status of a plant, determined using a Minolta SPAD meter, was slightly higher in the leaves of NAA-treated branches. The chlorophyll content in leaves from all the treated branches were higher than the control branch, up to 33% higher, in the 10 mg/L treated branch branches. However, the differences were not significant (p <0.05) (Table 4.8).

4.3.3 Fruit growth (length and diameter)

With regard to fruit growth the results showed that NAA-treated branches exhibited higher fruit growth rate from the first week till the 7^{th} week, with regard to fruit length and diameter (Figs. 4.16, 4.17 and 4.18).

Table 4.8 Effects of NAA treatment on bud number, fruit set, fruit drop, yield, dry matter and chlorophyll content of wax apple.

Treatment (mg/L)	Bud number	Fruit set (%)	Fruit drop (%)	Yield (kg/ branch)	DM in fruits (g/100g)	Chlorophyll (SPAD value)
Control	49[c]	27[b]	52[a]	0.31[b]	2.28[b]	53.96[c]
NAA 5	62[a]	41[a]	30[a]	0.50[a]	3.77[a]	66.10[a]
NAA 10	57[b]	46[a]	41[a]	0.54[a]	3.84[a]	71.76[a]
NAA 20	53[b]	47[a]	42[a]	0.42[a]	2.65[b]	63.90[b]

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

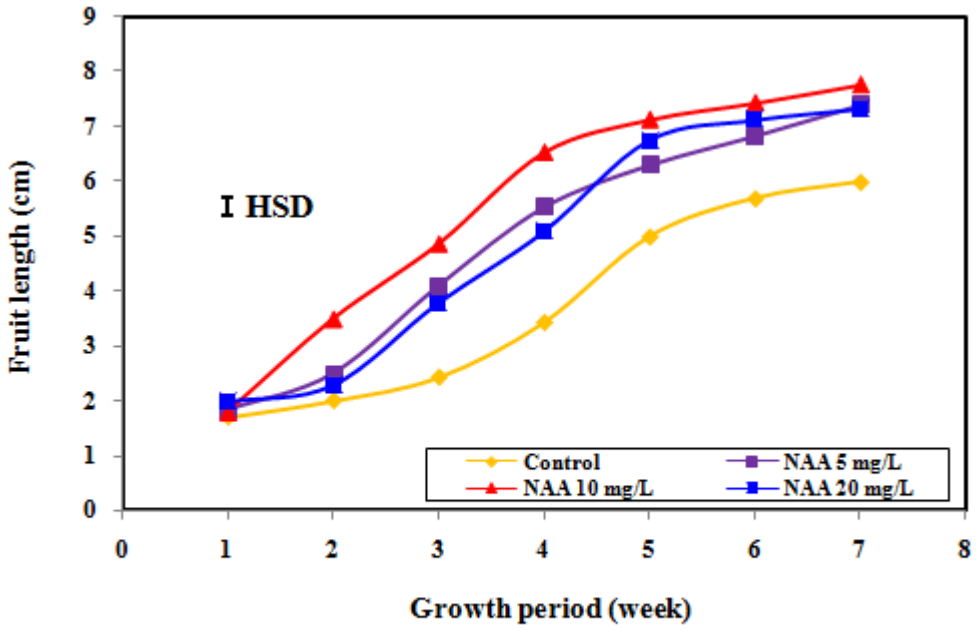


Fig. 4.16. The effects of different treatments of NAA on fruit growth length (cm) of wax apple (n = 12).

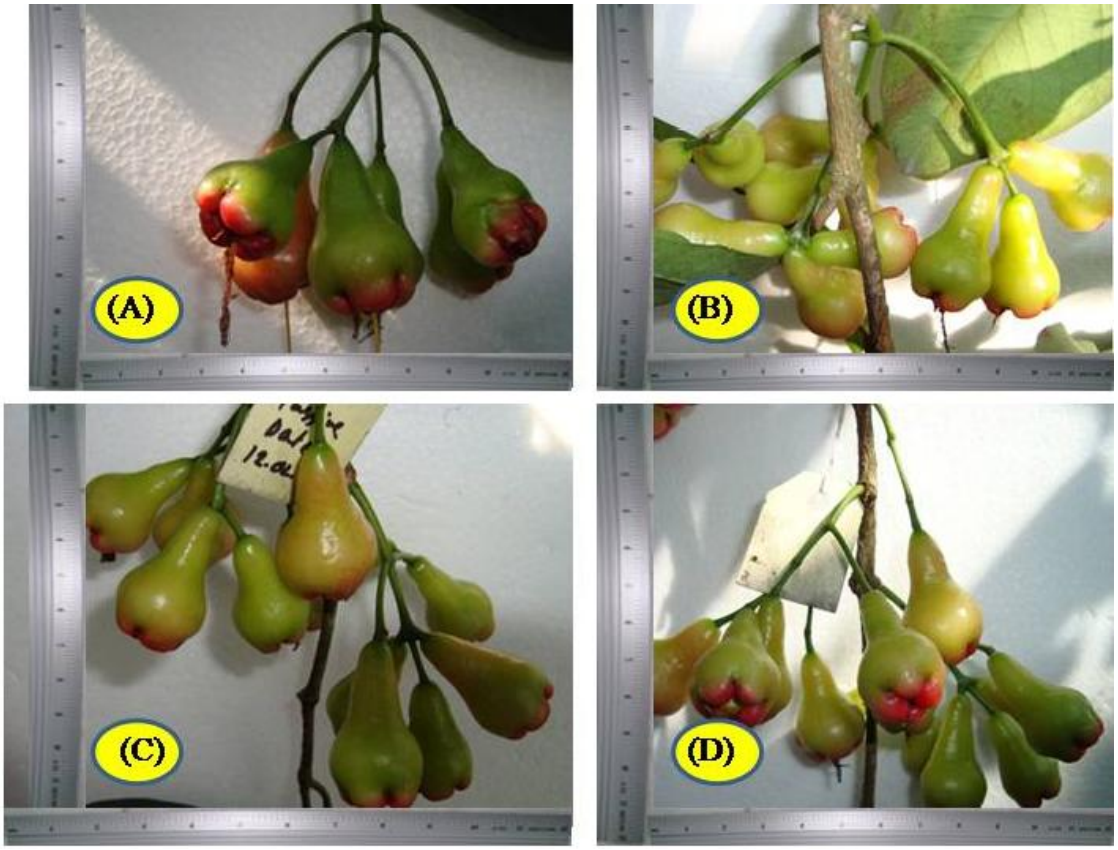


Fig. 4.17. Fruit size of wax apple at two week after anthesis as affected by NAA treatments (A): Control, (B): 5 mg/L, (C): 10 mg/L and (D): 20 mg/L NAA.

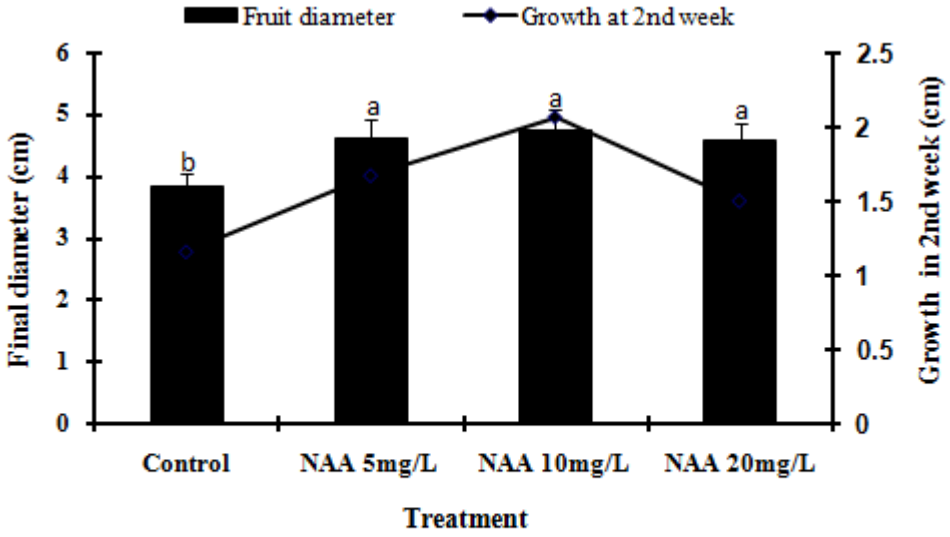


Fig. 4.18. Final fruit diameter (cm) and fruit growth of wax apple at 2nd week as affected by different treatments of NAA. Bars indicate ± S.E. and values (n = 12) followed by the same letter were not significantly different at $p < 0.05$.

Fig. 4.19a. Fruit size of wax apple as affected by NAA treatments (A): Control, (B): 5 mg/L, (C): 10 mg/L and (D): 20 mg/L NAA.

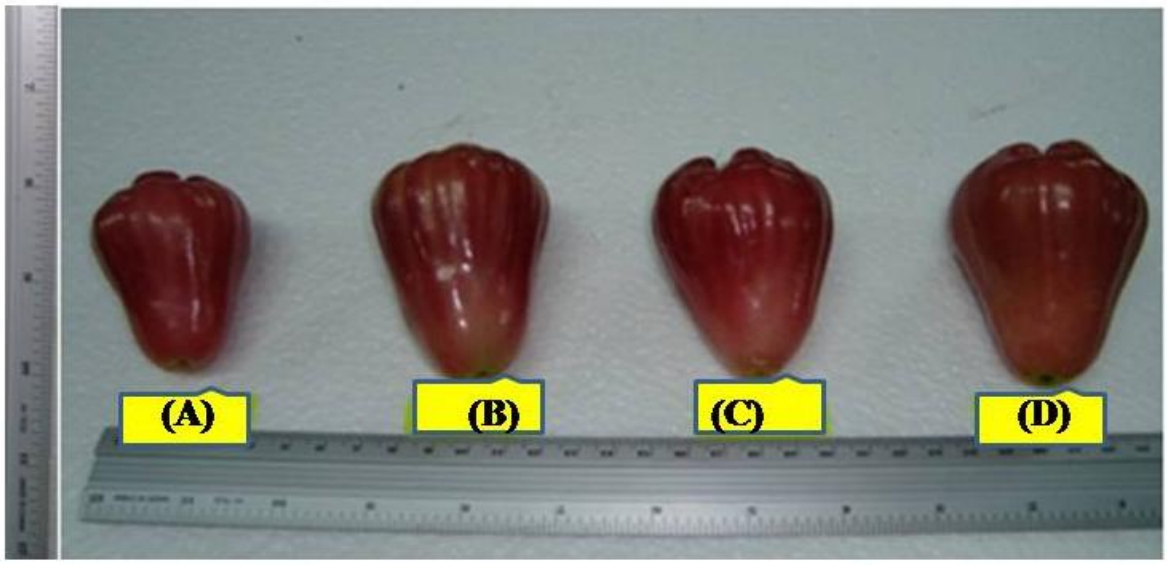


Fig. 4.19b. Fruit size of wax apple as affected by NAA treatments (A): Control, (B): 5 mg/L, (C): 10 mg/L and (D): 20 mg/L NAA.

At the 3rd week of observation, fruit length was 4.86 cm and 4.10 cm in 10 and 5 mg/L NAA treatments, respectively, whereas it was 2.4 cm for the control fruit. Similarly, 10 and 5 mg/L NAA treatments produced fruit diameter of 2.06 and 1.67 cm respectively, at 2nd week, whereas it was 1.16 cm among in control fruits. From the results, it can be seen that all the treated fruits grew at a faster rate and were larger than the untreated control fruits (Fig. 4.19). At 7th week of observation, fruit growth (diameter) was found to be significantly (p <0.05) different between the treatments and control.

4.3.4 NAA on color development

Fruit color development was greatly enhanced by the NAA treatments used in this study, with the 5 and 10 mg/L NAA treated fruits increased 110 % and 86% color cover from day 14 till 28 (Fig. 4.20). Furthermore, on day 14 after anthesis, the red color of the fruits had already started to show in the treated branches compared to the control fruits, which only started coloring one week later. At the 28th day of observation, the 10 mg/L treated fruits increased 115 % higher red color compared to control. It can be seen that significant difference was observed in peel color development between different NAA treatments and control (Fig. 4.21).

4.3.5 Total number of fruits/branch

In the first season, it was found that there was a significant difference between the treatments and the control. Similarly in the second growing season the treated branches produced more fruits compared to the untreated control. As shown in Figure 4.22, fruit number was 1.5 times higher in 5 mg/L NAA treatment compared to control. The same trend was also observed in the third season. All the treated branches produced the highest number of fruits compared to the untreated branches.

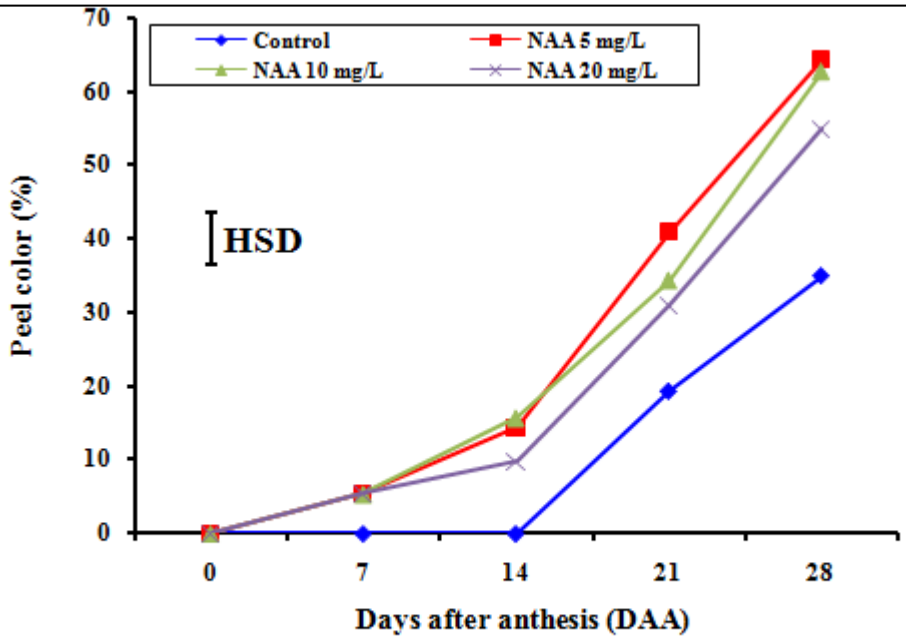


Fig. 4.20. The effect of NAA treatments on color development (%) of wax apple fruit after anthesis (n =12).

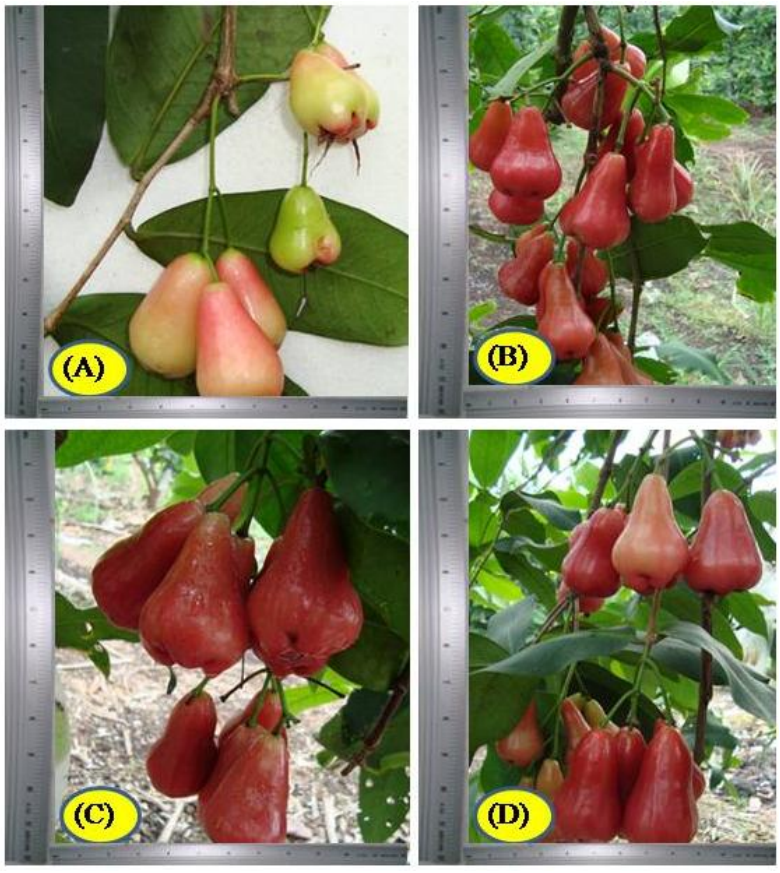


Fig. 4.21. Fruit size of wax apple as affected by NAA treatments (A): Control, (B): 5 mg/L, (C): 10 mg/L and (D): 20 mg/L NAA.

4.3.6 Chlorophyll fluorescence, photosynthetic yield, stomatal conductance and dry matter content

Naphthalene Acetic Acid had a significant effect on chlorophyll fluorescence of wax apple leaves (Table 4.9). The highest maximum fluorescence (Fm) was observed in 10 mg/L NAA treatment, followed by 5 and 20 mg/L NAA treatments respectively, whereas, the lowest (Fm) value was recorded in the leaves of control branches. Ground state fluorescence (F_0) was highest in 20 mg/L treatment followed by 10 and 5 mg/L NAA treatments respectively, whilst, leaves of control branches produced the lowest fluorescence (F_0). Similarly, variable fluorescence (Fv) was also the highest in the leaves of 10 mg/L NAA treated branches followed by 20 and 5 mg/L treatments, whereas the control exhibited the least variable fluorescence (Table 4.9). With regard to quantum yield, NAA treatments also showed a significant effect.

Optimum quantum or photosynthetic yield (Fv/Fm) was significantly different between the treatments and control. The highest optimum quantum yield (0.82) was in the leaves of 10 mg/L NAA treated branches, while, control produced the least (0.60) photosynthetic yield (Table 4.9).

NAA had a significant effect on stomatal conductivity in treated leaves. Results showed that the highest stomatal conductivity (591 mmol m^{-2} s^{-1}) was observed in 10 mg/L NAA treated leaves followed by 20 and 5 mg/L treatments with stomatal conductivity of 509 and 432 mmol m^{-2} s^{-1}, respectively, whereas, the control showed the least stomatal conductance (225 mmol m^{-2} s^{-1}) (Table 4.9). Furthermore, NAA treatments had a significant effect on dry matter accumulation in the leaves.

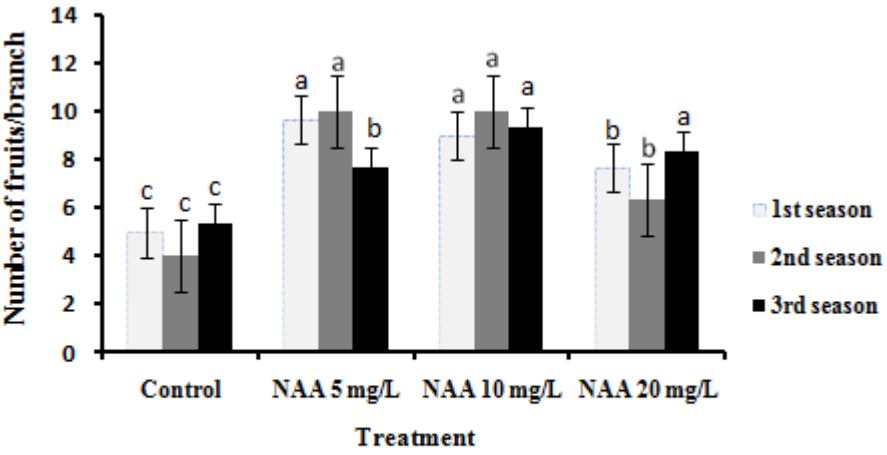


Fig. 4.22. The effects of NAA treatments on total number of fruits in wax apple through out the three growing seasons. Bars indicate ± S.E. and values (n = 30) followed by the same letter were not significantly different at $p < 0.05$ (LSD test).

Table 4.9 Effects of different treatments of NAA on chlorophyll fluorescence, photosynthetic yield, stomatal conductance, leaf DM and TSS (°Brix) of wax apple.

Treatment	Chlorophyll fluorescence			Quantum	Stomatal	DM	TSS
(mg/L)	F_0	F_M	F_v	yield $F_v\backslash F_M$	cond. (mmol $m^{-2}s^{-1}$)	(g/leaf)	(°Brix)
Control	419[c]	1852[c]	1433[c]	0.82[b]	225[d]	1.3[b]	5.6[c]
NAA 5	503[b]	2244[b]	1741[b]	0.85[a]	432[c]	1.8[a]	9.4[b]
NAA 10	585[a]	3540[a]	2955[a]	0.84[a]	591[a]	2.2[a]	10.7[a]
NAA 20	600[a]	3450[a]	2850[a]	0.83[a]	509 [b]	1.3[b]	9.7[b]

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

The highest amount of dry matter content was found in leaves of 10 mg/L NAA treated branches followed by 5 and 20 mg/L NAA treatment, whereas the control treatment produced the least amount of dry matter (Table 4.9) and these were found to be statistically significant (p <0.05) between the treatments.

4.3.7 Correlation between stomatal conductance and yield

As can be seen in Figure 4.23, it was observed that fruit yield showed a strong relationship (r = 0.91) with stomatal conductance of the leaves. From the 10 mg/L NAA treated branches, it was observed that fruit yield (kg) increased simultaneously with the stomatal conductance of leaves.

4.3.8 TSS content and correlation between TSS and peel color in fruit

Total soluble solids (TSS) of fruit is an important parameter that can strongly affect consumer acceptability of a variety of fruits. The TSS content of NAA treated fruit was found statistically significant at 1% level from the control treatment. Results showed that the highest TSS value of 10.70 °Brix was observed in 10 mg/L NAA treated fruits followed by 20 and 5 mg/L with a TSS value of 9.66 and 9.36 °Brix, respectively, whilst, the lowest TSS was in the control fruits (5.63 °Brix) (Table 4.9).

From Figure 4.24, it can be seen that fruit peel color showed a strong relationship (r = 0.988) with TSS. The results with 10 mg/L NAA treated fruits showed that fruit peel color correlated with its TSS content.

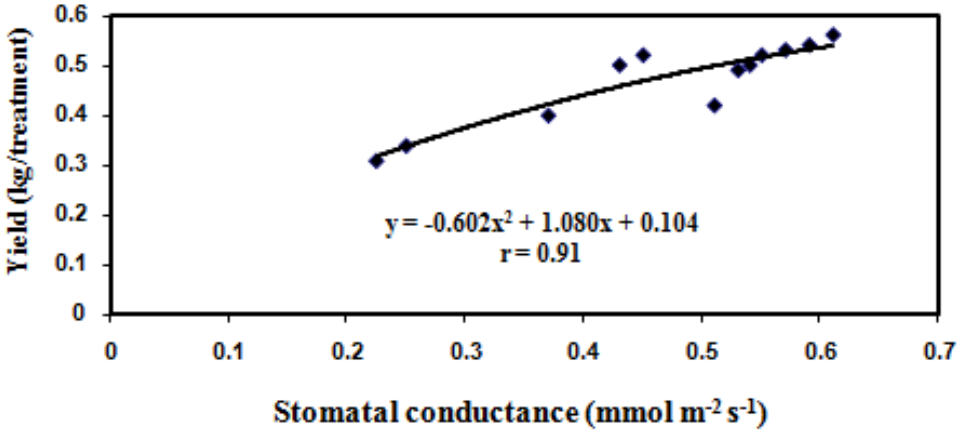


Fig. 4.23. Correlation between stomatal conductance and yield of wax apple fruits as affected by NAA treatments.

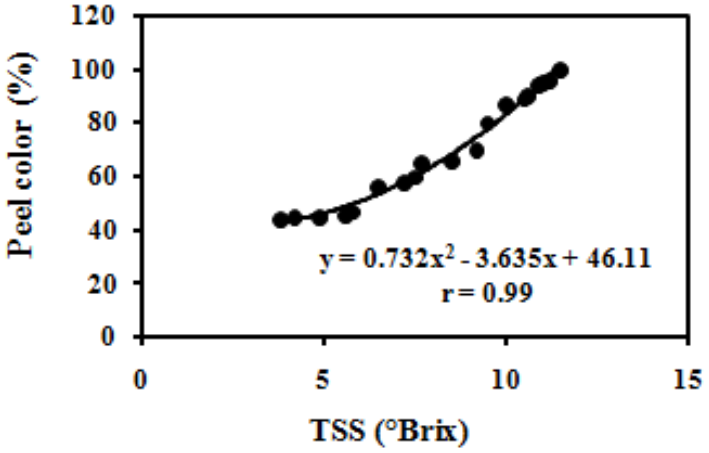


Fig. 4.24. Correlation between peel color and TSS of 10 mg/L NAA treated wax apple fruits.

4.3.9 Total sugar content

In the first season (2008) the sugar content was a significant ($p < 0.05$) difference among the treatments and the control. Similarly, in the second growing season, the total sugar content was significant ($p < 0.05$) difference between the treatments and control. This is shown in Figure 4.25, where the highest sugar content of 6.35 g was recorded in 10 mg/L NAA treated fruits, whereas, control fruits showed the lowest sugar content of 3.32 g.

The same trend was also observed in the third season. All the treated fruit produced higher amounts of total sugar content than the control fruits.

4.3.10 K^+ content of fruit

In this study it was observed that the different treatments of NAA produced significant differences in the K^+ content in treated and non-treated fruits (Table 4.10). Results showed that the K^+ content of fruit juice was the highest in 10 mg/L treated fruits followed by 5 and 20 mg/L NAA treated fruits, whereas control fruits produced the lowest value.

4.3.11 Total phenolic and flavonoid content in fruit

The application of different concentrations of NAA had a significant effect on the total phenolic content of wax apple fruits (Table 4.10). Fruits from 5 mg/L NAA treated branches exhibited the highest amount in the range of 681 mg GAE/100g of phenol followed by 10 and 20 mg/L treated fruits with phenolic content of 537 and 423 mg GAE/100g respectively, whilst, control fruits showed the least amount of 311 mg GAE/100g phenol content.

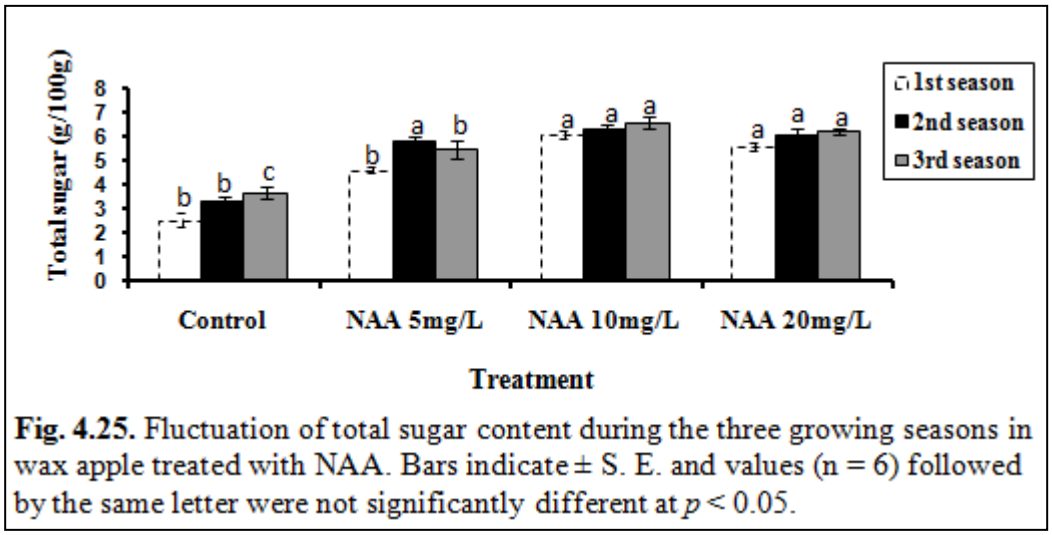


Fig. 4.25. Fluctuation of total sugar content during the three growing seasons in wax apple treated with NAA. Bars indicate ± S. E. and values (n = 6) followed by the same letter were not significantly different at $p < 0.05$.

Table 4.10 Effects of different treatments of NAA on K^+, total phenol, total flavonoid, antioxidant activity and anthocyanin content of wax apple.

Treatment (mg/L)	K^+ content (mg/kg)	Total phenol (mg GAE/100g)	Total flavonoid (mg CE/100g)	TEAC (mg/100g)	DPPH (mg/100g)
Control	15.3^c	311^c	12.55^b	8.06^b	13.16^b
NAA 5	67.3^a	681^a	27.40^a	8.52^a	14.23^a
NAA 10	76.0^a	537^a	28.87^a	8.37^a	13.86^a
NAA 20	28.3^b	423^b	27.38^a	8.33^a	13.95^a

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

Similarly from the results shown in Table 4.10, flavonoid content of wax apple fruit was found significantly difference between the NAA treatments and control. Results showed that 10 mg/L NAA treated fruits had the highest flavonoids content (28.87 mg CE/100g), while, the control fruits exhibited less than half amount found in treated fruits, recording the least flavonoid content of 12.55 mg CE/100g.

4.3.12 Antioxidant activity and correlation between total phenol and antioxidant activity

Antioxidant activity was significantly higher in NAA-treated fruits compared to those of the control fruits (Table 4.10). The antioxidant activity in the fruits was the highest in 5 mg/L NAA treated fruits, whereas, the fruits of the control treatment showed the lowest antioxidant activity. Figure 4.26 shows the relationship between antioxidant activity via TEAC method and the total phenols content of the *S. samarangense* fruits studied. A high correlation between the total phenolic content and antioxidant activity was observed ($r = 0.96$).

4.3.13 NAA on leaf soluble protein

Figure 4.27 shows that NAA treatments did not produced significant effect on leaf soluble protein among themselves, although, it was significantly different between the treatments and control. The highest leaf soluble protein was recorded in the 10 mg/L NAA treatment, whereas, control had the lowest protein content (Fig. 4.27)

4.3.14 Anthocyanin content in fruit

As shown in Figure 4.28, the NAA treatment had a significant effect on the anthocyanin content in wax apple fruits. The highest amount of anthocyanin (5.50 mg/g) was observed in 10 mg/L NAA treated fruits followed by 20 and 5 mg/L NAA treatment

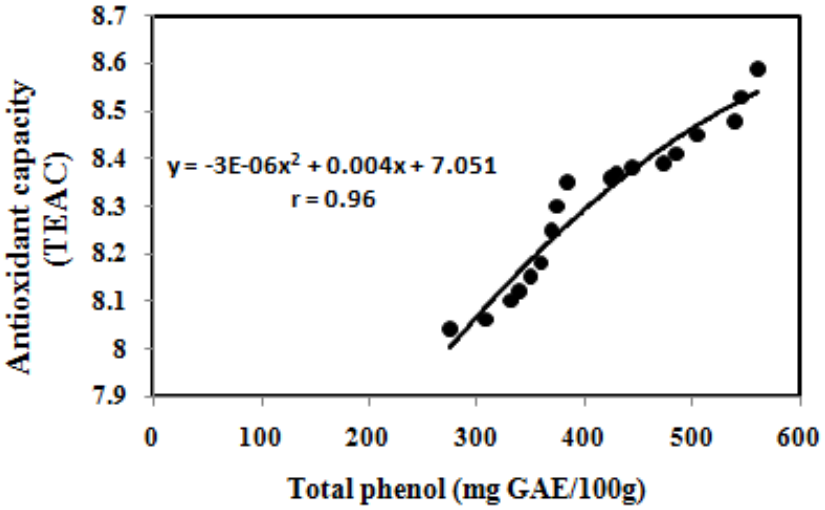


Fig. 4.26. Correlation between total phenols and antioxidant capacity via TEAC methods in 10 mg/L NAA-treated wax apple fruits.

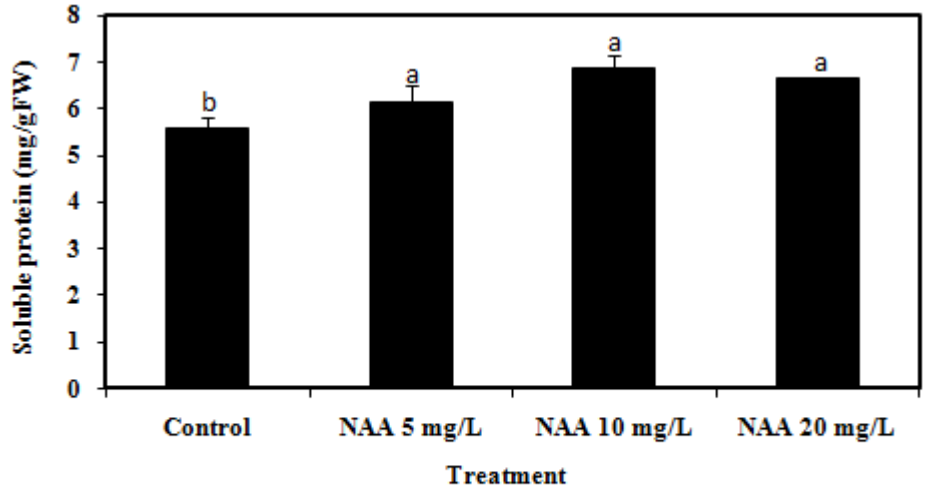


Fig. 4.27. The effect of different treatment of NAA on soluble protein (mg/g FW) in wax apple leaves. Bars indicate ± S.E. and values (n = 6) followed by same letter were not significantly different at $p < 0.05$.

with a value of 4.53 and 4.23 mg/g, respectively, whereas untreated control fruits showed the least amount of anthocyanin content (2.09 mg/g).

4.3.15 NAA on PAL activity

In the case of PAL activity in the wax apple fruit, it was found significantly difference between the treatments and control. The highest PAL activity in terms of cinnamic acid yield (14.29 nmol-cinnamic acid min^{-1} mg $protein^{-1}$) was recorded in 10 mg/L treatment followed by 5 and 20 mg/L NAA treatments, respectively, while, the lowest cinnamic acid yield Control yield (8.61 nmol-cinnamic acid min^{-1} mg $protein^{-1}$) (Fig. 4.29).

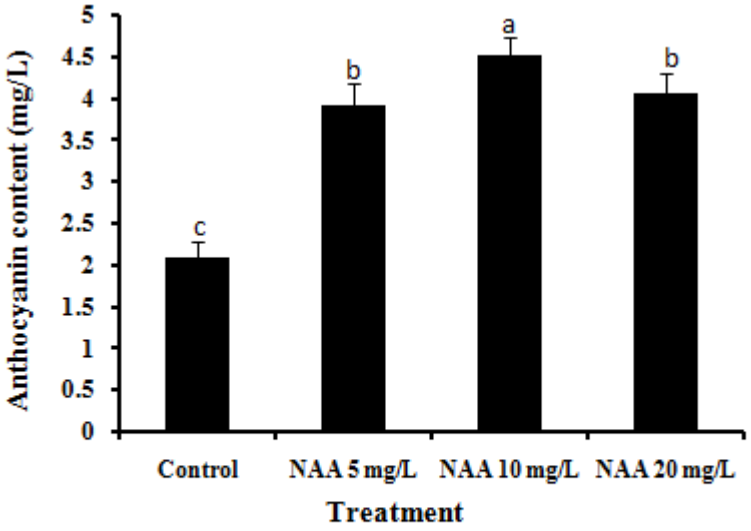


Fig. 4.28. The effect of NAA treatments on anthocyanin (mg/L) content of wax apple fruits. Bars indicate ± S. E. and values (n = 6) followed by same letter were not significantly different at $p < 0.05$.

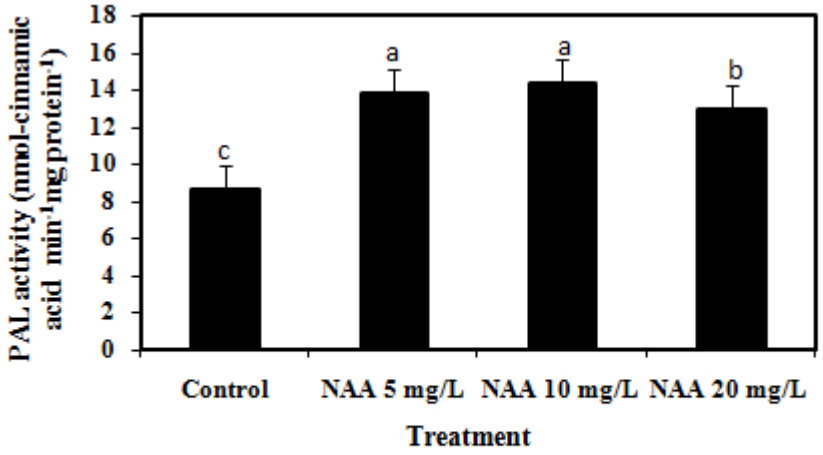


Fig. 4.29. PAL activity at 15 min of observation as affected by different treatment of NAA in wax apple fruits. Bars indicate ± S. E. and values (n = 6) followed by same letter were not significantly different at $p < 0.05$.

4.4 Effect of girdling on growth, development and quality of wax apple (*S. samarangense)*

Results of different quality paprameters of wax apple as affected by girdling treatments are reported below. Some correlations among the parameters studied were also carried out.

4.4.1 Bud number

As shown in Table 4.11, the different girdling techniques did not significantly increase total bud number. It was slightly higher in the V-shape phloemic stress.

4.4.2 Inflorescence development and fruit retention

The different types of girdling techniques significantly ($p < 0.05$) reduced the time needed for inflorescence emergence and the time of flowering (Table 4.12). The C-shape, 100% and V-shape girdling produced flowering on day 9, 11 and 12 followed by 50% and I-shape girdling which produced flowering on day 13 and 15, whilst it took 21 days for flowering in the control treatment. Different girdling treatments increased the C/N ratio in the treated branch, it might be enhanced the inflorescence development. Girdling treatment had a significant effect on fruit retention capacity of the plant. Table 4.11 shows that the fruit retention was almost 1.35 fold in C-shape girdling treatment compared to control. All the girdling treatments posted higher fruit retention values compared to than the control which was about 48% fruit retention per branch.

4.4.3 Bud drop and fruit set (%)

The V-shape girdling exhibited the lowest bud abscission number, averaging about 10 followed by C-shape girdling, 50% stress, 100% stress and I-shape girdling (Table

4.11). Non- treated control branches recorded around 48 % bud dropped. Almost five times more buds dropped in untreated branches compared with the V-shape girdled branch, which was statistically significant. Table 4.11 shows that fruit set was almost doubled in C-shape girdled branches compared to control branches. All the girdling treatments posted significantly ($p < 0.05$) higher fruit set values compared with the control which recorded about 32 % fruits set per branch.

4.4.4 Fruit drop (%)

The different girdling techniques did not significantly ($p < 0.05$) reduced fruit drop (Table 4.11). It was slightly higher in control treatment followed by the 50% phloemic stress and others.

4.4.5 Fruit growth (length and diameter)

As can be seen in Figures 4.30, 4.31 and 4.32, all the girdling treatments exhibited a higher fruit growth rate from the first week till the 7^{th} week, with regard to fruit length and diameter, compared to the control. At the 5^{th} week of after treatments, fruit length was 33% and 30% higher in C and V-shape girdling, respectively, whereas, it was lowest in control and these difference was found to statistically significant ($p < 0.05$).

This growth trend was observed throughout the fruit developmental period until the harvesting period. In the case of fruit diameter, a similar trend was observed during fruit development (Fig. 4.32). Between the 5^{th} and 7^{th} week of growth period, fruit growth (diameter) was found to be significantly different between the treatments and control. Results showed that fruit diameter was 4.23 cm in C-shape stress whereas it was 3.26 in the control treatment at week 7. The control fruits showed the lowest growth rate compared to all the other treatments.

Table 4.11 Effect of different types of girdling on bud number, bud drop, fruit set, fruit drop and yield per branch in wax apple trees.

Treatment (girdling)	Number of bud	Bud drop (%)	Fruit set (%)	Fruit drop (%)	Yield (kg/ treatment)	Fruit DM (g/100g)
Control	35[a]	48[a]	32[c]	55[a]	0.31[d]	2.81[b]
I-S	35 [a]	36[b]	43[bc]	40[a]	0.50[c]	3.76[a]
50%	35[a]	30[b]	45[b]	35[a]	0.41[c]	3.16[a]
100 %	35[a]	30[b]	40[bc]	40[a]	0.45[c]	3.03[a]
C-S	36[a]	16[c]	62[a]	36[a]	0.76[a]	3.72[a]
V-S	38[a]	10[c]	56 [b]	36[a]	0.56[b]	3.63[a]

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (HSD test).

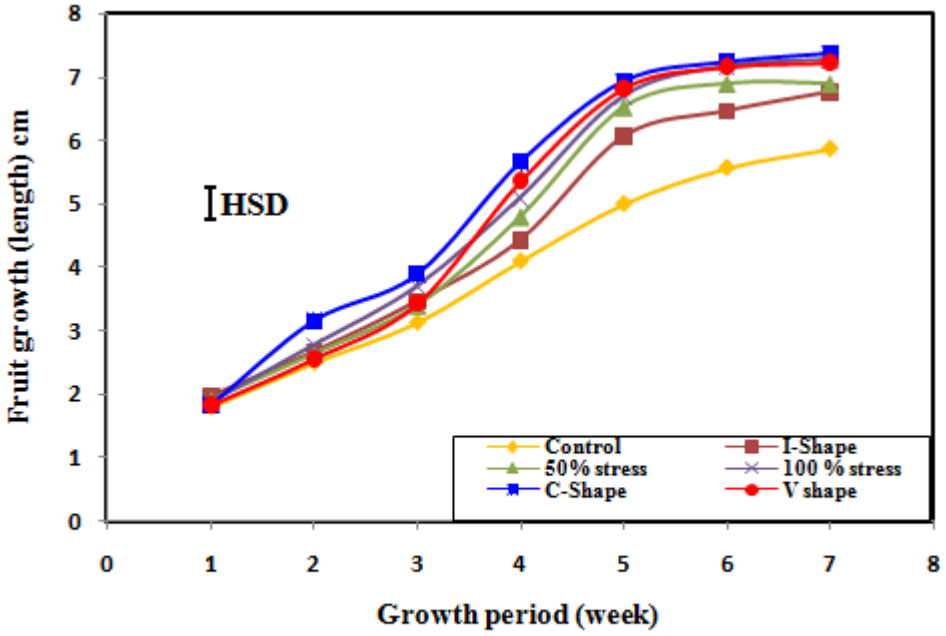


Fig. 4.30. Fruit growth (length) of wax apple as affected by different girdling treatments (n = 6).

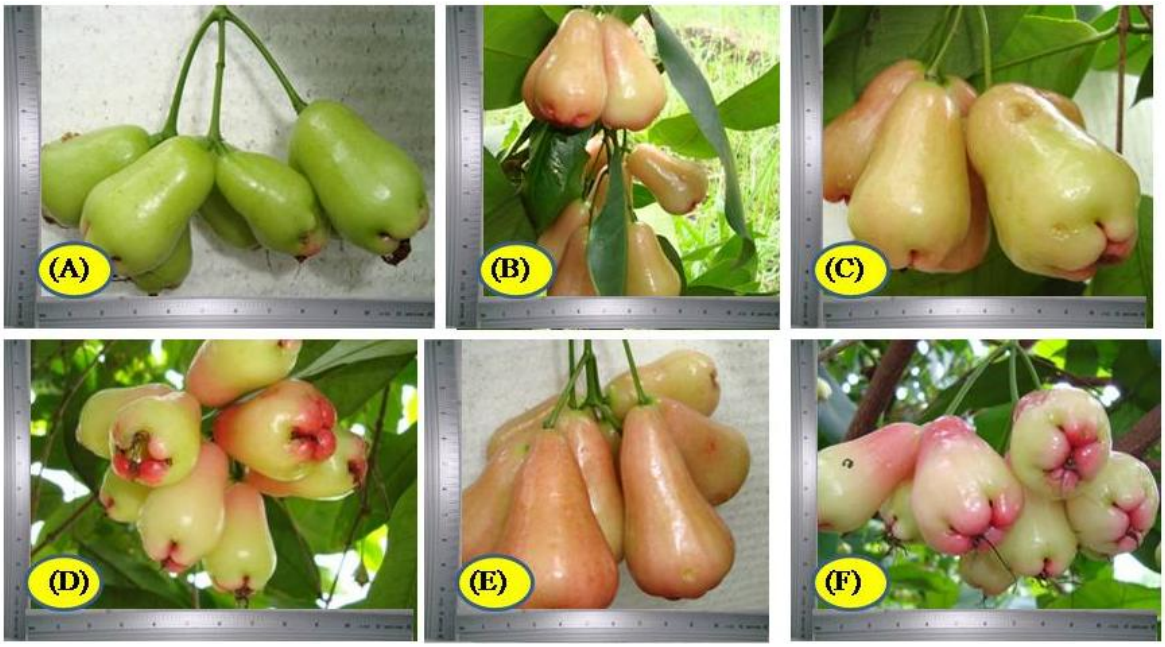


Fig. 4.31. Fruit growth and color development of wax apple as affected by different types of girdling. (A): Control, (B): I-shaped, (C): 50%, (D): 100%, (E): C-shaped and (F): V-shaped girdling.

4.4.6 Fruit size (length and diameter) and L/D ratio

As shown in Figure 4.33 and Table 4.12 fruit size (length) was significantly (p <0.05) influenced by different girdling treatments but fruit diameter was not affected significantly. As shown in Table 4.12, the different girdling techniques did not significantly increase length and diameter ratio of fruit, while, it was significantly different between the treatments and control. Results showed that length and diameter ratio was slightly higher in the 50 % girdling followed by the others.

4.4.7 Fruit yield (kg) and dry matter

As shown in Table 4.11 all the girdled branches in this study yielded significantly (p <0.05) higher fruit weight than the untreated control. The yield, on a fruit weight basis, was almost 50% higher in the treated branches compared to the control.

From the results, it can be seen that fruit matter content was significantly (p <0.05) affected by the different types of girdling methods carried out. C-shape girdling posted the highest amount of fruit drymatter with a value of 3.72 (Table 4.11). This was followed by V-shape and the other treatments, whilst the control treatment produced the lowest value for fruit drymatter.

4.4.8 Average fruit weight and number of fruits

The data on fruit weight showed significant (p <0.05) differences. In the first season, the highest fruit weight (51 g) was recorded in C-shape stress treatment followed by I-shape, 50% and 100% stress, with a weight of 49, 47 and 46 g, respectively, whereas, the lowest fruit weight (35 g) was observed in the control (Fig. 4.34).

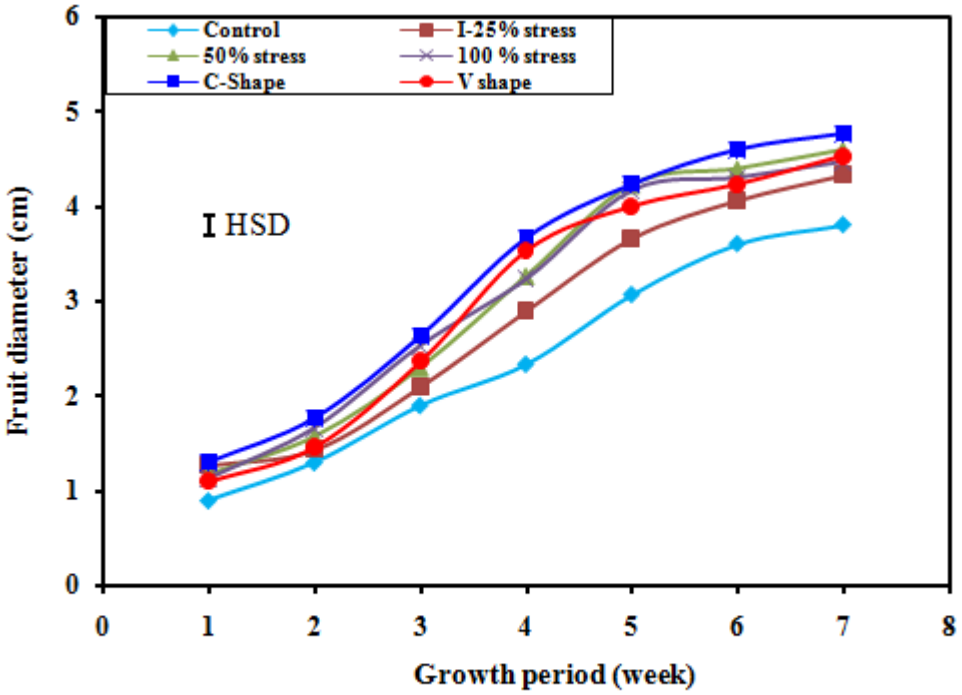


Fig. 4.32. Fruit growth (diameter) of wax apple as affected by different girdling treatments (n = 6).

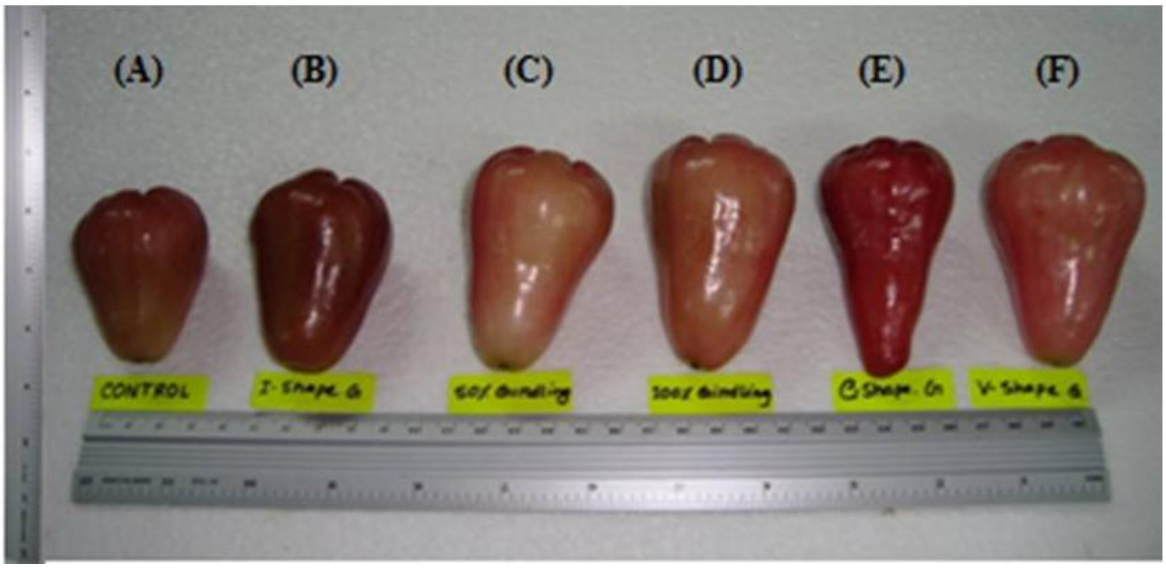


Fig. 4.33a. Fruit size of wax apple as affected by different types of girdling. (A): Control, (B): I-shaped, (C): 50%, (D): 100%, (E): C-shaped and (F): V-shaped girdling.

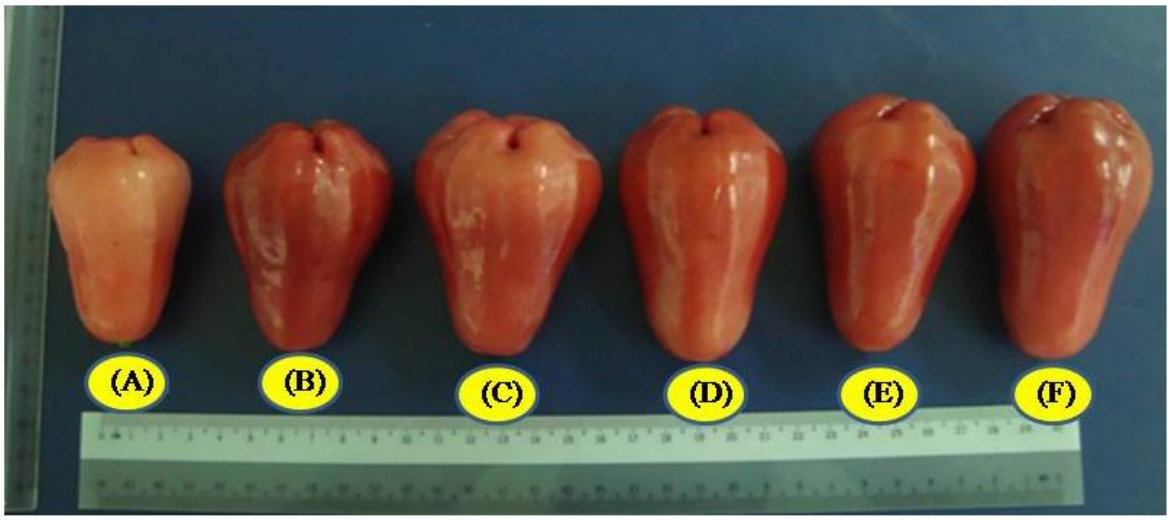


Fig. 4.33b. Fruit size of wax apple as affected by different types of girdling. (A): Control, (B): I-shaped, (C): 50%, (D): 100%, (E): C-shaped and (F): V-shaped girdling.

Table 4.12 Effects of different types of girdling on inflorescence development, fruit retention, fruit size, leaf and fruit drymatter of wax apple fruits.

Treatment (girdling)	Inflorescence development (d)	Fruit retention (%)	Fruit length (cm)	Fruit diameter (cm)	Length/diam. ratio
Control	21^{a}	48^{c}	5.43^{b}	3.93^{b}	1.36^{b}
I-S.	15^{b}	59^{ab}	6.49^{ab}	4.50^{ab}	1.44^{ab}
50%	13^{bc}	61^{ab}	6.46^{ab}	4.43^{ab}	1.45^{a}
100%	11^{bc}	58^{b}	6.40^{ab}	4.60^{ab}	1.40^{ab}
C-S.	9^{c}	65^{a}	6.90^{a}	4.83^{a}	1.43^{ab}
V-S.	12^{bc}	64^{ab}	6.63^{a}	4.66^{ab}	1.42^{ab}

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (HSD test).

During the 2nd season analysis, the highest mean fruit weight (50 g) was recorded in C-shape stress followed by V-shape, I-shape and 50% stress respectively, whereas, lowest fruit weight (34 g) was found in the control. Similar observations were also recorded in the 3rd season. From the Figure 4.34, it is clear that the different girdling treatment had a significant effect on average fruit weight.

In this study, different girdling techniques were employed to observe their effect on fruit number. From the results of first season, it was found that there was a significant (p <0.05) difference between the girdling techniques used and the control (Fig. 4.30). Similarly in the second and third growing seasons, fruits of the different girdled branches produced significantly more number of fruits (Fig. 4.30).

As can be seen from Figure 4.35, in the first season the highest number of fruits (11) was recorded in C-shaped girdling followed by V-shape, 50% and I-shape girdling, whereas the number of fruits recorded in the control treatment was 4. During the 2nd season the highest number of fruits (12) was recorded in C-shape stress followed by V-shape, I-shape and 50% stress, respectively, whereas, the lowest fruit number (4) was found in the control. Similarly, in the third season, the results showed the positive effects of the girdling treatments.

4.4.9 Peel color

The results in Figure 4.36 shows that fruit color was greatly enhanced by the various girdling techniques used in this study, with the C-shape and 100% girdling treated fruits exhibiting the greatest percentage color cover from day 14 till 28. Furthermore it was observed that on 14 days after anthesis, the red color of the fruits had already started to show in the treated branches compared to the control fruits, which only started coloring

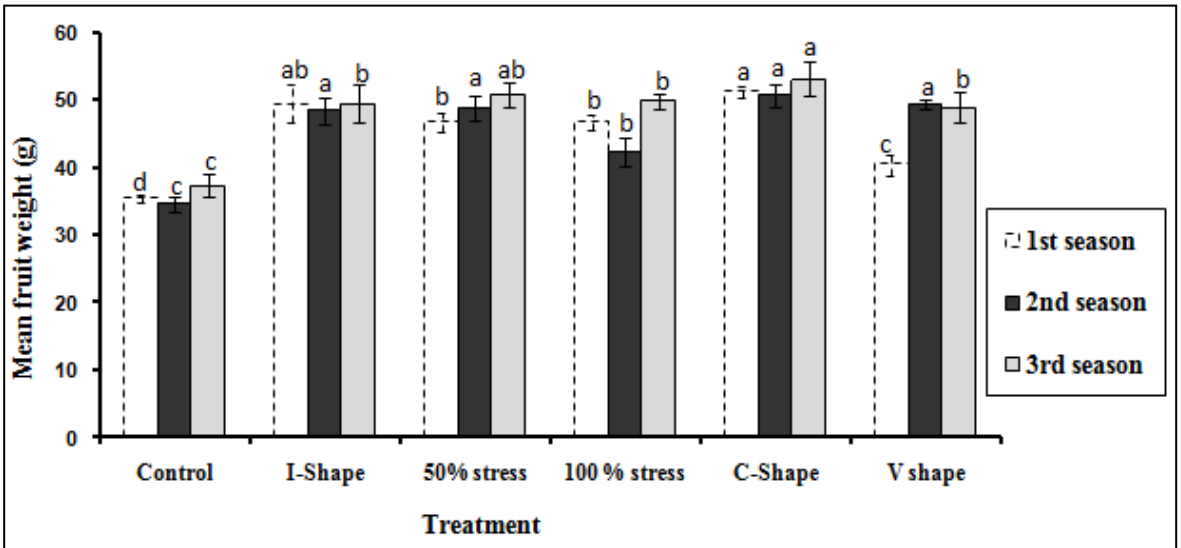


Fig. 4.34. Mean fruit weight of wax apple as affected by different girdling techniques. Bars indicate ± S. E. and values (n = 18) followed by the same letter were not significantly different at p <0.05.

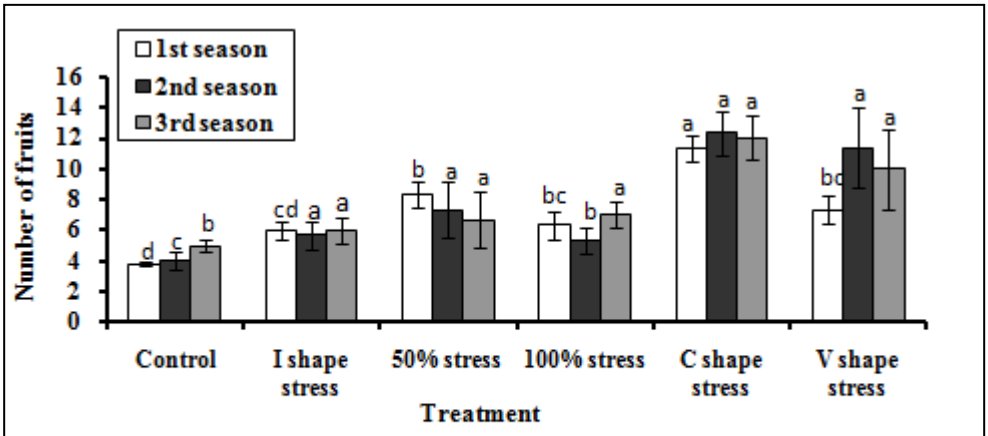


Fig. 4.35. Total number of fruits as affected by different types of girdling techniques. Bars indicate ± S. E. and values (n = 18) followed by the same letter were not signifcantly different at p < 0.05.

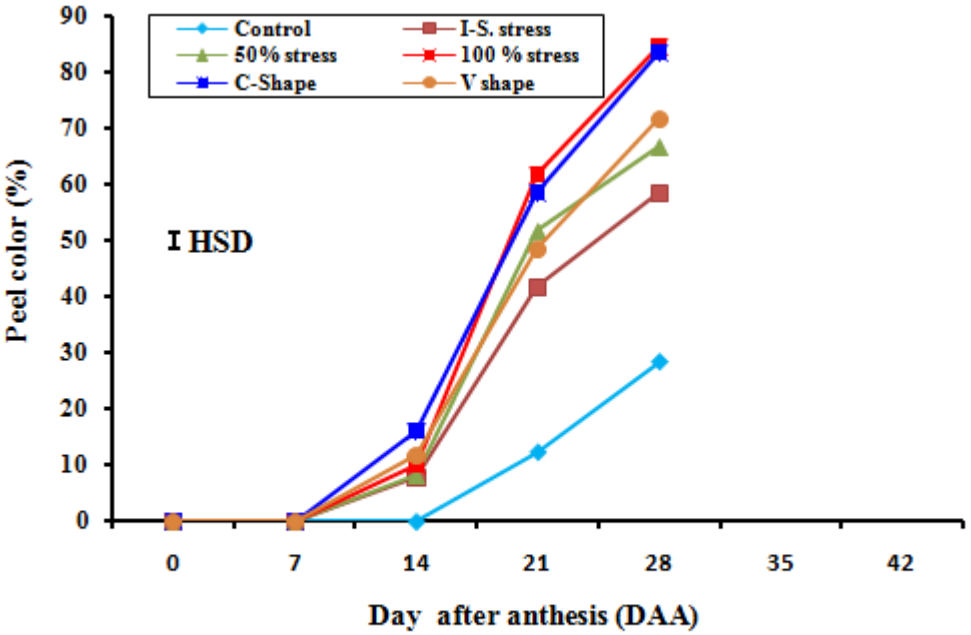


Fig. 4.36. The effect of different type of girdling on peel color (%) of wax apple fruits after anthesis (n = 6).

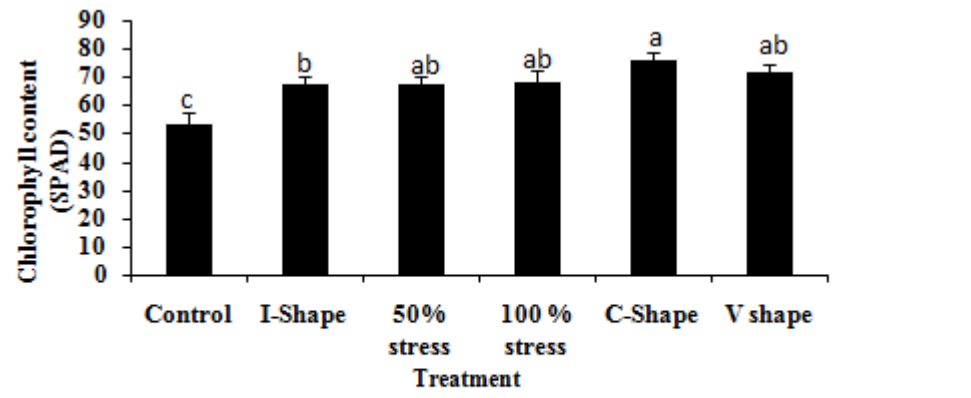


Fig. 4.37. Chlorophyll content (SPAD) of leaves as affected by different girdling techniques. Bars indicate ± S. E. and values (n = 18) followed by the same letter were not significantly different at $p < 0.05$.

one week later. From the graph it can be seen that a significant difference was observed in peel color development among the different treatments and the control.

4.4.10 Leaf chlorophyll content (SPAD)

In this study the chlorophyll content was determined using a Minolta SPAD meter. As shown in Figure 4.37 the chlorophyll readings in leaves from all the treated branches were significantly (p <0.05) higher than the control branch, with up to 43 % higher, in the C-shape treated branches.

4.4.11 Leaf size, dry matter chlorophyll, and quantum yield

Different girdling treatments had no negative effects on leaf length (Table 4.13). All the girdling treatments increased leaf width from the control, and their differences were statistically significant (p <0.05) (Table 4.13). Results showed that leaf dry matter content was significantly affected by different girdling treatments (Table 4.13). Leaves of C-shaped girdling yielded 57% higher dry matter content compared to control. In this study the chlorophyll *a* chlorophyll *b* and total chlorophyll content was determined using the methods described in Hendry and Price (1993).

From this study, it could be seen that different girdling treatment had a significant (p <0.05) effect on chlorophyll a, chlorophyll b and total chlorophyll in the leaves. As shown in Table 4.13 C-shape girdled branches produced the highest amount of chlorophyll a (4.51mg/g), chlorophyll b (3.11mg/g) and total chlorophyll (7.62 mg/g) followed by V-shape, 50% stress and I-shape stress, whereas, control treatment produced the lowest amount of chlorophyll content. From the results it was also found that the chlorophyll content decreased with the increasing of removal of phloem surface area. Leaves of 100%

girdled branches produced the lowest amount of chlorophyll among the different treatments.

Different girdling treatments also produced the significant (p <0.05) effects on the quantum yield or photosynthetic yield. From the Figure 4.38, it could be seen that the highest quantum yield was observed in the C-shape girdling treatment followed by V-shape, 50% stress, and I-shape with a value of 0.85, 0.84, 0.84 and 0.82, respectively, while, control produced the least Fv/Fm value of 0.80.

4.4.12 Fruit juice, fruit biomass and pH content

There was no significant difference for juice content between the different treatments. Table 4.14 shows that juice content (ml/100g) was 89 ml in C-shape girdling followed by the control, I-shape and V-shape and 50% stress treatments, with a juice content of 88, 87 and 82 ml, respectively. The results revealed that fruit dry matter (DM) content increased with the different girdling practices. From Table 4.14, it can be seen that the highest DM content of fruit/100g (18.00g) was observed in C-shape stress followed by I-shape, V-shape and 50% stress, with a DM content of 17.50, 17.36 and 14.50 g, respectively, whereas, control was 9.21 g. However the results for the four stress treatments were not statistically significant between each other, although they were significantly different from the control.

The leaf DM content analyzed on the 5^{th} week of fruit development also showed significant (p <0.05) differences between the treatments and control. It was observed that the highest leaf DM content (1.92 g) was recorded in C-shape stress treatment followed by I-shape, 50 % and V-shape treatments with a value of 1.88, 1.68 and 1.59 g, respectively. Control leaves gave the lowest value. Result showed that different girdling treatment did not produce significant effects on pH of fruit juice (Table 4.14).

Table 4.13: The effects of different types of girdling on leaf chlorophyll content and leaf size of wax apple

Treatment (girdling)	leaf length (cm)	leaf width (cm)	Leaf DM (g/leaf)	Leaf chlo *a* (mg/g)	Leaf chlo *b* (mg/g)	Total chlo (mg/g)
Control	19.5[a]	6.9[b]	1.22[b]	2.99[b]	1.91[bc]	5.01[d]
I-S.	20.9[a]	8.5[a]	1.88[a]	3.28[b]	2.18[bc]	5.46[cd]
50%	20.5[a]	7.9[a]	1.68[ab]	3.53[ab]	2.63[ab]	6.16[bc]
100%	19.6[a]	8.1[a]	1.51[ab]	3.19[b]	2.02[c]	5.10[d]
C-S.	21.3[a]	8.7[a]	1.92[a]	4.51[a]	3.11[a]	7.62[a]
V-S.	20.7[a]	8.1[a]	1.74[a]	4.09[ab]	2.71[ab]	6.80[b]

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (HSD test).

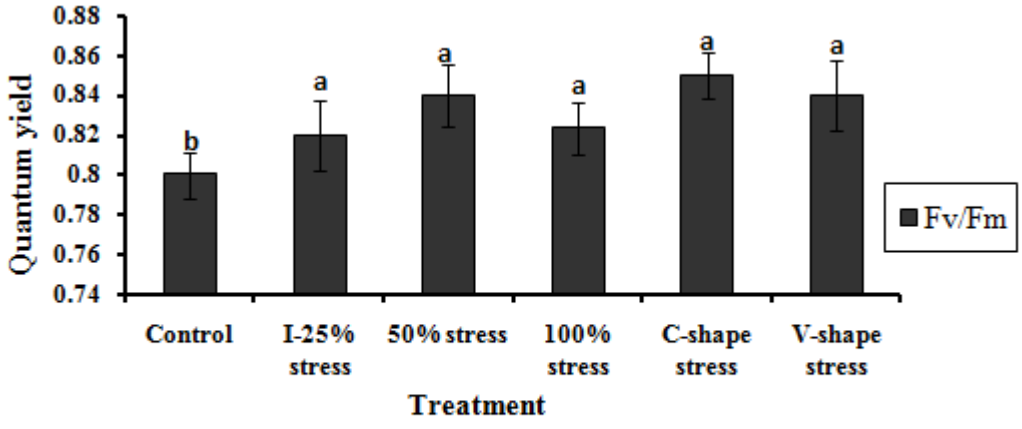


Fig. 4.38. Quantum yield (Fv/Fm) as affected by different treatments of girdling. Bars indicate ± S. E. and values (n = 18) followed by the same letter were not significantly different at $p < 0.05$.

4.4.13 Potassium and TSS content

In the case of potassium content, the different girdling techniques produced significantly (p <0.05) higher potassium content in treated fruits. Results showed that the highest K^+ content (48.67 mg/kg) in fruit was recorded in I-shaped stress followed by 50% stress, V-shape and C-shape girdle fruits with a value of 46.67, 41.33 and 38.00 mg/kg, respectively, whereas, the lowest value of potassium 15.33 mg/kg recording the control (Table 4.14).

The TSS content of fruit juice was found to be statistically significant (p <0.05) between the different treatments and the control treatment. From the results, it was observed that the highest (13.33°Brix) TSS was recorded in C-shape stress fruits followed by V-shape, 100 %, and 50 % stress with a TSS of 13.00, 12.13 and 11.36 (°Brix), respectively, while, the lowest TSS 5.63 (°Brix) was recorded in untreated fruits (Table 4.14). It was also observed that fruit peel color correlated with its TSS content. In the case of C-shape girdled fruits the TSS content of fruit juice increased positively with the peel color development (data not shown).

4.4.14 Total sugars content

The total sugars content of fruit juice was found to be statistically significant (p <0.05) different between the different treatments and the control treatment. From the results of 1st season, it can be seen that different girdling treatments produced significantly different amount of total sugar between the treatments and the control (Table 4.15). Similarly in the 2nd season, fruits of different girdled branches produced significantly (p <0.05) more total sugar in the fruits than the control (Table 4.15).

Table 4.14 Effects of different types of girdling on juice content, dry matter, K^+ and TSS (°Brix) content of wax apple.

Treatment (girdling)	Juice (ml/100g)	Fruit biomass (g/100g)	pH value	K^+ content (mg/kg)	TSS (°Brix)
Control	88^{a}	9.2^{d}	4.92^{a}	15.33^{b}	5.6^{c}
I-S.	87^{ab}	17.5^{ab}	4.98^{a}	48.67^{a}	10.7^{b}
50%	84^{b}	14.5^{c}	5.10^{a}	46.67^{a}	11.4^{ab}
100%	78^{c}	14.9^{bc}	5.08^{a}	37.33^{a}	12.1^{ab}
C-S.	89^{a}	18.0^{a}	5.14^{a}	38.00^{a}	13.3^{a}
V-S.	87^{ab}	17.4^{ab}	5.06^{a}	41.33^{a}	13.0^{b}

[s=stress, DM=Dry matter] Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (HSD test).

From the results, it was observed that the highest total sugar (6.8 g/100g) was recorded in C-shape stress fruits followed by V-shape, I-shaped, 50 %, and 100 % stress with a sugar content (g/100g) of 6.5, 6.2 ,4.75 and 4.6 g/100g, respectively (Table 4.15), while, the lowest total sugar (3.63 g/100g) was recorded in untreated fruits. In the third season again, the results showed the positive effects of the girdling treatment on the total sugar content in the fruits.

4.4.15 Total phenolics and flavonoids

The application of different types of girdling had a significant (p <0.05) effect on the total phenolic content in wax apple fruits (Table 4.15). Fruits from C-shaped girdled branches exhibited the highest amount in the region of 635 mg/100g of phenols followed by 100%, 50%, V and I-shaped branch with phenols content of 626, 585, 508 and 455 mg/100g, respectively. Control fruits exhibited the lowest phenol content 396 mg/100g. From the results shown in Table 4.15, it was observed that fruits of different girdling treatments produced higher flavonoid content than the control treatment and their difference was statistically significant (p <0.05). As can be seen from the Table 4.15, branches with V-shape girdles produced fruits with the highest flavonoids content (55 mg CE/100g) followed by C-shape, I-shape and 50% stress treatments which recorded values of 42, 34 and 32 mg CE/100g, respectively, while, the control fruits produced the lowest flavonoid content (22 mg CE/100g).

4.4.16 Correlation between antioxidant activity and total flavonoid content

Antioxidant activity, via the TEAC assay, was found to be statistically significantly different between the treatments and the control fruits (see; appendix 16). Among the different girdle techniques, C and V-shaped girdle treated fruit showed higher antioxidant

Table 4.15 Effects of different types of girdling on total sugar, total phenol, total flavonoid and DPPH concentrations of wax apple fruits.

Treatment	Total sugar (g/100g pulp)			Total phenols	Total flavonoids	DPPH	Anthocyanin
(girdling)	1st seas.	2nd seas.	3rd seas.	(mg GAE/100g)	(mg CE/100g)	(mg/100g)	(mg/g)
Control	3.1^{c}	3.6^{b}	3.9^{c}	396^{b}	22.0^{c}	13.7^{a}	2.03^{c}
I-S.	3.7^{bc}	6.2^{a}	5.4^{ab}	455^{ab}	34.9^{b}	14.2^{a}	4.00^{b}
50%	4.8^{ab}	4.7^{b}	4.4^{bc}	585^{ab}	32.7^{bc}	14.3^{a}	4.32^{ab}
100%	4.7^{ab}	4.6^{b}	4.6^{bc}	626^{a}	32.7^{b}	14.3^{a}	3.87^{b}
C-S.	5.5^{a}	6.8^{a}	6.5^{a}	635^{a}	42.8^{a}	14.4^{a}	4.84^{a}
V-S.	5.1^{a}	6.6^{a}	6.4^{a}	508^{ab}	55.0^{a}	14.42^{a}	4.56^{a}

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (HSD test).

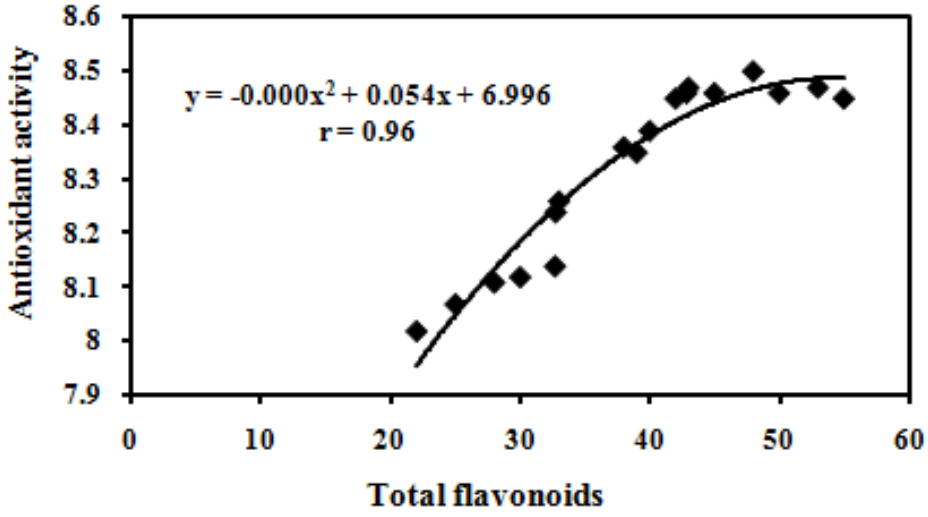


Fig. 4. 39. Correlation between total flavonoids and antioxidant activity via TEAC methods in the girdled fruits of wax apple.

activity via DPPH assay compare to other girdle techniques, although, these differences was not significant among the treatments and control (Table 4.15).

As shown in Table 4.15, different types of girdling had a significant (p <0.05) effect on the anthocyanin content in wax apple fruits. The highest (4.84 mg/g) amount of anthocyanin was observed in C-shape girdled fruit followed by V-shape, 50 % and I shaped girdling with a value of 4.56, 4.32 and 4.00 mg/g, respectively, whereas, the untreated control fruit showed the lowest amount of anthocyanin content (2.03 mg/g).

A high correlation between the total flavonoids content and antioxidant activity (TEAC measurements) was observed (r = 0. 96) in fruits of C-shaped girdle (Fig. 4.39).

4.5 Influence of hydrogen peroxide on growth, yield, biochemical and phytochemical properties of wax apple

Results of physiological, biochemical and phytochemical quality of wax apple fruits as affected by hydrogen peroxide treatments are presented below.

4.5.1 Leaf drymatter and chlorophyll content

As shown in Table 4.16, hydrogen peroxide treatment had a significant effect on leaf dry matter contents of wax apple in all the seasons studied. In the first season, it was found that the branches treated with 5 mM hydrogen peroxide appeared healthier than those of the control and exhibited a higher leaf dry matter content, 1.23-fold than that of the control. This was followed by 20 and 50 mM hydrogen peroxide treatments. Leaves from the control treatment showed the lowest leaf dry matter content. Similar findings were recorded in second and third seasons.

With regard to chlorophyll content, which indirectly indicates the health status of a plant, it was slightly higher in the leaves of the treated branches. As shown in Table 4.16, in the second season, the chlorophyll levels in all treated leaves were higher than in the control, up to 24% higher for the 5 mM H_2O_2 treatment. The differences between the treatments and the control were statistically significant in all three seasons.

4.5.2 Photosynthesis, stomatal conductance and leaf transpiration

Hydrogen peroxide treatments increased the leaf photosynthesis activity considerably. This effect was statistically significant in the 2010–2011 season.

Table 4.16 Effects of H_2O_2 treatment on leaf dry weight, chlorophyll content, bud drop, fruit set and fruit drop in *Syzygium samarangense* based on harvest values for all seasons.

Treatment (mM)	Leaf (g/DW)	Chlorophyll SPAD value	Bud drop (%)	Fruit set (%)	Fruit drop (%)
Season 1 (2008/2009)					
Control	1.20^{c}	55^{c}	42^{a}	27^{d}	43^{a}
H_2O_2 5	1.53^{a}	68^{a}	40^{a}	30^{c}	30^{d}
H_2O_2 20	1.51^{a}	64^{b}	31^{c}	33^{a}	35^{c}
H_2O_2 50	1.38^{b}	65^{ab}	34^{b}	35^{b}	39^{b}
Season 2 (2009/2010)					
Control	1.0^{c}	53^{b}	40^{a}	26^{c}	40^{a}
H_2O_2 5	1.30^{a}	67^{a}	39^{a}	29^{b}	28^{b}
H_2O_2 20	1.24^{b}	66^{a}	33^{a}	37^{a}	38^{a}
H_2O_2 50	1.15^{bc}	65^{a}	35^{a}	32^{a}	40^{a}
Season 3 (2010/2011)					
Control	1.0^{c}	56^{c}	48^{a}	25^{b}	42^{a}
H_2O_2 5	1.41^{b}	75^{a}	39^{b}	27^{b}	33^{c}
H_2O_2 20	1.46^{a}	67^{b}	29^{d}	37^{a}	35^{bc}
H_2O_2 50	1.44^{ab}	68^{b}	33^{c}	40^{a}	38^{b}

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

The activities were 1.60-, 2.36- and 2.38-fold higher than the control at 350 ppm CO_2 and light intensities of 400, 800 and 2,000 µmol m^{-2} s^{-1} respectively, in the leaves treated with 20 mM H_2O_2 (Fig. 4.40). Leaf photosynthesis was highest in leaves under the 20 mM H_2O_2 treatment, followed by the 5 and 50 mM H_2O_2 treatments, in that order, whereas the control leaves evidenced the least photosynthesis at light saturation.

Hydrogen peroxide treatment also produced a significant effect on the stomatal conductance of the leaves. The highest stomatal conductance was observed with the 50 mM treatment, followed by the 5 and 20 mM H_2O_2 treatments, with values of 0.09, 0.08 and 0.07 mol H_2O m^{-2} s^{-1}, respectively, at a light intensity of 400 µmol m^{-2} s^{-1}. The control recorded the lowest value (Fig. 4.41).

Similarly to photosynthesis, leaf transpiration was also affected by hydrogen peroxide treatment. The transpiration rates of the hydrogen peroxide-treated leaves were significantly higher than that of the untreated leaves (Fig. 4.42). The leaf transpiration rates were 3.30-, 3.90- and 3.73-fold higher than the control with the 5, 20 and 50 mM treatments at a light intensity of 800 µmol $m^{-2}s^{-1}$ (Fig. 4.42). As shown in Figure 4.43, we observed that transpiration had a strong relationship ($r = 0.89$) with the stomatal conductance of the 20 mM H_2O_2 treated leaves: transpiration increased proportionally with stomatal conductance.

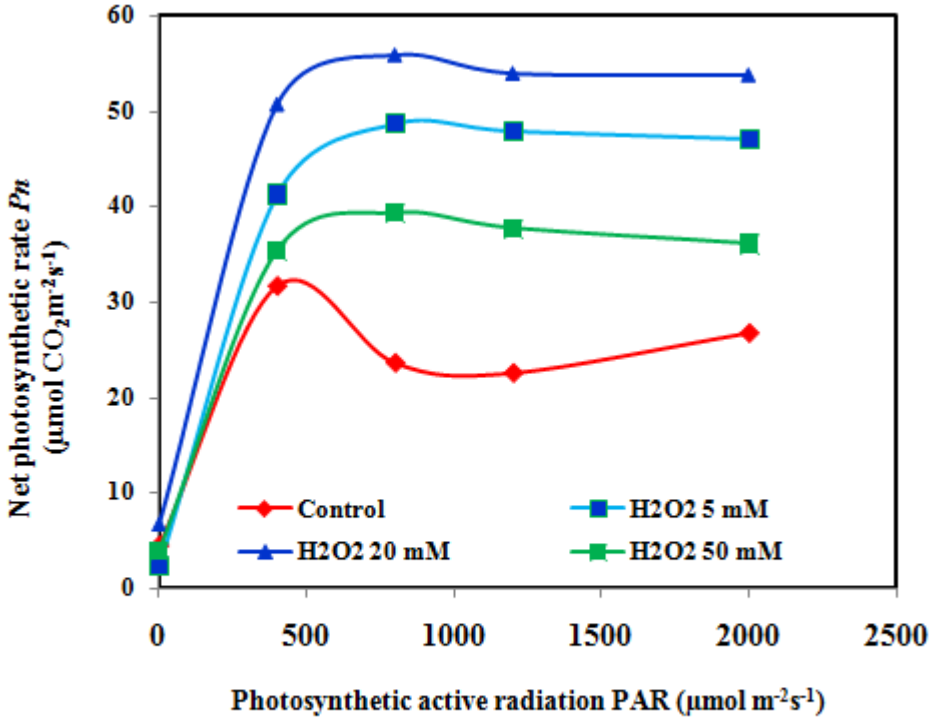


Fig. 4.40. Effects H_2O_2 treatments on net photosynthesis of wax apple trees under field condition (n = 12).

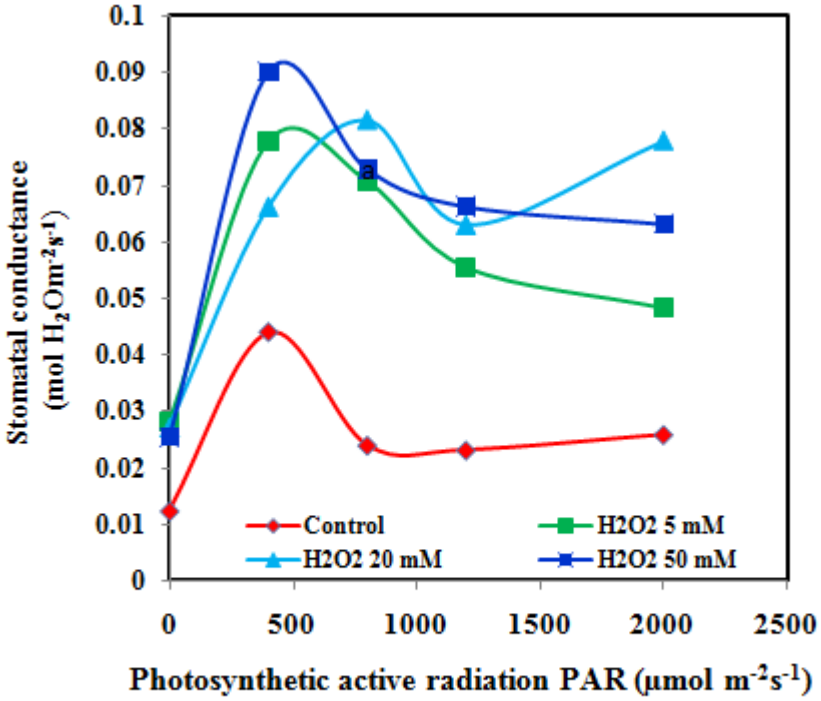


Fig. 4.41. Effects H_2O_2 treatments on stomatal conductance of wax apple leaves under field condition (n = 12).

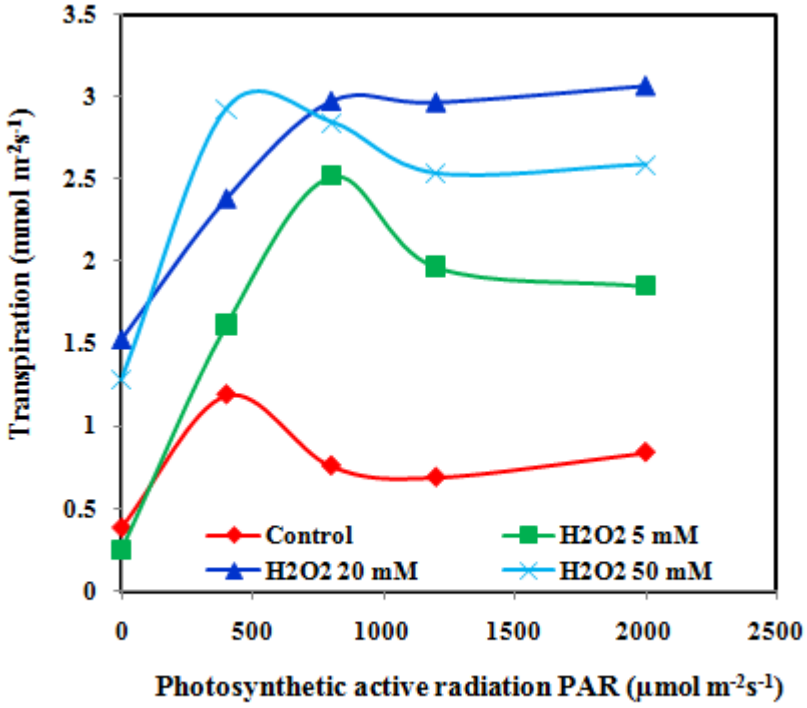


Fig. 4.42. Effects H_2O_2 treatments on transpiration of wax apple leaves under field condition (n = 12).

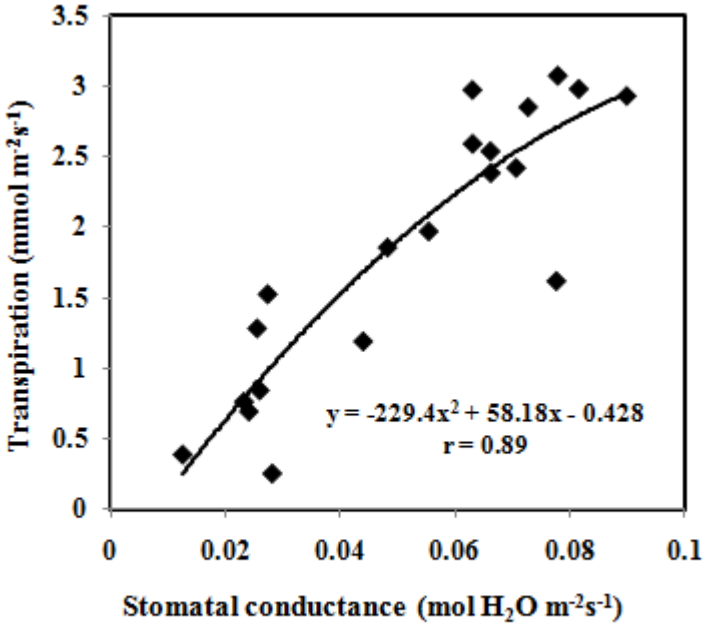


Fig. 4.43. The relationship between stomatal conductance and transpiration of H_2O_2 treated wax apple trees.

4.5.3 Bud drop, fruit set and fruit drop (%)

In all seasons, hydrogen peroxide treatment reduced bud abscission numbers, averaging approximately 36%, although their differences were not significant in second season (Table 4.16). In the three successive growing seasons, treatment with 20 mM hydrogen peroxide yielded the best results, followed in order the 50 and 5 mM H_2O_2 treatments. In the 2010–2011 season, almost 1.65 times as many buds dropped from the untreated branches as the treated branches, where the control branches had a bud drop of approximately 48%. Hydrogen peroxide had a positive effect on bud development and reduced bud drop. Furthermore, Table 4.16 shows that fruit set was increased almost 1.46-fold on the 20 mM H_2O_2-treated branches compared with the control, followed in order by the 50 and 5 mM H_2O_2 treatments. This effect was statistically significant ($p < 0.05$) in the second and third seasons but not in the first season. Hydrogen peroxide treatment had a significant effect on fruit drop in all the seasons. For the 2009–2010 season, these results showed that the control branches experienced the highest (40%) premature fruit drop whereas the 5 mM H_2O_2 treatment had the lowest (28%) percentage of fruit drop, followed by the 20 and 50 mM H_2O_2 treatments. Similar observations were recorded in the 2008–2009 and 2010–2011 seasons.

On the basis of these results, it can be seen that all the treated fruits grew at a faster rate and were larger than the untreated control fruits (Fig. 4.48). Between the 3rd and 7^{th} week of observation, fruit growth (length and diameter) showed significant differences between the treatments and the control. Similar growth trends were also recorded in the first and second seasons (data not shown).

4.5.4 Fruit growth (length and diameter)

The next parameter studied was fruit size, which included fruit length and diameter. As shown in Figures 4.44, 4.45 and 4.46, all the H_2O_2-treated branches exhibited higher fruit growth rates from the 1st week to the 7th week. During the 2009–2010 season, the average fruit length was 3.8 cm and 3.3 cm for the 20 and 50 mM H_2O_2 treatments, respectively, at the 3rd week of observation whereas the average fruit length for the control fruits was 2.3 cm.

Similarly, the average fruit diameters were 2.23 and 2.03 cm for the 20 and 50 mM H_2O_2-treated fruits, whereas the average fruit diameter was 1.6 cm for the control fruits. This growth trend was observed throughout the whole fruit developmental period until harvest.

4.5.5 Color development

In the 2009–2010 season, the fruit color development was significantly enhanced by the H_2O_2 treatments used in this study, with the 20 and 5 mM H_2O_2 treatments exhibiting the greatest percentage color cover from days 14 to 28 (Fig. 4.47). Furthermore, we observed that at 14 days after anthesis, the red color of the fruits had already started to show on the treated branches whereas the control fruits only began coloring one week later. On the 28th day of observation, the 20 mM-treated fruits showed approximately 46% red colour cover whereas the control was only 20% color covered (Fig. 4.46). Similarly, in the 2008–2009 and 2010–2011 seasons, H_2O_2 treatments also showed significant effect on peel color development.

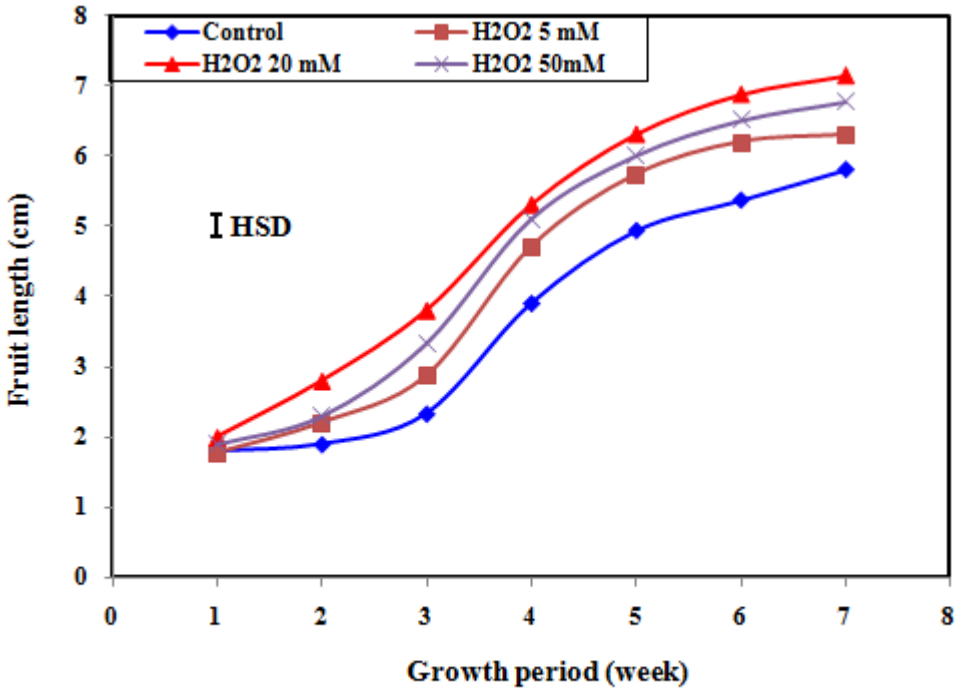


Fig. 4.44. Fruit growth (length) of wax apple as affected by different treatments of H_2O_2 (n = 12).

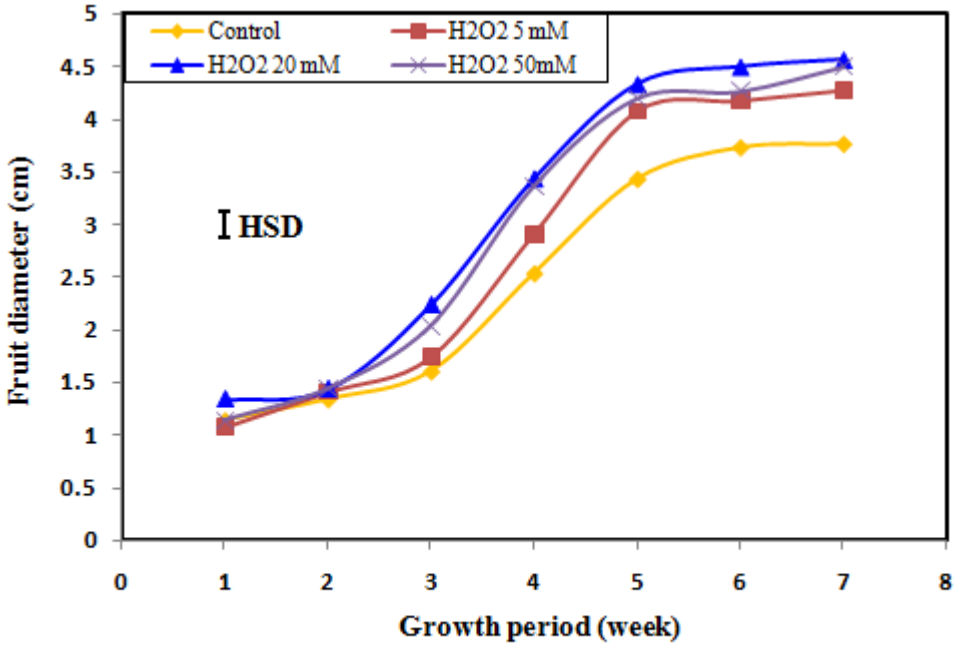


Fig. 4.45. Fruit growth diameter (cm) of wax apple as affected by H_2O_2 treatments (n =12).

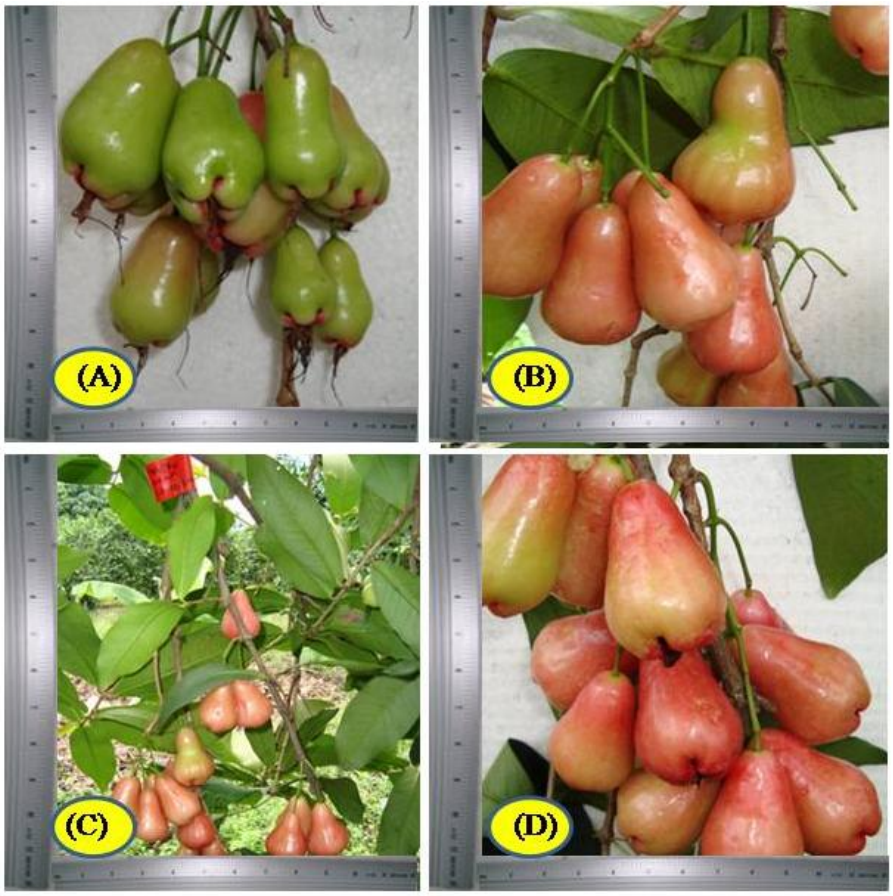


Fig.4.46. Fruit growth and color development of wax apple at 4th week after anthesis. (A): Control, (B): 5 mM, (C): 20 mM and (D): 50 mM H_2O_2.

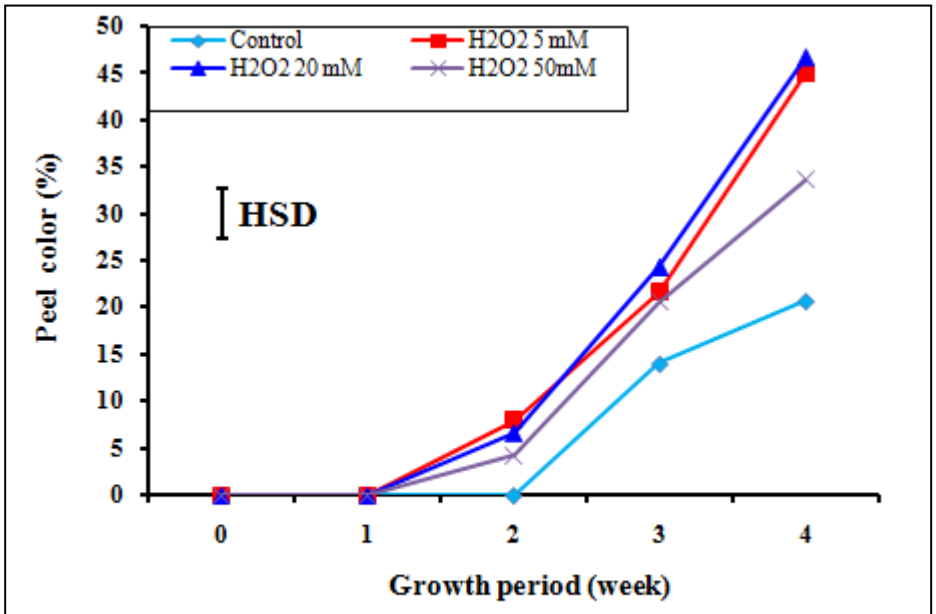


Fig. 4.47. The effect of different concentrations of H_2O_2 on peel color of wax apple fruit after anthesis (n = 12).

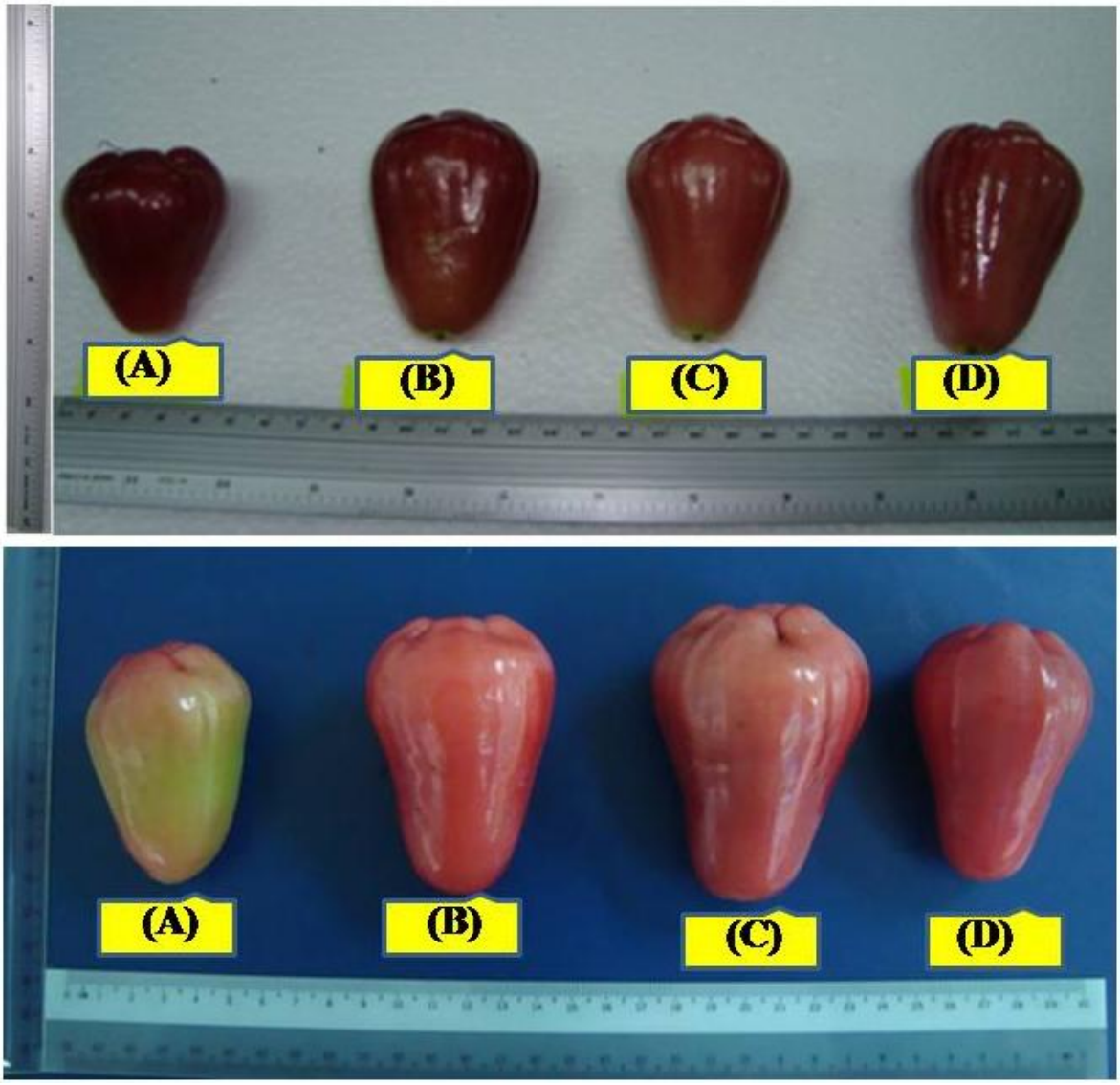


Fig. 4.48. Fruit size of wax apple as affected by H_2O_2 treatments. (A): Control, (B): 5 mM, (C): 20 mM and (D): 50 mM H_2O_2.

4.5.6 Fruit yield (kg), drymatter content and total number of fruits

For the three growing seasons, the treated branches exhibited a significantly ($p < 0.05$) higher yield of fruits and fruit drymatter content compared with the untreated control (Table 4.17). During the 2008–2009 season, the yield calculated on the basis of fresh fruit weight was almost1.56 times higher on the 20 mM-treated branches than on the control, producing the highest yield, followed by the 5 and 50 mM treatments. The results were found to be statistically significant between treatments. Similarly, in the last two seasons, hydrogen peroxide treatment also had a significant effect on yield.

Furthermore, hydrogen peroxide had a significant effect on drymatter accumulation in the fruits in all the seasons (Table 4.17).

Hydrogen peroxide treatment had a significant effect on the total number of fruits in the three successive growing seasons from 2008 to 2011. As shown in Table 4.18, for the first season (2008–2009), 20 mM hydrogen peroxide treatment produced on average, the highest number of fruits (15), followed by the 5 and 50 mM treatments, each with 14 and 11 fruits, respectively, whereas the control branches produced the lowest number of fruits (7). Similarly, in the second (2009–2010) and third growing seasons (2010–2011), the results showed significant differences between the H_2O_2 treatments and controls. It was observed that in all the experiments performed, 20 mM hydrogen peroxide-treated branches produced the highest number of fruits compared with the other treatments and the untreated control.

Hydrogen peroxide treatment also significantly increased the fruit juice content of wax apple throughout all the growing seasons (Table 4.18). Fruit biomass also significantly affected by hydrogen peroxide treatments (data not shown).

Table 4.17 Effects of H_2O_2 treatment on yield, fruit dry matter, K+ content and phenolic content in *Syzygium samarangense* based on harvest values for all seasons.

Treatment (mM)	Yield (kg/ treatment)	Fruit DM (g/100 g)	K^+ content (mg /kg)	Phenol (mg GAE/100g)
Season 1 (2008/2009)				
Control	0.25^{c}	2.22^{c}	25^{b}	300^{c}
H_2O_2 5	0.36^{a}	2.94^{ab}	28^{b}	415^{b}
H_2O_2 20	0.39^{a}	3.16^{a}	36^{a}	446^{a}
H_2O_2 50	0.30^{b}	2.86^{b}	33^{a}	423^{b}
Season 2 (2009/2010)				
Control	0.23^{c}	2.28^{c}	23^{c}	307^{b}
H_2O_2 5	0.38^{b}	2.78^{b}	29^{b}	350^{a}
H_2O_2 20	0.43^{a}	3.07^{a}	38^{a}	418^{a}
H_2O_2 50	0.39^{b}	2.42^{c}	28^{b}	413^{a}
Season 3 (2010/2011)				
Control	0.28^{c}	1.97^{c}	25^{c}	296^{c}
H_2O_2 5	0.36^{b}	2.69^{a}	35^{b}	339^{b}
H_2O_2 20	0.41^{a}	2.70^{a}	32^{a}	404^{a}
H_2O_2 50	0.34^{b}	2.53^{b}	26^{b}	372^{a}

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

4.5.7 TSS content of fruit

In these studies of the three growing seasons, it was found that the TSS content of the fruit juice showed statistically significant differences between the different H_2O_2 treatments and the control treatment. In the first two seasons, the hydrogen peroxide treatments produced higher TSS contents than the control (Table 4.19). As shown in Table 4.19, in the third season, the highest TSS value of 10.23 °Brix was observed in the 5 mM H_2O_2-treated fruits, followed by the 20 and 50 mM H_2O_2 treatments, with TSS values of 9.26 and 8.0, respectively, while the minimum TSS in the control samples was 5.63°Brix.

4.5.8 Total sugar

Subsequent to the above experiments, the sugar contents of the *wax* apple fruits was also determined. On the basis of the results for all seasons, it was observed that there were significant differences between the treatments and the control. In the 2nd season, shown in Table 4.19, the highest total sugar content of 5.9 g/100g was recorded for the 5 mM H_2O_2-treated fruits, followed by the 20 and 50 mM H_2O_2 treatments, with sugar contents of 5.60 and 5.54 g/100g, respectively, whereas the untreated control fruits showed the lowest sugar content of 3.65 g/100g. The same trend was also observed in the third seasons (Table 4.19).

4.5.9 K^+ content

H_2O_2 treatment resulted in significant differences in the K^+ content of the fruits for all the three growing seasons (Table 4.17). In the 2008–2009 season, the K^+ content of the fruit juice was highest in the 20 mM-treated fruits, followed by the 50 and 5 mM H_2O_2 treatments, with the control fruits having the lowest value. Similar trends were recorded for the 2nd and 3rd seasons (Table 4.17).

Table 4.18 Effects of H_2O_2 treatments on the number of fruits and their juice content in *Syzygium samarangense* for three different harvest seasons.

H_2O_2	Number of fruits			Fruit juice (ml/100g fruit)		
(mM)	1st season	2nd season	3rd season	1st season	2nd season	3rd season
0	8^{c}	5^{b}	6^{c}	62^{b}	69^{c}	65^{c}
5	14^{a}	9^{a}	10^{ab}	70^{a}	78^{b}	76^{ab}
20	15^{a}	9^{a}	11^{a}	73^{a}	87^{a}	80^{a}
50	11^{b}	7^{ab}	8^{bc}	76^{a}	79^{b}	75^{b}

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

Table 4.19: Effects of H_2O_2 treatments on total soluble solids (TSS) and total sugar content in *Syzygium samarangense* for three different harvest seasons.

H_2O_2	TSS (°Brix)			Total sugar		
(mM)	1st season	2nd season	3rd season	1st season	2nd season	3rd season
0	5.1^{d}	6.2^{d}	5.63^{c}	3.0^{d}	3.6^{c}	3.9^{c}
5	6.6^{b}	10.4^{b}	10.2^{a}	5.7^{a}	5.9^{a}	5.8^{a}
20	7.2^{a}	11.0^{a}	9.26^{a}	4.5^{b}	5.6^{a}	5.5^{a}
50	6.2^{c}	8.5^{a}	8.00^{b}	4.2^{c}	4.7^{b}	4.7^{b}

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

4.5.10 Total phenolic and flavonoid content

The application of the various concentrations of H_2O_2 had a significant effect on the total phenolic content of wax apple fruits (Table 4.17). In the 2008–2009 season, all the treated fruits produced higher amounts of total phenolics than the control treatment. Similarly, in the second season, fruits from the 20 mM H_2O_2-treated branches exhibited the highest content of phenols, in the region of 418 mg/100g, followed by the 5 and 50 mM-treated fruits, with phenolic contents of 413 and 350 mg/100g, respectively. The control fruits had lower amounts than the treated fruits, at 307 mg/100 g. Similar significant effects were also recorded in the third season (Table 4.17).

For all three growing seasons, it was observed that fruits with H_2O_2 treatment produced higher flavonoid contents than the controls, and the differences were significant (Table 4.20). In the first season, as shown in Table 4.20, 20 mM H_2O_2-treated fruits had the highest flavonoids content (37.0 mg CE/100 g), followed by the 5 and 50 mM H_2O_2 treatments, with recorded values of 30.5 and 25.1 mg CE/100g, respectively. The control fruits had the lowest flavonoid content. Treated fruits from the second and third seasons also contained more flavonoids than the controls.

4.5.11 Antioxidant activity in the fruit

The TEAC results showed that the hydrogen peroxide-treated fruits had a slightly higher antioxidant activities than the control, although these differences were not statistically significant ($p < 0.05$) in all the seasons. Antioxidant activities determined by the DPPH method were significantly higher in the hydrogen peroxide-treated fruits from all three seasons than in the untreated control.

Table 4.20 Effects of different H_2O_2 treatments on K^+, total phenol, total flavonoid, TEAC and DPPH concentrations of wax apple at harvest for the three growing seasons.

Treatments (mM)	Flavonoid (mg CE/100g)	TEAC (mg/100g)	DPPH (mg/100g)	Anthocyanin (mg/g)	Carotene (μg/g)
Season 1(2008/2009)					
Control	21.0^{c}	8.00^{a}	12.5^{b}	3.0^{c}	7.63^{b}
H_2O_2 5	30.5^{a}	8.17^{a}	14.2^{ab}	4.2^{b}	9.58^{a}
H_2O_2 20	37.0^{a}	8.13^{a}	14.7^{a}	4.6^{a}	10.0^{a}
H_2O_2 50	25.1^{b}	8.11^{a}	14.4^{bc}	3.9^{b}	9.20^{a}
Season 2 (2009/2010)					
Control	20.8^{b}	8.07^{a}	13.8^{c}	2.7^{c}	7.8^{d}
H_2O_2 5	22.4^{b}	8.57^{a}	14.3^{b}	4.0^{ab}	9.3^{b}
H_2O_2 20	28.6^{a}	8.22^{a}	14.6^{a}	4.5^{a}	10^{a}
H_2O_2 50	23.9^{b}	8.10^{a}	14.4^{a}	3.1^{b}	8.5^{c}
Season 3 (20010/2011)					
Control	25^{c}	311^{b}	12.7^{b}	2.05^{c}	7.6^{c}
H_2O_2 5	33.0^{a}	8.62^{a}	13.9^{a}	3.2^{b}	9.5^{ab}
H_2O_2 20	29.2^{b}	8.20^{a}	14.5^{a}	4.0^{a}	10^{a}
H_2O_2 50	27.7^{b}	8.14^{a}	13.8^{a}	3.5^{b}	9.2^{b}

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

The highest value (14.7 mg/100g) of antioxidant activity was found with the 20 mM H_2O_2 treatment, followed by the 50 and 5 mM H_2O_2 treatments, with values of 14.4 and 14.2 mg/100g, respectively, whereas the control had the lowest antioxidant activity (12.5 mg/100 g) (see Table 4.20). Similar trends were also observed for the second and third seasons.

4.5.12 Correlation between flavonoid content and antioxidant activity

Similarly to the phenols and flavonoids contents, the antioxidant activity determined by the TEAC and DPPH methods was slightly higher in the hydrogen peroxide-treated fruits than in the control fruits (Table 4.20). Figure 4.49 shows the relationship between antioxidant activity by the DPPH method and the flavonoid contents in the *S. samarangense* fruits being analysed.

A high degree of correlation was observed between the total phenolic content and DPPH measurements (r = 0.93). Similarly, a high degree of correlation (r = 0.87) was observed between total phenols and the TEAC measurements (Fig. 4.50).

4.5.13 Carotenoid contents

During the 2009–2010 season, the highest carotene content of 10.0 μg/g was found in the 20 mM-treated fruits, followed by the 5 and 50 mM H_2O_2-treated fruits, with values of 9.3 and 8.5 μg/g, respectively, whereas the lowest amount of carotene (7.8 μg/g) was found in the control fruits. In addition, hydrogen peroxide treatments in the first and third seasons also resulted in similar trends for the carotene contents of the fruits (Table 4.20).

4.5.14 PAL activity and anthocyanin formation

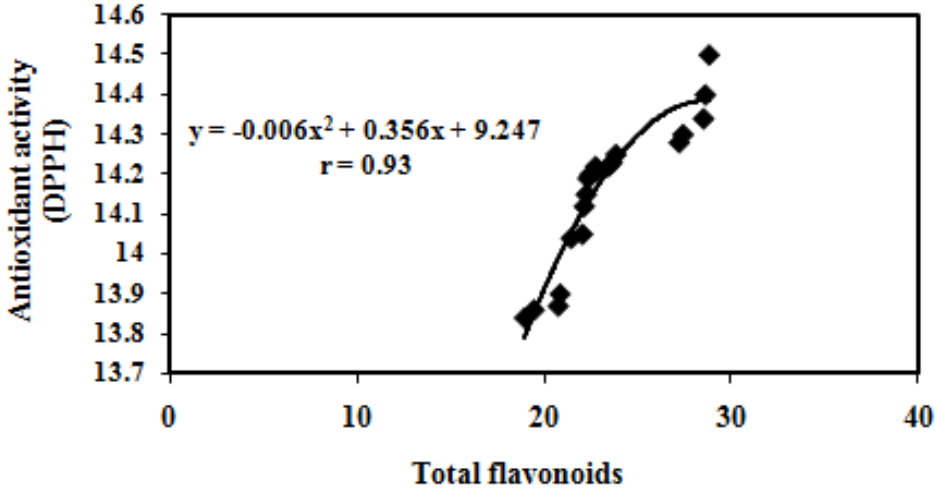


Fig. 4.49. The correlation between total flavonoids and antioxidant activity via DPPH method in H_2O_2 treated wax apple fruits.

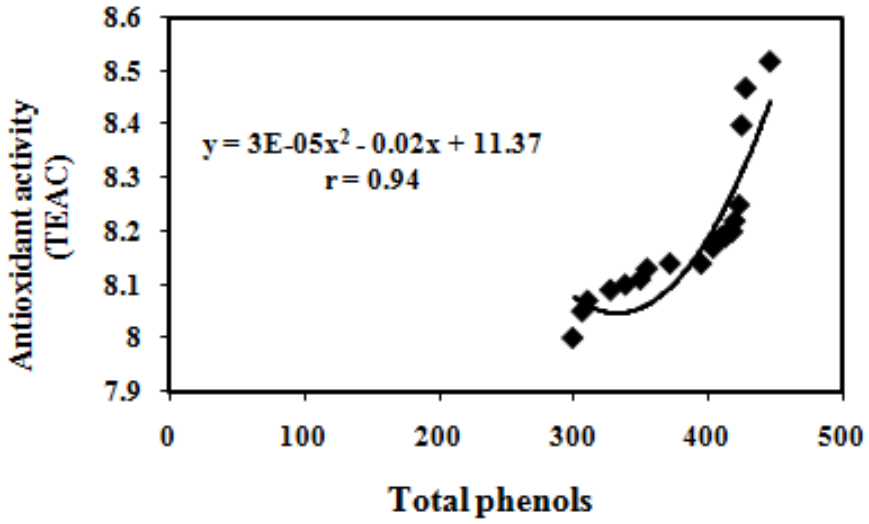


Fig. 4.50. The correlation between total phenols and antioxidant activity via TEAC method in H_2O_2 treated wax apple fruits.

Results showed that hydrogen peroxide treatment had a significant ($p < 0.05$) effect on PAL actives in the fruits. As can be seen in Figure 4.51, after 30 min of incubation, PAL activity in expression of the cinnamic acid yield was the highest (36.97 nmol min^{-1} mg $protein^{-1}$) for the 20 mM treatment, followed the 5 and 50 mM H_2O_2 treatments, with values of 35.87 and 29.76 nmol min^{-1} mg $protein^{-1}$, respectively; the control fruits produced the lowest amount of cinnamic acid (20.37 nmol min^{-1} mg $protein^{-1}$).

As shown in Table 4.20, the application of various concentrations of H_2O_2 had significant effects on the anthocyanin content in wax apple fruits in all three growing seasons. During the 2008–2009 season, the highest (4.6 mg) amount of anthocyanin was observed in the 20 mM H_2O_2-treated fruits, followed by the 5 and 50 mM treatments, with values of 4.2 and 3.9 mg, respectively, whereas the control fruits showed the lowest anthocyanin content (3.0 mg/g). In the second and third seasons, H_2O_2 treatment also produced higher anthocyanin contents in the fruits (Table 4.20).

4.5.15 Soluble sugars, fruit protein, endogenous hydrogen peroxide concentration and SPS activity

The H_2O_2 treatments had a significant ($p < 0.05$) effect on fructose content of wax apple fruits (Table 4.21). The sucrose content of wax apple fruits was significantly elevated by H_2O_2 application. The highest sucrose content (7.37% Brix) was observed in the 20 mM H_2O_2-treated fruits, while the control samples contained the lowest amount of sucrose (5.53% Brix).

As shown in Table 4.21 fruit protein of wax apple also significantly ($p < 0.05$) affected by H_2O_2 treatments. The highest amount of protein (6.1 mg/g) was recorded in 20 mM H_2O_2 treatment followed by 5 and 50 mM H_2O_2 treatments with a value of 6.0 and 5.8 mg/g

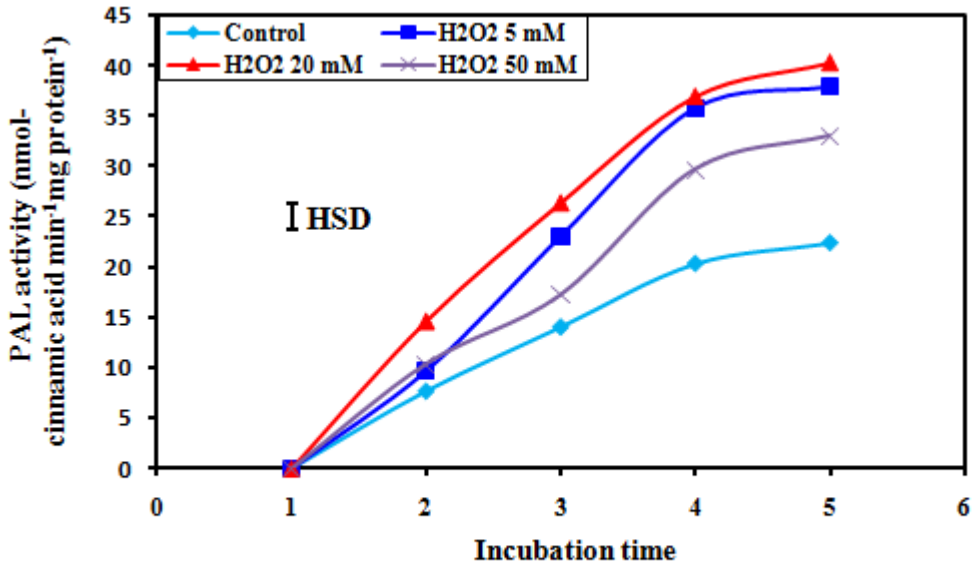


Fig. 4.51. PAL activity (nmol-cinnamic acid min^{-1} mg $protein^{-1}$) as affected by different treatment of H_2O_2 in wax apple fruit (n = 12).

Fig.4.52a. Fruit size and color of wax apple as affected by GA_3, NAA, 2, 4-D and H_2O_2 treatments.

Table 4.21 Effects of H_2O_2 treatments on the number of fruits and their juice content in *Syzygium samarangense* for three different harvest seasons.

Treatment (mM)	Fructose (%)	Sucrose (%)	Fruit protein (mg/g)	Endogenous H_2O_2 con.	SPS activity (μmol suc.mg^{-1}protein^{-1})
H_2O_2 0	5.73^{c}	5.53^{b}	5.13^{b}	0.67^{c}	8.2^{d}
H_2O_2 5	8.01^{a}	6.80^{a}	6.0^{a}	0.75^{b}	13.36^{c}
H_2O_2 20	7.28^{a}	7.37^{a}	6.1^{a}	0.99^{b}	16.33^{a}
H_2O_2 50	7.10^{b}	7.13^{a}	5.8^{a}	1.18^{a}	11.96^{b}

Values in a column sharing the same lower case letters are not significantly different at $p < 0.05$ (LSD test).

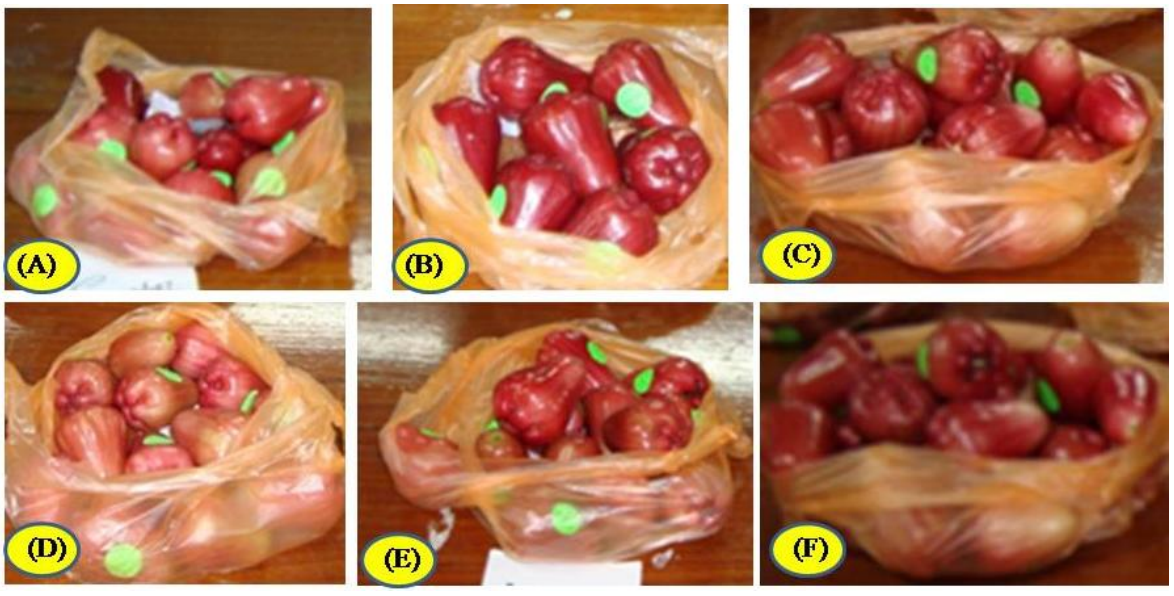


Fig. 4.52b. Fruit quality of wax apple as affected by GA_3, NAA, 2, 4-D, Girdling and H_2O_2 treatments. (A): Control, (B): 50 mg/L GA_3, (C): 10 mg/L NAA, (D): 10 mg/L 2, 4-D, (E): C-shaped girdling and (F): 20 mM H_2O_2 treatments.

protein respectively, whereas, the lowest amount of protein (5.13 mg/g) found in control fruits (Table 4.21).

The exogenous application of hydrogen peroxide significantly elevated its endogenous level in wax apple leaves (Table 4.21). These results show that, the levels were 1.12-, 1.48-, and 1.76-fold higher than the control in 5 mM, 20 mM and 50 mM H_2O_2- treated leaves.

The SPS activity of treated plant leaf was significantly elevated by H_2O_2 treatments. The SPS activity increased about 1.99, 1.63 and 1.46 fold higher than the control with the 20, 5 and 50 mM H_2O_2 treatments (Table 4.21).

4.6 Effect of GA_3 on selected physiological processes of wax apple (*Syzygium samarangense*)

4.6.1. GA_3 on PAL enzyme activity

Gibberellic acid treatment had a significant effect on PAL activity in the treated fruits (Fig. 4.53). GA_3 at 50 mg/L treatment increased the PAL activity (14.67 nmol-cinnamic acid min^{-1} mg $protein^{-1}$) followed by 100 mg/L and 20 mg/L GA_3 treatments with a PAL activities of 10.59 and 9.39 nmol-cinnamic acid min^{-1} mg $protein^{-1}$) yield respectively, whilst, the control treatments produced the lowest PAL activity 8.15 nmol-cinnamic acid min^{-1} mg $protein^{-1}$).

The application of various concentrations of GA_3 had a significant effect on the PAL activity as a function of time (Fig. 4.54). As can be seen in Figures 4.54 gibberellic acid treated fruit produced significantly the highest amount of cinnamic acid yield over time. Thirty minutes after treatment, the highest cinnamic acid yield was 36 nmol-cinnamic acid min^{-1} mg $protein^{-1}$ observed in the 50 mg/L GA_3 treated fruits followed by 20 and 100 mg/L GA_3 treatments with cinnamic acid yields of 34.98 and 30.59 nmol-cinnamic acid min^{-1} mg $protein^{-1}$ respectively, whereas, the lowest cinnamic acid yield of 20.37 nmol-cinnamic acid min^{-1} mg $protein^{-1}$ was observed in the control treatment (Fig. 4.54).

Higher concentration of GA_3 showed the negative effects on PAL activity, probably as a result of decrease in the respiratory activity and a delay in anthocyanin synthesis and chlorophyll degradation. From the results, it was observed that at different stages of fruit development on PAL activity varied (Fig. 4.55). As can be seen in Figure 4.51, at the early fruit developmental stage PAL activity a little bit high, then decreased up to mature green stage and subsequently increase up to fully ripened red stage. From the results, it can be

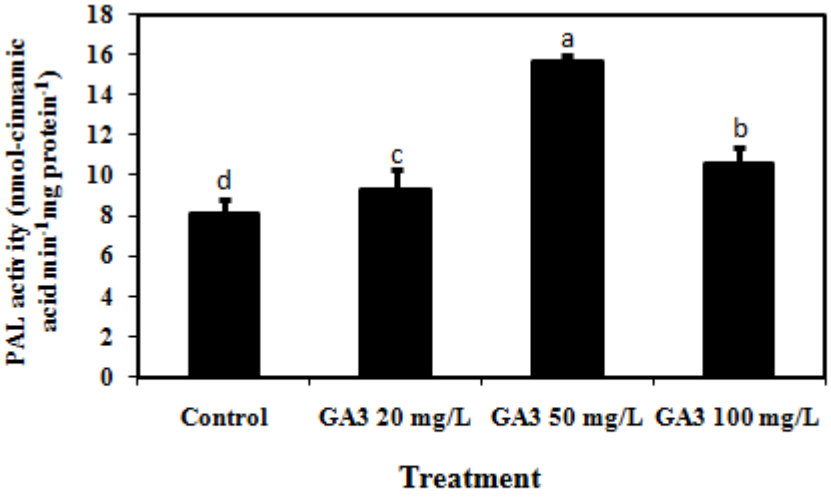


Fig. 4.53. PAL enzyme activity of wax apple fruit (nmol-cinnamic acid min^{-1}mg $protein^{-1}$) at 1 min of observation with GA_3 treatments. Bars indicate ± S.E. and values (n = 6) followed by the same letter were not significantly different at $p < 0.05$.

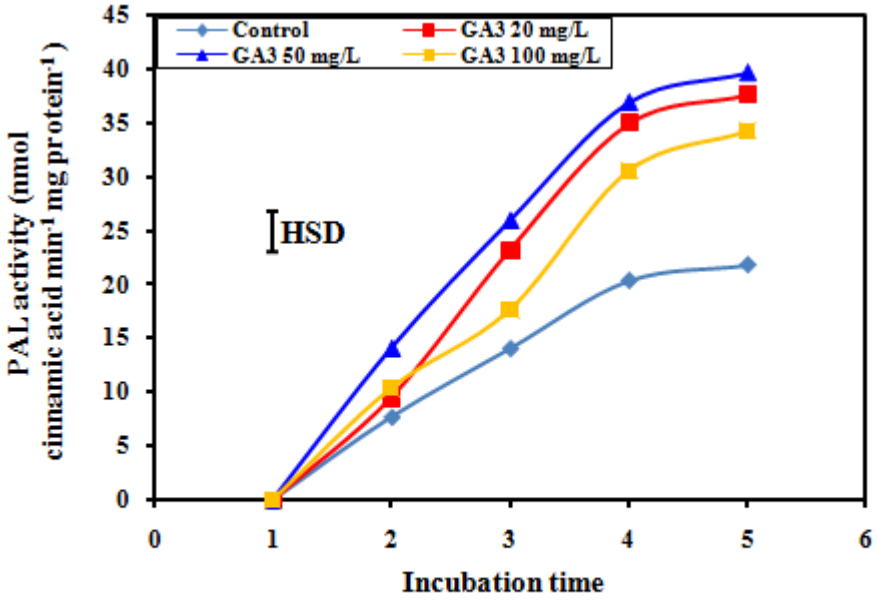


Fig. 4.54. PAL activity (nmol-cinnamic acid min^{-1} mg $protein^{-1}$) as affected by different treatment of GA_3 in wax apple fruits (n = 6).

seen that PAL activity was highest in ripening stage and the lowest PAL activity were recorded in mature green stage (Fig. 4.55).

Fortyfive minutes after treatment, the highest cinnamic acid yield was 28.85 nmol-cinnamic acid min^{-1} mg $protein^{-1}$, observed in the red ripen fruits followed by green and color turning stage with cinnamic acid yields of 24.38 and 22.98 nmol-cinnamic acid min^{-1} mg $protein^{-1}$ respectively, where as the lowest cinnamic acid yield of 21.28 nmol-cinnamic acid min^{-1} mg $protein^{-1}$ was observed in the mature green fruit (Fig. 4.56).

4.6. 2 GA_3 on anthocyanin content

The application of various concentrations of GA_3 had a significant effect on the anthocyanin content of wax apple fruits (Fig. 4.57). The anthocyanin contents of fruits showed a strong correlation with the GA_3 concentrations applied. The highest amount of anthocyanin was observed in 50 mg/L treated fruits followed by treatment with 100 and 20 mg/L, whereas untreated control fruits showed the lowest anthocyanin content. It can be seen that different stages of fruit development had a significant effects of anthocyanin accumulation in the fruits. In early fruit developmental stage anthocyanin accumulation was very low until 3 weeks after anthesis, then accumulation started to increased until full ripen stage (Fig. 4.58).

4.6.3 Correlation between anthocyanin and PAL activity

Figure 4.59 shows the relationship between PAL activity and the anthocyanin content of the *S. samarangense* fruits studied. A high correlation between the PAL activity and anthocyanin content was observed (r = 0.93).

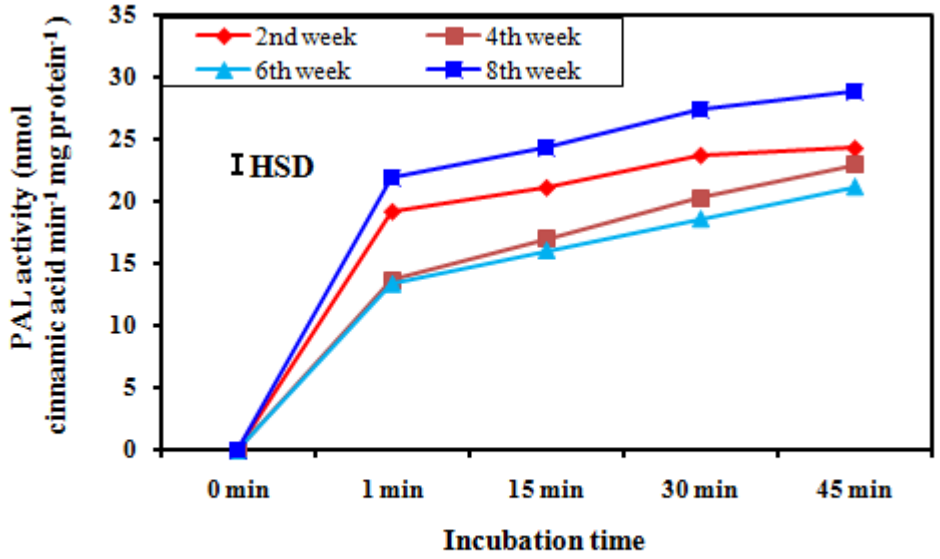


Fig. 4.55. PAL activity (nmol-cinnamic acid min^{-1} mg $protein^{-1}$) as affected by different growth stage of wax apple fruits (n = 6).

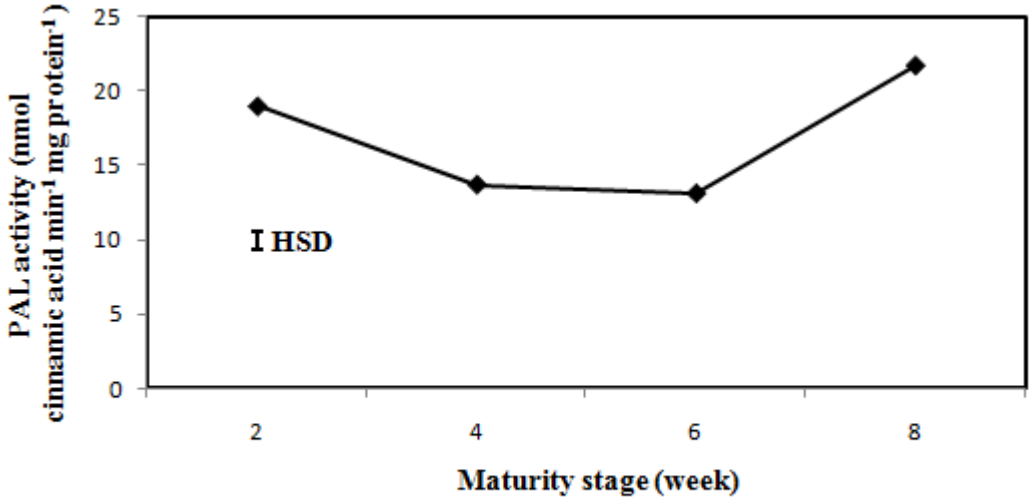


Fig. 4.56. PAL activity (nmol-cinnamic acid min^{-1} mg $protein^{-1}$) at different maturity stages of GA_3- treated wax apple fruit (n = 6).

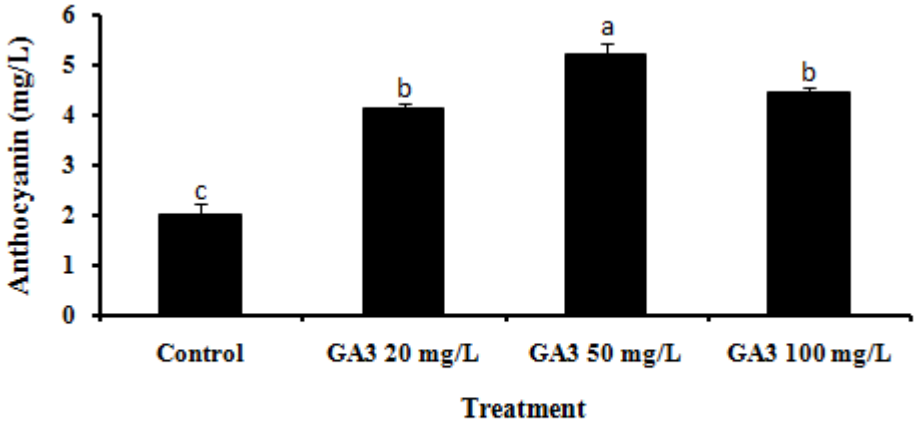


Fig. 4.57. The effect of treatments of GA_3 on anthocyanin content in wax apple fruits. Bars indicate ± S.E. and values (n = 6) followed by same letter were not significantly different at $p < 0.05$.

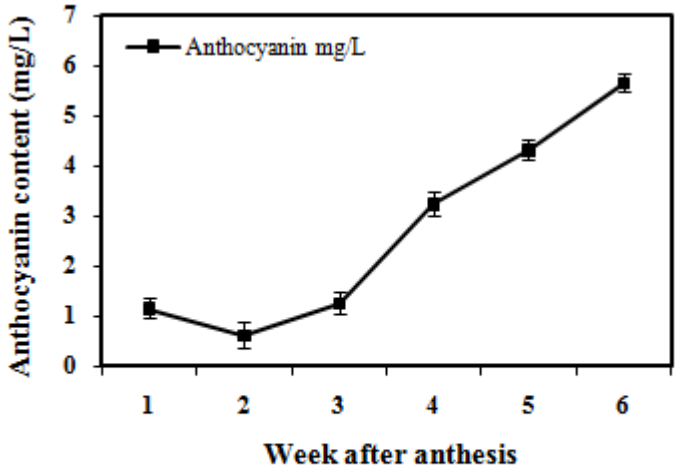


Fig. 4.58. The effect of different stages of development on anthocyanin content in wax apple fruits. Bars indicate ± S. E. and n = 6.

4.6.4 GA_3 on photosynthetical characteristics

GA_3 treatments increased the photosynthesis activity considerably and these were statistically significant ($p < 0.05$). The activities were 1.67, 2.54 and 2.60- fold higher than control at 350 ppm CO_2 and light intensities of 400, 800 and 2000 µmol m^{-2} s^{-1} respectively, in the leaves treated with 100 mg/L GA_3 (Fig. 4.60). Leaf photosynthesis was the highest with the 50 mg/L treatment followed by the 100 and 20 mg/L GA_3 treatments, whereas the control leaves produced the lowest photosynthesis activity.

As can be seen in Figure 4.61, GA_3 treatments produced a significant effect on the stomatal conductance of leaves. The highest stomatal conductance (0.052 mol H_2O $m^{-2}s^{-1}$) was recorded with 50 mg/L GA_3 treatment, followed by 100 and 20 mg/L treatments, with a value of 0.045 and 0.040 mol H_2O $m^{-2}s^{-1}$ respectively, at a light intensity of 400 µmol m^{-2} s^{-1}; whereas the control was the lowest (0.023 mol H_2O $m^{-2}s^{-1}$). Similar trends of stomatal conductivity were also recorded at 800, 1200 and 2000 µmol m^{-2} s^{-1} of light intensities.

From the results, it can be seen that GA_3 treatments had a significant effects on transpiration rate of leaves of wax apple. The highest transpiration rate was recorded in 100 mg/L GA_3 treatment followed by 20 and 50 mg/L treatments, whilst the control leaves showed the lowest transpiration rate at a light intensity of 400 µmol m^{-2} s^{-1}. Similar trends were also recorded in different light intensities (Fig. 4.62).

A strong correlation ($r = 0.80$) was observed between the stomatal conductance and net photosynthesis in GA_3 50 mg/L treatment. Results showed that net photosynthesis rate increased proportionally with stomatal conductance (Fig. 4.63).

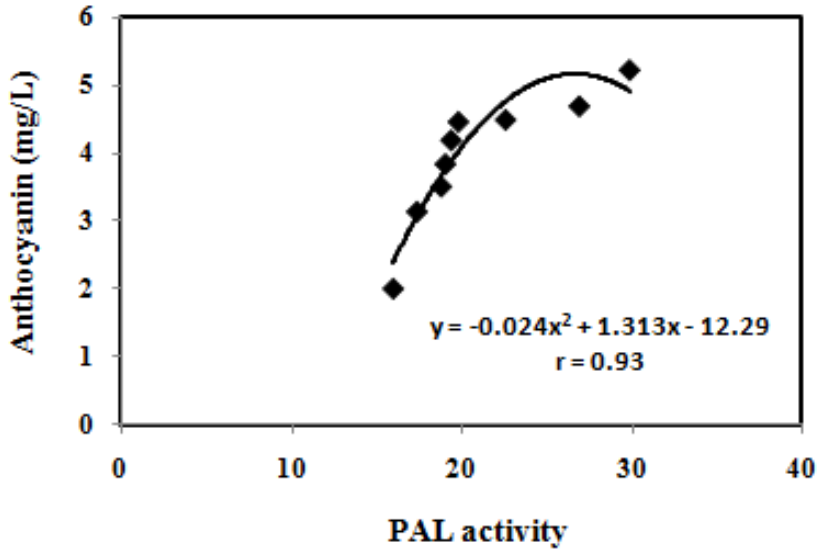


Fig. 4.59. Correlation between PAL activity (nmol-cinnamic acid min^{-1} mg $protein^{-1}$) and anthocyanin content in 50 mg/L GA_3-treated wax apple fruits.

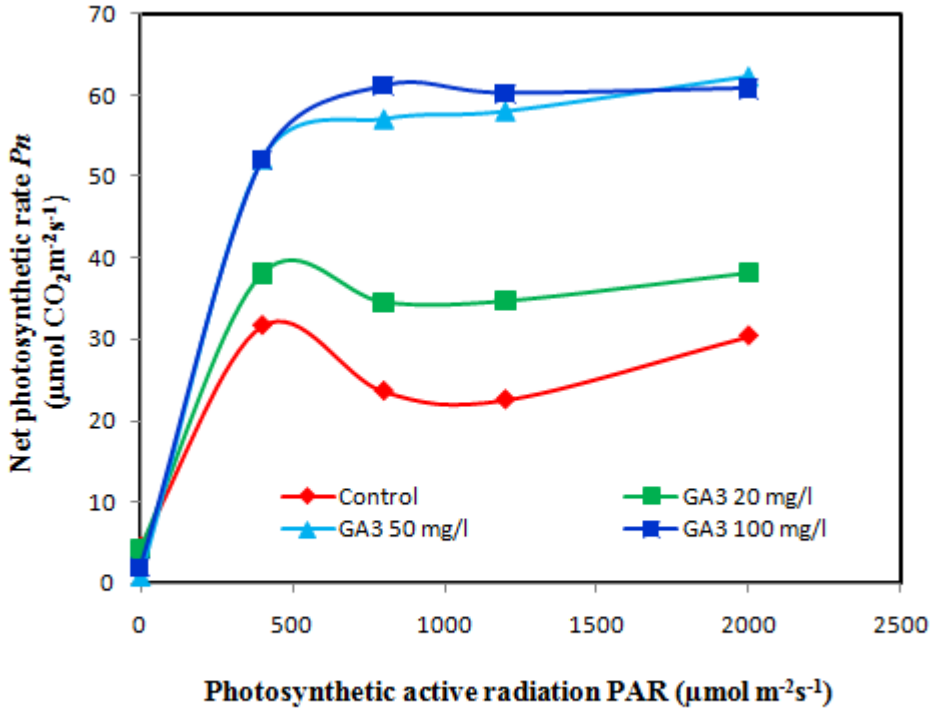


Fig. 4.60. Effects GA_3 treatments on net photosynthesis of wax apple trees under field condition (n = 12).

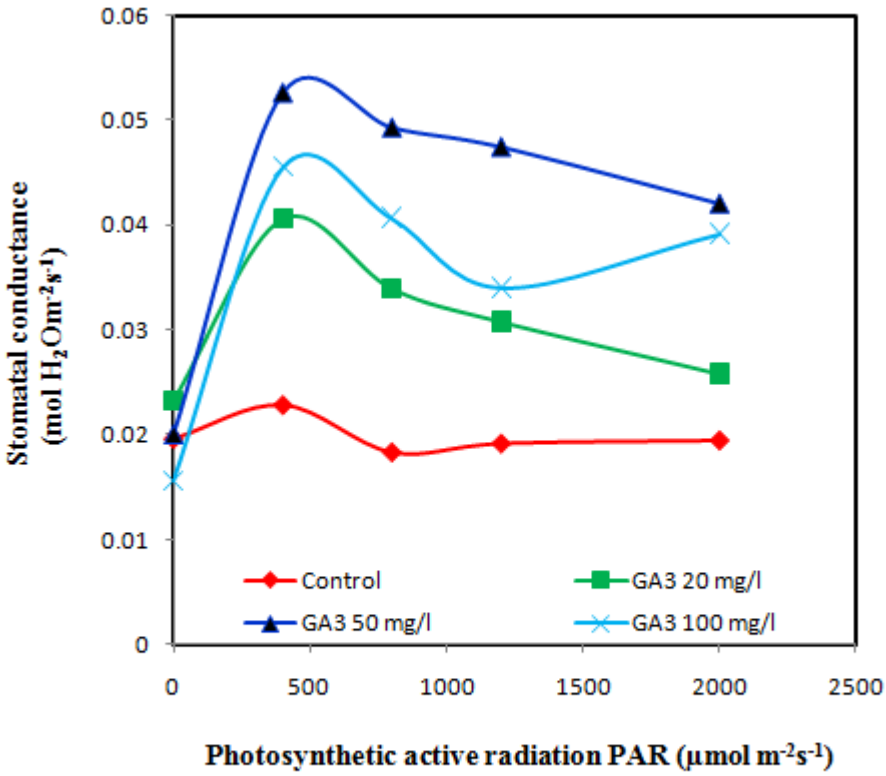


Fig. 4.61. Effects GA_3 treatments on stomatal conductance of wax apple trees under field condition (n = 12).

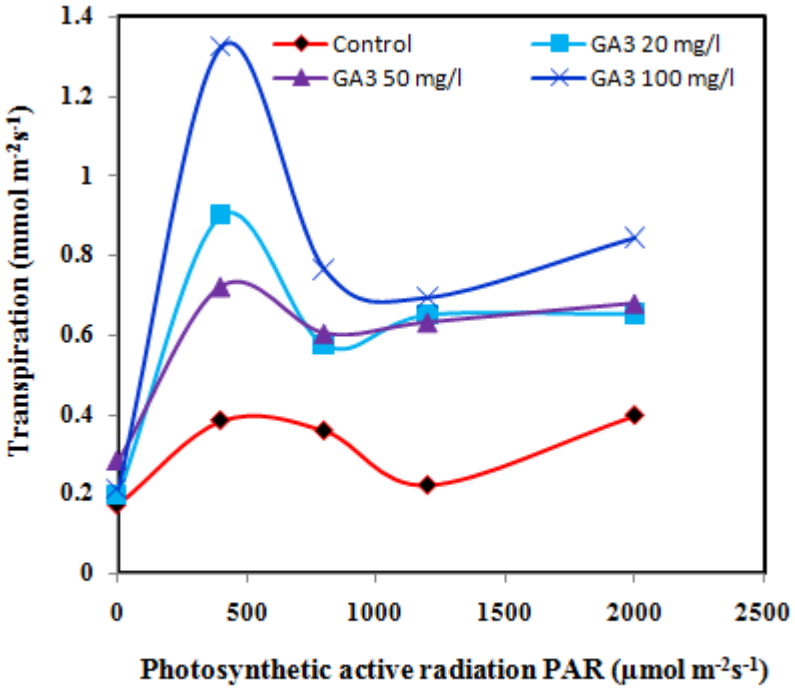


Fig. 4.62. Effects of GA_3 treatments on transpiration of wax apple trees under field condition (n = 12).

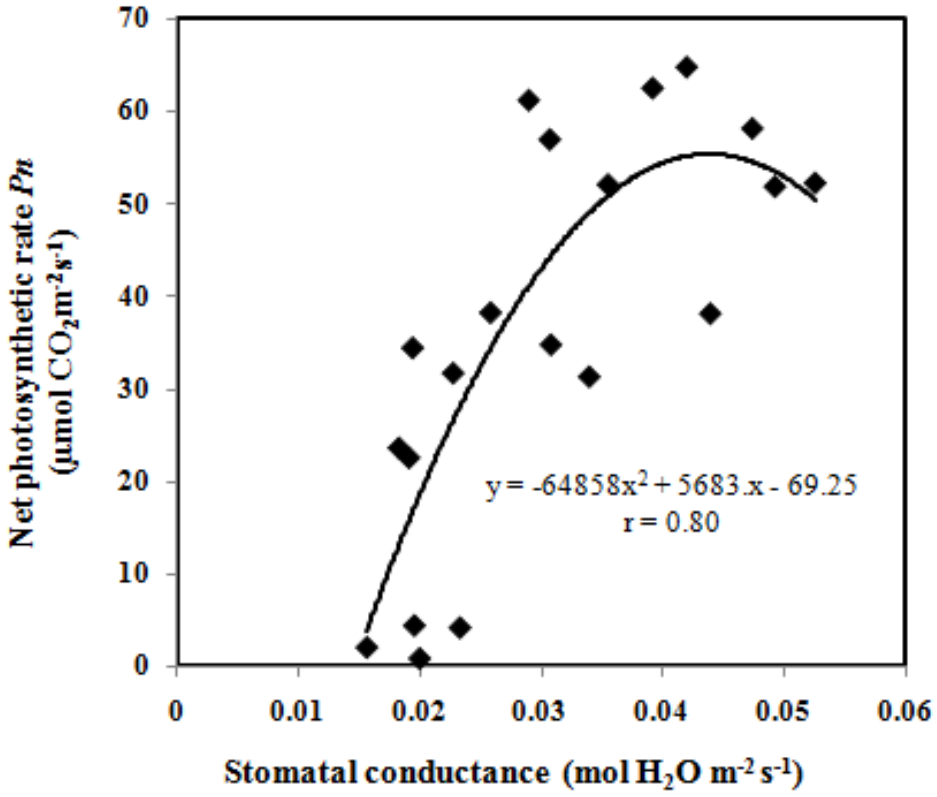


Fig. 4.63. The correlation between stomatal conductance and net photosynthetic rate in GA_3-treated wax apple trees.

As can be seen in Figure 4.64, transpiration had a strong relation (r = 0.88) with the net photosynthesis in GA_3 50 mg/L treatment. It also observed that photosynthesis rate increased proportionally with the transpiration.

4.6.5 GA_3 on sucrose phosphate synthase (SPS) activity

The sucrose phosphate synthase activity (SPS) was also significantly affected by GA_3 application (Fig. 4.65). The highest SPS activity (20.67 µ mol mg^{-1} protein h^{-1}) was recorded in 50 mg/L GA_3 treatment followed by100 and 20 mg/L GA_3 treatments, respectively, with a parallel enzyme activities of 18.33 and 15.00 µ mol mg^{-1} protein h^{-1}. The lowest SPS activity (10.67 µ mol mg^{-1} protein h^{-1}) observed in control treatment.

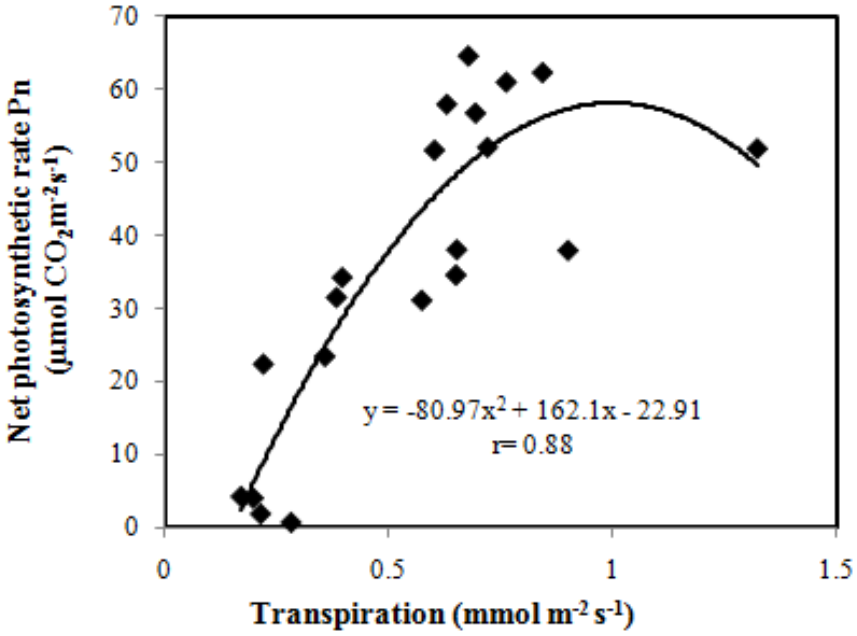


Fig. 4.64. The correlation between transpiration and net photosynthetic rate in GA_3-treated wax apple trees.

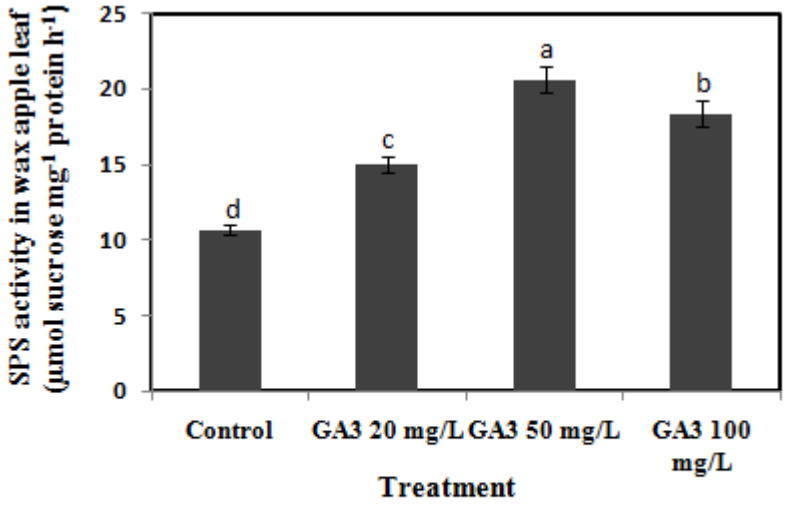


Fig. 4.65. Effect of GA_3 treatment on SPS activity in wax apple leaves. Bars indicate ± S.E. and values (n = 6) followed by the same letter were not significantly different at $p < 0.05$ (LSD test).

CHAPTER 5

DISCUSSION

The effect of gibberellic acid

Nowadays, plant growth regulators are becoming very important as an effective and often relatively low cost means of improving crop production (Ali *et al.*, 2004). From this study, it was observed that GA_3 treatments produced siginificant effects on reduction of bud drop. This result for the reduce of bud drop were in agreement with the reports of Almeida *et al.* (2004). They reported that endogenous hormones and their balance play a modulating role in the mobilization of nutrients to the developing organs and can influence the longevity of a bud in oranges. It is well documented in the literature that gibberellic acid is used widely in horticultural crops for improving fruit set. In this study, all the GA_3 treated branch posted significantly higher fruit set values compared with the control which recorded about 29% fruits set per branch. Choi *et al. (*2002) also reported that gibberellic acids (GA_3) treatments significantly increased fruit set and growth in apple, pear and cherry fruit. All the GA_3 treated branches registered significantly higher fruit set values compared with the control. Recently, Davies and Zalman (2006) reported that 2, 4-D and GA_3 significantly increased the total number of fruits, fruit weight per plant by reducing pre-harvest fruit drop. Similarly, the positive effects of GA_3 on reducing fruit drop on wax apple fruit was also observed in the current experiment.

Previous studies have reported that GA_3 treatment can increase fruit weight, peel thickness and fruit diameter of Valencia oranges (Almeida *et al.*, 2004). Leyla *et al.* (2006) reported that application of GA_3 increased the biomass yield, seed yield and yield of soybean. In this study, GA_3 significantly affect the fruit weight and yield of wax apple. These findings for the fruit weight and yield were in agreement with that of Basak *et al.*

(1998) who observed that GA_3 significantly influenced the fruit weight as well as yield in cherry. Spraying GA_3 once at full bloom to some Indian litchi cultivars at 50 - 100 mg/L of GA_3 were effective in improving fruit size (Singh and Lal, 1980). The 50 mg/L GA_3 treated fruits grew at a faster rate and were larger than the control fruits. Singh and Lal (1980) also described a similar trend in Yu Her Pau' litchi over two years in Taiwan. They also hypothesized that sprays of GA_3 during stage I (growth of pericarp and seed coat) of fruit growth would increase fruit and aril weight. In this study the chlorophyll *a,* chlorophyll *b* and total chlorophyll contents were determined using the methods described in Hendry and Price (1993). GA_3 had a significant effect on chlorophyll content in the leaves. These results are supported by the findings of Lim *et al.* (2003). They reported that mepiquat chloride and GA_3 alone or combined, increased leaf area and chlorophyll content in apple. Fruit juice content, which is related to fruit size is an important parameter in industrial processing. Fruit size depends on genetic characteristics and cultural practices such as application of plant growth regulators. GA_3 treatment had a positive effect on juice content of wax apple fruits. These results were in agreement with that of Wang *et al.* (2004), who reported that the application of gibberellic acid at flowering and pre harvest significantly increased the juice percentage in various Citrus species. Potassium regulates the translocation of photosynthates, protein synthesis, ionic balance and plant stomatal opening and also known as a quality nutrient because of its important effects on fruit quality factors such as size, shape, color, taste, shelf life and fiber quality (Almeselmani *et al.,* 2010). The results in this study suggested that GA_3 have positive effects on K^+ content in the fruits.

One indirect measure of increase in sugar content is looking at the total soluble solids (TSS) *in* fruits. Fruits from GA_3 treatment branches yielded a higher soluble solids

content due to accumulation of sucrose. Application of growth regulators like auxin and gibberellins can significantly increased the total soluble solids contents of the fruit in sweet cherry species **(**Basak *et al.,* 1998*).* It has been well acknowledged in the literature that there is a correlation between fruit peel color and total soluble solids in many fruits. The TSS content starts to increase slightly during a period of 2 to 6 weeks after anthesis, showing a log phase increase until 8 weeks after anthesis followed by a rapid increase during fruit maturity and ripening in sweet pepper (Tadesse *et al.,* 2002). The TSS content of tomato fruits increased with gradual advancement of fruit maturity and the highest TSS was recorded in fully ripened tomatoes (Moneruzzaman *et al.,* 2008). Fruit color had a positive correlation with total soluble solids in tomato (Moneruzzaman *et al.,* 2009) and in cranberries (Mustafa *et al.,* 1995*).* The argument for this (peel color positively correlated with TSS) is that TSS is indicative of higher sugar content in the fruits and this in turn supplies the energy for the synthesis of the red colour pigments found in these fruits, as ripening sets in. It was found that peel color correlated with TSS content of wax apple. Accordingly, in our study, GA_3 treatments produced a significant effect on total sugar content of wax apple fruits. These results were similar to the findings of Wang *et al.* (2004), who found that application of 2, 4-D, GA_3 and some other growth regulators increased the sugar contents in various mandarin and sweet orange cultivars.

Fruits are rich source of phytochemicals such as vitamins, minerals and phenolic compounds. Phenolic compounds are important because they can exhibit antioxidant properties. The antioxidant properties of poly phenolic compounds is mainly due to their redox properties, which allows them to acts as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Ronald, 2001). From this study, it was clear that GA_3 treatments had a significant effect on the total flavonoids and phenolic content of wax

apple fruits. These results also showed that phenolic content had a positive correlation with antioxidant activity in GA_3 treated fruits. This statement are in agreement with the findings of Pourmorad *et al.* (2006), who reported that the extracts of *Mellilotus officinalis*, which contained the highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity. During ripening degradation of chlorophyll takes place and is accompanied by the synthesis of other pigments usually either anthocyanin or carotenoids. In this study it was observed that chlorophyll loss gradually took place with the GA_3 application at color turning stage. Similar results were reported by Perez *et al.* (1993) that the plant growth regulator methyl jasmonate promoted the chlorophyll degradation of the skin of Golden Delicious apple fruit.

Anthocyanin pigments are responsible for the red, purple and blue colors of many fruits, vegetables, cereal grains and flowers and as a result, research on anthocyanin pigments has intensified recently because of their possible health benefits as dietary antioxidants (Ronald, 2001). Carotenoids are the precursors of vitamin A and those commonly occuring in nature include, α, β and γ carotene, lycopene and cryptoxanthin (Goodwin, 1986). Moneruzzaman *et. al.* (2011) reported that the highest amount (2.75 mg/L) of anthocyanin was observed in 'Jambu madu Red' cultivar fruits followed by 'Masam manis pink' cultivar with a anthocyanins content of 2.67 mg/L, whereas, 'Giant green' cultivar fruits showed the least amount (0.95 mg/L) of anthocyanin content. Results showed that GA_3 treatment significantly increased the anthocyanin and carotene content in the fruits. These results concur with the findings of Roussos *et al.* (2009), who observed that anthocyanin content in strawberry fruit increased significantly when the plants were treated with GA_3. It is suggested that GA_3 could also play a role in the accumulation of pigments in the fruits.

An increase in ascorbic acid content in fruit is thought to be an indication that the fruit is still in the ripening stage, while a decrease indicates a senescent fruit (Esteves *et al.*, 1984). Miller and Rice Evans (1997) reported that phenolic substances have been found to play a protective effect on the ascorbic acid. The presence of phenolics in the fruit cells may help to maintain the ascorbic acid content. Pila *et al*. (2010) reported that application of GA_3 was found to be effective in reducing the rate of respiration and ethylene production and yielding higher amount of ascorbic acid. In this study, it was found that GA_3 treatments had a significant effect on ascorbic acid content in wax apple. This finding is in agreement with the results of Wahdan et al. (2011) who reported that trees sprayed with 40 ppm of GA_3 at two months after full bloom significantly increased Vitamin C in 'Succary Abiad' mango. Thakur *et al.* (1996) indicated that acidity of tomato fruits was reduced when the whole plant was sprayed GA_3 and 2, 4-D. In this study, it was observed that application of GA_3 reduced the titrable acidity in apple fruit. Andrew *et al.* (2006) also reported a similar finding that titrable acidity decreased with increasing GA in table grapes.

Finally, it can be concluded that the application of 20 and 50 mg/l GA_3 had enhanced the bud number, stimulated the fruit growth, color development and also increased the yield. Furthermore, GA_3 also reduced bud and fruit drop and increased the leaf chlorophyll contents. With regard to fruit quality, GA_3 treatment increased amount of juice, increased the TSS, total sugars, total phenols and flavonoids content in the fruits (Fig. 6.1). Anthocyanin content and antioxidant activity via the DPPH assay also observed in GA_3 treated fruits. GA_3 treated fruit also showed increased K^+ and carotenoid content.

The effect of 2, 4-Dichlorophenoxy acetic acid

Synthetic auxin (2, 4-D) enhanced the inflorescence development; fruit set and increased

the number of fruit per plant in strawberry and raspberry (Mezzetti *et al.,* 2004). These studies showed that synthetic auxin had positive effects on flower bud development. The application of 2, 4-D treatments had a significant effect on fruit drooping of wax apple. The results found are in agreement with the findings of Davies and Zalman (2006) who observed that synthetic auxin (2, 4-D) significantly reduce the fruit drop in citrus fruits. Average fruit weight was also significantly affected by 2, 4-D application. These results supported by the findings of Modise *et al.* (2008) who reported that preharvest application of 2, 4-D significantly increased the average weight by enlarging fruit size in the naval orange. Leaf dry matter (DM) was also significantly increased with 2, 4-D treatments. These results are supported by the findings of Rony (2006) who reported that auxin had positive effects on flower bud formation in *Arabidopsis thaliana*. It also can be seen that 2, 4-D treatments significantly reduced the bud dropping but at a higher doses of 2, 4-D increase abscission of flower bud. Auxin (NAA and 2, 4-D) is general growth factors involved in many developmental processes throughout the plant, nevertheless, they can play a major role in the fruit set and development (Maaike *et al.,* 2009). Results regarding with fruit set were found to be in agreement with that of Lopez-Galarza *et al.* (2004) who reported that a localized application of 2, 4-D to ovaries increased the fruit set of triploid watermelon. Plant growth regulators (PGR's) are known to have a great influence on fruit drop and fruit retention in fruit trees. An imbalance of auxins, cytokinins and gibberellins for example may lead to the formation of abscission layer at the stem point and eventually fruit drop (Chen *et al.,* 2006). The use of auxins prevents dropping of fruit by maintaining the cells at zone of abscission, preventing the synthesis of hydrolytic enzymes such as cellulase, which decompose the cell wall.

Leaf chlorophyll content is one of the most important factors that's affects the plant productivity. It has been reported that foliar spray of 2, 4-D increased the chlorophyll content of leaves, leaf area index and interception of photo synthetically active radiation in paddy (Grewal *et al.,* 2006). In this study the chlorophyll content was determined using a Minolta SPAD meter. From the results, it can be seen that 2, 4-D treated-leaf contain a higher chlorophyll content. These results supported by the results of Gutam *et al.* (2009) who reported that foliar spray of synthetic auxin increased the chlorophyll content in Bell pepper. They also observed greener leaves had a linked to possible increase in sugars resulting from the 2, 4-D and NAA treatments.

Auxin affects the fruit growth at cell division and cell enlargement phase. During the cell enlargement period, synthetic auxin increases photosynthesis and carbohydrate availability causes cell enlargement and also increases final fruit size (Agustí *et al.,* 1994). 2, 4-D treatments stimulate the fruit growth of wax apple though the fruit developmental stage and it was significantly different from control. These results found in concurrent with findings of Arteca (1996) who observed that 2, 4-D application stimulate carbohydrate translocation to the fruits and increasing cell wall elasticity. Likewise fruit length, fruit diameter was also significantly increased by 2, 4-D treatments. Similar findings was reported by Raphael *et al. (*2007) who reported that exogenous application of synthetic auxin raised the carbohydrate level in the fruit and increased fruit size in Bing cherry.

Chlorophyll fluorescence has become one of the most powerful and widely used techniques available to plant physiologist and ecophysiologist. Chlorophyll fluorescence gives information about the state of photosystem II. Our results of this study showed that 2, 4-D treatment had a significant effect on leaf photochemical efficiency. Results regarding photochemical efficiency were found to be in consonance with that of Zhang *et al.* (2003)

who reported that plant growth regulators increased the photochemical efficiency (Fv/Fm). Stomatal conductance affects the photosynthesis rate by regulating CO_2 fixation in leaf mesophyll tissue also accumulation of drymatter content in plants. Stomatal conductance was measured by a Leaf Porometer in a sunny day (800- 1500 µE $m^{-1}s^{-1}$) at fruit developmental period. Results showed that application of 2, 4-D treatments significantly increased the stomatal conductance of leaves. These results were found to be in concurrence with that of Nahar and Takeshi (2002) who observed that synthetic auxin (figaron) increase the stomatal conductance at a lower concentrations but it decreased at a higher concentrations in soybean.

It has been reported that 2, 4-D treatments increased the fruit yield at a lower concentration (20 mg/L) but at a higher concentration (30 and 40 mg/L) it reduced the yield by increasing the premature dropping (Modise *et al.*, 2009). In the case of wax apple, synthetic auxin also had a significant effect on yield and it was positively correlated with stomatal conductance. These finding were found to be in agreement with those of Raphael *et al.* (2007) who reported that 2, 4-D treatment significantly increased the fruit yield in loquat fruit.

Total soluble solids (TSS) of fruit is one of the important parameters that strongly affect consumer acceptability of a variety. The application of 2,4-D treatments significantly increased the TSS content of fruit juice, which was positively correlated with peel colour of fruits and the highest TSS was observed at a fully red ripen stage. These results showed an agreement with the results of Raphael *et al.* (2007) who reported that 2, 4-D increased the TSS contents in Bing cherry. Mustafa *et al.* (1995) also reported that fruit color had positive relation with TSS content in cranberries.

In this study, growth regulators had positive effects on the firmness of wax apple fruits. Similar findings were reported by Iqbal *et al.* (2009), who showed that application of synthetic auxin significantly increased pulp firmness in loquat fruit.

Soluble sugars such as glucose and sucrose play a central role in metabolism also regulate many developmental and physiological processes in plants (Yu, 1999). Fruit development and sugar content depend on sugar accumulation and metabolism within the fruit. Sugar accumulation within a fruit, or translocation of photosynthate, is driven by the sucrose concentration gradient from source to sink (Islam, 2001). Kataoka *et al.* (2009) reported that auxin application at the anthesis period increased the amount of sugar content in tomato fruit. The total sugars results obtained were in an agreement with the results of Baogang *et al.* (2007) who observed that treatments of 2, 4-D increased the total sugars contents in mango fruits.

Potassium promotes root growth, and provides key metabolic features including the formation of starch, protein synthesis, translocation of sugars, stomata regulation, and the formation of xylem vessels. It also increased the size, shape, color, taste and improved the shelf life of fruits (Almesemani *et al.,* 2000). It was observed that 2, 4-D treatments has a positive effects on potassium content of fruit juice.

It has been reported that the concentration of phenolic compounds, such as flavanols and anthocyanins in fruits differ with cultivars, maturity stage, environmental conditions and the part of the fruit (Drogoudi *et al.,* 2008)**.** Phenolics posses a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability to modify the gene expression (Floridi *et al.,* 2003). Flavonoids have diverse beneficial biochemical and antioxidant effects (Donald and Cristobal, 2000). In fruits, flavonoids may contribute in a number of ways to fruit quality, for instance to traits

such as color, flavor, bitterness or texture. The results showed that application of 2, 4-D increased phenols and flavonoids content in wax apple fruits.

Different parts of the plant and plants at different stages of maturity yield different levels of antioxidants. The antioxidants that are present in fruits and vegetables plays an important role in the maintenance of human health and prevention of disease. It has been reported that application of 2, 4-D and kinetin *in vitro* culture increased the antioxidant activity of sorghum (Awika and Rooney**,** 2004). It can be seen that 2, 4-D had a positive effect on antioxidant of wax apple fruits. These results are supported by the findings of Baogang *et al.* (2008) who reported that 2, 4-D treatment during postharvest storage of Mango moderately increased the antioxidant enzyme activity. A high correlation between flavonoids and antioxidant activity was found in the 10 mg/L 2, 4-D treated wax apple fruits. These results showed a concurrence with the findings of Pourmorad *et al.* (2006) who reported that the extract of *M. officinalis*, which contain the highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity. Anthocyanin are responsible for attractive colors of fruit and vegetables such as the red, purple, and blue and research on anthocyanin has intensified recently because of their possible health benefits as dietary antioxidants (Ronald, 2001). It is acknowledged in the results, that 10 mg/L 2, 4-D produced significant effect on anthocyanin content in wax apple fruits. Similar results were reported by Tsukasa *et al.* (1994) who observed that 2, 4-D treatments increase the anthocyanin content in strawberry fruits.

The effect of naphthalene acetic acid

These studies showed that 5 mg/L NAA treatment increased the flower bud formation in the treated branch of wax apple, which was statistically significant between

the treatments and control. Aloni (2006) also reported similar effects of NAA on the flower bud formation in *Arabidopsis thaliana*. As can be seen from the results, the application of 10 mg/L NAA significantly increased the fruit set in wax apple trees. Similar results reported by Maaike *et al.* (2009) in tomato fruits.

The use of growth regulators has become an important component of agrotechnical routings practices for most of the cultivated plants and especially for fruit plants (Monselise, 1979). Auxins and gibberellins are used to control the fruit drop in citrus and to improve the quality of fruits (Almeida *et al.*, 2004). Treatments with NAA reduced the fruit drop from the control treatment, although their differences were not significant. This may be effect due to environmental factor; especially heavy rain before harvesting that enhanced fruit drop significantly. Davies and Zalman (2006) reported that NAA significantly reduced the fruit drop in citrus fruits.

Dubay *et al.* (2002) reported that NAA sprayed at 250 mg/L resulted in a higher yield and improve the quality in Allahabad Sufeda guava. Treatment with 10 mg/L NAA had a significant effect on total yield. These results were found to be in agreement with that of Amorós *et al.* (2003) who reported that NAA treatment significantly increased the fruit yield in loquat fruit. Furthermore, NAA had a significant effect on dry matter accumulation in the fruits.

It has been reported that foliar spray with NAA increased the chlorophyll content in the leaves (Grewal *et al.*, 2006). This study shows that chlorophyll content, which can indirectly indicate the health status of a plant, was slightly higher in the leaves of NAA treated branches. These results supported by the findings of Gutam *et al.* (2009) who reported that foliar spray of NAA increased the chlorophyll content in Bell pepper. It has been reported that NAA affects the inflorescence development and number of fruits per

plant (Mezzetti *et al.,* 2004). The application of NAA significantly increased wax apple fruits number and 1.7 times yield than the control. These results were found to be in an agreement with that of Amorós *et al.* (2003) who reported that 10 mg/L NAA treatment increased the total number fruits as well as yield in loquat.

Several previous studies have reported that auxins level in the fruit that can promote the sink potential of the fruits, which is in direct proportion of to the rate of fruit growth (Miller *et al.,* 1987). From the study it was observed that, NAA treatment enhanced the wax apple fruit growth though the whole developmental period, resulted bigger fruit size, which was statistically difference from the control. These results were supported by the findings of Agusti *et al.* (2000). They reported that exogenous application of synthetic auxin raised the carbohydrate level in the fruit and as a result increased fruit size in citrus.

Additionally, colors or the pigments in fruits and vegetables reflect the presence of certain biologically active phytochemical compounds and antioxidants that reportedly can promote good health. Results revealed that wax apple fruit color development was greatly enhanced by the NAA treatments used in this study, and it was significantly difference from control treatment. Similar findings reported by Agusti *et al.* (2000) who observed that synthetic auxin enhanced the fruit color development in loquat fruit.

It is documented in the results that, NAA had a significant effect on chlorophyll fluorescence of wax apple leaves. Similar findings are reported by *C*zerpak *et al.* (2002) who found that synthetic auxin stimulated the chlorophyll synthesis as well as increased the chlorophyll fluorescence. NAA-treated leaves showed the highest stomatal conductance then the control one, this was statistically significant. These results were found to be in agreement with that of Nahar and Takeshi (2002) who observed that

synthetic auxins increase the stomatal conductance in soybean. It was also observed that NAA-treated leaves maintain the highest drymatter content. From the results of 10 mg/L treatment, it was observed that stomatal conductance had a strong correlation with wax apple fruit yield. Similar results were observed by Lu *et al.* (1998). They reported that yield had a positive correlation with stomatal conductance in Pima cotton (*Gossypium barbadense*) and bread wheat (*Triticum aestivum*).

Total soluble solids (TSS) of fruit is an important sweeten indicator that can strongly affect the fruit quality. Results showed that NAA treatments increased wax apple fruits TSS significantly from the control treatment. These results showed an agreement with that of Yaday *et al.* (2002) who reported that application of NAA at 60 mg/L can significantly increased the TSS contents in guava fruits. It has also been found that peel colour of NAA-treated wax apple fruit was positively correlated with TSS content of fruits. Similar results were observed by Mustafa *et al.* (1995) who reported that fruit color had a positive correlation with total soluble solids in cranberries.

As it is well known during and after photosynthesis, sugars namely sucrose, are exported from the source leaves to other plant parts (Islam, 2001). Sugar is an important parameter of quality measurement in fruits as they are main and ready source of energy when used by human. Kataoka *et al.* (2009) reported that auxin application at the anthesis period increased the amount of sugar content in tomato fruit. In this study, it was found that 10 mg/L NAA treatments increased the total sugar content in the fruits and which was significantly different from the control. These results are in an agreement with the findings of Yaday *et al.* (2002) who observe that treatment of guava trees with 60 mg/L NAA increased the total sugar contents in fruits relatively to untreated control trees.

It is well documented that potassium plays several important regulatory roles in the plant cell (Ismail, 2005). It is involved in and regulates the translocation of photosynthates, protein synthesis, ionic balance and plant stomata opening. It is also involved in the activation of plant enzymes and several other processes. Potassium is also known as a quality nutrient because of its important effects on fruit quality factors such as size, shape, color, taste, shelf life and fiber quality (Almeselmani *et al.,* 2010). Results showed that NAA application increased the potassium content in the fruit juice significantly.

Fruits are rich source of phytochemicals such as vitamins, minerals and phenolic compounds and they can exhibit antioxidant properties. Rice-Evans *et al.* (1997) stated that the antioxidant properties of phenolics is mainly due to their redox properties, which allow them to acts as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators. It has also been reported that the total polyphenolic content as well as nutritional content in the berries can be increased with auxins (Elisa *et al.,* 2007). From our results, it was observed that synthetic auxin had a significant effect on total phenolics content in the fruits of wax apple. Donald and Cristobal (2000) reported that flavonoids are polyphenolic compounds found in fruits and vegetables that have diverse beneficial biochemical and antioxidant effects. It is well acknowledged that flavonoids impart color and taste to flowers and fruits, and it is expected that humans consume between a few hundred milligrams and one gram of flavonoid everyday (Pieta, 2000). The results showed that application of NAA significantly increased the flavonoids content of wax apple fruits. It has been reported that NAA treatment increased the total antioxidant capacity as well as nutritional quality in transgenic silcora seedless grape (Elisa *et al.,* 2007). In this study, the antioxidant capacity of fruits was determined using TEAC and DPPH methods and significant differences were recorded in treated and control fruits. It also found that total

phenols and antioxidant activity were positively correlated in the fruits. These results are in synchronization with the results of Pourmorad *et al.* (2006) who reported that the extract of *M. officinalis*, which contain highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity.

It has been reported that anthocyanin pigments are responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers and as a result, research on anthocyanin pigments has intensified recently because of their possible health benefits as dietary antioxidants (Ronald, 2001). From these results, it can be seen that NAA application enhanced the anthocyanin formation significantly as well as improved the quality of fruits of wax apple. Our findings were supported by the results of Shulman and Lavee (2005) who reported that NAA enhanced the accumulation of anthocyanin content in olive fruits.

Girdling effects

It is well documented in the literature, from an agronomic point of view, that practices such as girdling can improve carbohydrate availability to fruits, consequently leading to an increase in fruit-set and yield (Goren *et al*., 2003; Rivas *et al*., 2004). The immediate effect of a girdle is to interrupt the movement through the phloem of photosynthates produced by leaves. Girdling treatment increased the accumulation of carbohydrate content in the upper part of girdle (Chun *et al.,* 2003). The increase in carbohydrate level in the leaves a well correlated with the fruit retention. Different girdling treatments especially C and V-shapes increased the fruit retention capacity from other treatments and control. The increase in fruit retention with girdling application may be ascribed to increased level of carbohydrates, especially during initial 4-6 weeks of heavy fruitlet abscission. Our results are supported by the findings of Shao *et al.* (1998). They

reported that girdling treatment increased the C/N ratio and carbohydrate content thus reduced the fruitlet abscission and increased the fruit retention of citrus. Girdling has been practiced to increase productivity in many fruit trees (McNeil, 2001). The increase in fruit size demonstrated here in response to girdling application at the three weeks before flowering may indicate their ability to stimulate carbohydrate translocation to the fruit in combination with their effect on increasing cell wall elasticity. Thus, our results could be in agreement with the finding of Mustafa and Saleh (2006), who reported that girdling alone or with potassium spray increase the fruit size and fruit weight in Balady mandarin orange. The girdle branch increased the leaf and fruit drymatter than the non-girdle branch (Famiani *et al.,* 2000). Consequently, we can conclude that girdling treatment increased the leaf and fruit drymatter of wax apple (Table 4.11 and 4.13). Chen *et al.* (2009) reported similar results in Oolong Tea. They stated that girdling significantly increased the dry matter content in the leaf as well as increased the quality of leaf. Fruit weight is an important quality parameter of fruit production. Bark ringing or girdling significantly increased the fruit weight as well as yield (Hossain *et al.,* 2007). By contrast, in the current study, different girdling treatment significantly increased the fruit weight (Fig.4.34). Our results are supported by the findings of Juan *et al.* (2009). They reported that scoring one type of girdling significantly increased the fruit weight in persimmon. Girdling can improve carbohydrate availability to fruits and as a consequent lead to an increase in fruit-set and yield as well as number of fruits (Goren *et al.,* 2003; Rivas *et al.,* 2004). In this context, all the girdled branches produced the higher number of fruits than the untreated control fruit (Fig.3). Thus, our results could be in agreement with the findings of Casanova *et al*. (2009), who observed that scoring (girdling) had no negative effect on the number of

harvested bunches per vine the year following the scoring year, both in ‘Emperatriz’ and ‘Aledo’ cultivars.

This increases foliar carbohydrates (sugars and starch) and plant hormones in above parts of the girdle which enhances the flowering (Roper and Williams, 1989). Our results showed that girdling treatments significantly reduced the flowering time as well as enhanced inflorescence development of wax apple. Similar observations have been reported by Arakawa *et al.* (1997) in apple, who reported that girdling enhanced the inflorescence development.

Girdling treatments increased the total number of bud from the control but their differences were not statistically significant. Similar observations have been reported by Arakawa *et al.* (1997) in apple. They reported that girdling significantly increased flowering the following spring. Different types of girdling exhibited the lowest bud abscission and increase the fruit set. Similar findings were reported previously in ‘Ponkan’ mandarins by Mataa *et al*. (1998). Branch girdling, which interrupts the phloem pathway and hence disrupts the transport of carbohydrates in and out of the branch, has been utilized experimentally for control dropping as well as increase the fruit setting of apple (Priestly, 1976). The increase in carbohydrate supply caused by girdling has been shown to correlate well with a transient reduction in fruitlet abscission. Among the other different factors, poor tree nutrition is a crucial factor which can cause bud and fruit drop (Stepenson *et al.,* 1986).

From the results, it can be seen that all the girdling treatments posted significantly higher fruit set and reduced the fruit drop compared to the control in wax apple. It is well recognized in the literature that girdling can improve photosynthesized availability and

increase fruit-set and yield in citrus fruit (Goren *et al*., 2003; Rivas *et al*., 2004). It is well documented that girdling is considered to be an important horticultural practice responsible for improving fruit setting, yield as well as the physical and chemical properties of various fruits through the accumulation of carbohydrates and natural plant hormones above the girdling rings. Regarding fruit growth (length and diameter), all the girdling treatments stimulate fruit growth significantly. This growth trend was observed throughout the fruit developmental period until the harvesting period. Similar results were reported by Carreño *et al.* (1998). They reported that girdling enhanced the fruit growth, colour development and ripening of 'Italia' grapes. Di Vaio *et al.* (2001) reported that the faster fruit growth rate observed could be the result of the accumulation of carbohydrates above the girdle. They also reported that girdling has a positive effect on berry size in grapes. One of the possible causes for the fruit enlargement is the increase in sucrose levels, a few days after girdling which would enhance its availability for cell division and growth of fruitlets (Iglesias *et al.*, 2006).

It has been reported that girdling had a positive effect on berry size (Carreño *et al.*, 1998). In this study, all the girdled-branch produced higher fruit weight and yield than the untreated control. Lahav *et al*. (1996) reported that a general increase in yield of the girdled branch was observed in the first season, after girdling in avocado trees. Allan *et al.* (1993) reported that girdling resulted in a greater number of fruits, larger and of desirable marketable size (>90 g) than the control, in the low chill peach cultivar, Florida prince. After the first season the girdled branches regenerated new phloem to fill the gap left by the girdle as a healing process. After a few months, regenerated phloem will make a connection between upper and lower part of girdling ring.

Girdling has been shown to alter the partitioning of photosynthates, mineral nutrients and plant growth regulators in trees (Mataa *et al*., 1998). It would be interesting to see how this in turn affects, if at all, the leaf chlorophyll content. As shown in the result, the leaf chlorophyll content from all the treated branches were higher than the control branch of wax apple. The greener leaves observed could be linked to possible increase in sugars resulting from the girdling and stress treatments, although this has to be investigated further. Furthermore the partitioning of photosynthates, mineral nutrients and plant growth regulators as a result of such treatments could also have had an effect.

Girdling treatments significantly increased both total and chlorophyll *a* compared with the untreated trees (Mostfa and Saleh, 2006). Accordingly, in this study, girdling increased the leaf chlorophyll *a*, *b* and *a*+*b*. In addition, several authors have proposed that total leaf carbohydrate content and starch increased as a result of girdling (Rivas *et al.*, 2008). It may be due to the accumulation of chlorophyll content and increased photosynthesis in the girdled branch. Chlorophyll fluorescence has become one of the most powerful and widely used techniques available to plant physiologist and ecophysiologist. Chlorophyll fluorescence gives information about the state of photosystem II (PS-II). In this study, different types of girdling showed a significant difference in case of chlorophyll fluorescence and quantum yield of wax apple. Rivas *et al.* (2008) reported similar results in citrus cultivars. They reported that girdling did not affect the chlorophyll fluorescence in mature leaf but in case of young leaves of chlorophyll fluorescence start to increase from 30 DAG. They also reported that girdling increased the quantum yield of PS-II.

It has been shown that the application of sugars, especially sucrose, improves the color of skin disks of wax apple (Liaw *et al.*, 1999). Recently it was reported that trunk girdling stimulated fruit color development and fruit softening after harvest of Japanese

persimmon (Kazutoshi *et al.,* 2009). From the results it can be seen that a significant difference was observed in peel color development among different treatments and control of wax apple. Matsui *et al.,* (1979) reported similar results in grapes. They reported that anthocyanin and sugar accumulation increased proportionately with increasing leaves per cluster of girdled shoots.

Application of girdling did not produce significant difference in case fruit juice of wax apple but fruit drymatter was significantly higher in treated fruits compared to control. The leaf DM content analyzed on the 5^{th} week of fruit development also showed significant differences between the treatments and control. Sharif *et al.* (2006) found similar results in peach trees. They reported that carbohydrate transport from leaves to roots through phloem was reduced with girdling. This in turn will suppress food movement to the lower parts of the plant or tree and increase growth in the upper regions of the plant.

In this study, the different types of girdling did not produce significant effect on potassium content in fruit juice. The TSS content of fruit juice was found to be statistically significant between the different treatments and control (Table 4.14). These results are in agreement with the findings of Iwahori *et al.* (1976). They reported that fruit quality enhancement in ponkan mandarin oranges, with regard to fruit color and soluble solids, in phloem ringed plants compared to the control. Verreynne *et al.* (2001) reported that girdling enhanced fruit color, total soluble solids and total sugar content in Marisol' Clementine's. Fruits from the girdle branch yielded the higher amount total sugars which may be due to carbohydrate availability and starch content high in upper part of girdle. Total sugar content in the fruits of wax apple greatly increased with the girdling treatments and it was statistically significant between the treatments and control. These results are in

agreement with the findings of Kazutoshi *et al.* (2009). They reported that sugar content increased in Japanese persimmon, in phloem ringed plants compared to the control.

Phenolic compounds in fruits are important because of their antioxidant properties. Girdling as a treatment had a significant effect on total phenolic content in wax apple fruit. These findings are in agreement with those of Kubota *et al.* (2001). They reported that girdling significantly increased the PAL enzyme activity and total phenolic content in the peach fruits. Flavonoids have diverse beneficial biochemical and antioxidant effects (Donald and Cristobal, 2000) and they impart color and taste to flowers and fruits (Pietta, 2000). In our study, it has been shown that girdling treatments increased total flavonoids content in the fruits of wax apple. These findings are supported by the results of Harsimranjit *et al*. (2008), who observed that girdling increased the flavanols content in the grape. In this context, we also analyzed the effects of girdling on antioxidant activity of the wax apple fruits. Girdling enhanced the antioxidant activity of fruits (Rivas *et al.,* 2008). Girdling enhanced color development, ripening and also had positive effects on anthocyanin accumulation in fruits (Downey *et al.*, 2006). Consequently, we also observed similar effects of girdling on anthocyanin accumulations in the wax apple fruits. This variation in the accumulation of anthocyanins in the berry skin due to the girdling effect, may be girdling stimulated the activity of F 30, 50-hydroxylase enzyme (a key *enzyme* for the expression of blue or purple color) thereby producing higher levels of trisubstituted anthocyanins in the berry skin compared to control. Perhaps, girdling of clone 314 enhanced the anthocyanin accumulation in the berry skin by stimulating the supply of photosynthates to the grape berries.

These results also showed that flavonoid with antioxidant activity had a positive correlation in the girdle fruits of wax apple. These findings are in agreement with the

results of Ghafar *et al.* (2010). They reported that polyphenolic compounds (phenols and flavonoids) had a strong correlation with antioxidant activity of *Citrus hystrix* fruits and flavonoid and phenolic contents can be used potentially as a readily accessible source of natural antioxidants.

The effect of hydrogen peroxide

The results for the changes in leaf dry matter of wax apple are in agreement with the report by Pilar *et al.* (2009), that injecting hydrogen peroxide into the soil significantly increased the biomass of the aerial portions of the avocado (*Persea americana* Mill.).The accumulation of chlorophyll content (SPAD) and soluble protein were also induced after treatment with 5 or 20 mM hydrogen peroxide. Higher concentrations of H_2O_2 decreased the leaf chlorophyll content in rice leaves (Upadhyaya *et al.,* 2007). However, the negative effect of hydrogen peroxide on chlorophyll content of wax apple was not observed in this study. The lowest incidence of bud drop in the hydrogen peroxide-treated plants is an indication of positive effects on bud development. Similar findings were obtained by Webber *et al.* (2007), who reported that the application of H_2O_2 to container-grown nasturtium flowers enhanced bud development and increased the number of flowers. Souza *et al.* (2004) also reported that hydrogen peroxide increased floral receptivity in passion fruits, which ultimately increased fruit set.

Hydrogen peroxide is involved in many developmental processes throughout a plant; nevertheless, it can play a major role in fruit set and premature fruit drop (Ozaki *et al.,* 2009). Recently, it was reported that the application of hydrogen peroxide improved yield as well as the physical and chemical properties of melon fruits through the accumulation of carbohydrates in the leaves (Ozaki *et al.,* 2009). Similar positive effect of hydrogen peroxide was also observed on wax apple yield (Table 4.17). Injecting H_2O_2

through the irrigation water has been reported to increase biomass and yield in zucchini, soybean, and cotton (Bhattarai *et al.*, 2004).

Several previous studies have reported that hydrogen peroxide treatment can increase fruit size (Bryce *et al.*, 1982); tomato fruit growth increased when H_2O_2 was added to the irrigation water, and fruit biomass was significantly increased by this treatment. H_2O_2 may function as a signaling molecule involved in the formation, growth and development of adventitious roots in cucumber (Shiweng *et al.*, 2007). The colours, or pigments, in fruits and vegetables reflect the presence of certain biologically active phytochemical compounds and antioxidants that reportedly promote good health. Hydrogen peroxide treatments significantly increased the peel colour of wax apple fruits in this study, possibly because of an increment in the accumulation of pigment in the skin. In this study also obtained positive effects on PAL enzyme activity with hydrogen peroxide (Fig. 4.51).

In rice seedlings, it has been shown that the activity of sucrose phosphate synthase (SPS), an enzyme important in the formation of sucrose from triose phosphates during and after photosynthesis, can be induced at the transcriptional level by treatment with hydrogen peroxide (Uchida *et al.*, 2002). Subsequently, Ozaki *et al.* (2009) reported sugar enrichment in melon fruits treated with hydrogen peroxide. In this study, it was also found that H_2O_2 treatment significantly increased photosynthetic characteristics, i.e., net photosynthesis, stomatal conductance and transpiration, in the wax apple plant (Figs. 4.40, 4.41 & 4.42). Ozaki *et al.* (2009) also reported similar positive effects of H_2O_2 on photosynthesis in melon plants. Stomatal conductance affects the photosynthetic rate by regulating CO_2 fixation in leaf mesophyll tissue and is positively correlated with photosynthesis. An indirect measure of increased sugar content in fruits is the total soluble solids (TSS) content. Fruits from hydrogen peroxide-treated branches had higher soluble

solids contents due to the accumulation of sucrose. The application of hydrogen peroxide can significantly increase the total soluble solids contents in melon fruits (Ozaki *et al.*, 2009). Shin *et al.* (1998) reported that increased photosynthetic activity enhances the yield and sugar content in fruits. It has been well documented in the literature that there is a correlation between skin colour and total soluble solids in many fruits. Moneruzzaman *et al.* (2008) reported that the TSS content of tomato fruits increased with the gradual development of fruit maturity, and the highest TSS was recorded in fully ripened tomatoes. Fruit colour also had a positive correlation with soluble solids in tomato (Moneruzzaman *et al.,* 2009). A likely explanation for this relation is that TSS is indicative of higher sugar content in the fruits, and this sugar in turn supplies the energy required for the synthesis of the red colour pigments found in these fruits as ripening sets in.

As mentioned earlier, during and after photosynthesis, sugars, namely sucrose, are exported from the source leaves to other plant parts. Sucrose is synthesised in the cytosol from triose phosphates made in the Calvin cycle and exported from the chloroplasts, where it is converted into fructose 6-phospate which combines with UDP-glucose to form sucrose phosphate, catalysed by sucrose phosphate synthase. As noted above, it has been shown that sucrose phosphate synthase (SPS) activity in rice seedlings can be induced at the transcriptional level by treatment with hydrogen peroxide (Uchida *et al.,* 2002), a nonspecific signalling molecule involved in the responses to various abiotic stresses (Prasad *et al.,* 1994). Furthermore, adaptations to abiotic stresses involve complex signal transduction pathways, which, along with its components, may lead to increased soluble sugar contents (Carvajal *et al.,* 2000). Accordingly, in our study, hydrogen peroxide had a significant effect on total sugar content in wax apple fruits. In melon plants treated with 20 mM hydrogen peroxide, the greatest increases in the contents of the soluble sugars

fructose, glucose, and sucrose in the leaves were 256, 294 and 288 %, respectively, those of the untreated control plants (Ozaki *et al.,* 2009).

Potassium regulates the protein synthesis, stomatal opening, maintain ionic balance and translocation of photosynthates from source to sink (Ismail, 2005). Potassium is also known as a quality-determining nutrient because of its important effects on fruit quality factors such as size, shape, colour, taste, shelf life and fibre quality (Almeselmani *et al.,* 2010). Our results suggested that hydrogen peroxide at low concentrations had positive effects on the K^+ content in the fruits (Table 4.17). Phenols and flavonoids are polyphenolic compounds found in fruits and vegetables that have diverse beneficial biochemical and antioxidant effects (Donald and Cristobal, 2000). From our findings, it is clear that hydrogen peroxide treatment had significant effects on the phenols and flavonoids contents of the wax apple fruits (Fig. 6.1). No supporting statements were found in the literature for this finding. Hydrogen peroxide has been reported to play an important role in regulating the course of ripening and senescence in fruits and vegetables (Hodges and Forney, 2000). This result also showed that the flavonoid contents were positively correlated with antioxidant activity in hydrogen peroxide treated fruits. This finding is in agreement with those of Pourmorad *et al.* (2006), who reported that extracts of *Melilotus officinalis* containing the highest amounts of flavonoid and phenolic compounds exhibited the greatest antioxidant activity.

H_2O_2 has been reported to be associated with fruit development, ripening and senescence (Woods *et al.,* 2005). Anthocyanin pigments are responsible for the red, purple, and blue colours of many fruits, vegetables, cereal grains, and flowers, and as a result, research on anthocyanin pigments has intensified recently because of their possible health

benefits as dietary antioxidants (Ronald, 2001). From our results, it is clear that H_2O_2 treatment enhanced PAL enzyme activity in the fruits; thus, it may stimulate anthocyanin accumulation in wax apple. Carotenoids are the precursors of vitamin A, and those commonly occurring in nature include α, β and γ carotene, lycopene and cryptoxanthin (Goodwin, 1986). Our results showed that hydrogen peroxide treatment significantly increased the anthocyanin and carotene contents in the fruits, suggesting that hydrogen peroxide may also play a role in the accumulation of fruit pigments of wax apple (Table 4.20).

Ozaki *et al.* (2009) reported that application of hydrogen peroxide to melon plants increased photosynthesis. They also reported that photosynthates are either converted into starch or exported from chloroplast to cytosol for soluble sugar synthesis resulting in an enhanced level of soluble sugar and starch in melon leaves as well as increased dry weight of shoots and fruits. Shin *et al.* (1998) observed that increased photosynthetic activity in melon leaves during CO_2 enrichment condition enhanced the yield and soluble sugar content in fruits. Hydrogen peroxide treatment increased the invertage activity and enhanced sucrose synthesis of melon plants (Ozaki *et al.*, 2009). As can be seen from the results hydrogen peroxide treatment produced the significant effects on photosynthesis, stomatal conductance and transpiration of leaves of wax apple. This finding are supported by the results of Ozaki *et al.* (2009) who reported that treatment of melon plants with a suitable concentration of hydrogen peroxide did not decrease the plant growth and fruit yield, albeit increased the soluble sugar content in leaves and fruits of the melon plants, thus improving the fruit quality.

An enhanced SPS activity can be closely associated with an increase in sucrose accumulation in plant parts. Uchida *et al.* (2002) reported that H_2O_2 treatment increased the

SPS activity and sugar content of tomato and melon plants without any negative effects on plant growth or fruit productivity. Similarly, our results showed that H_2O_2 treatment increased SPS activity of treated wax apple trees. We also got a positive correlation between net photosynthesis and SPS enzyme activity in treated wax apple leaves. This is not surprising since the Calvin cycle of photosynthesis provides the carbon skeletons for sucrose and starch synthesis during the day and the control both these pathways are tightly regulated. Higher rates of photosynthesis provide higher amounts of phosphoglycerate (PGA) which in turn provide more fructose 6-phosphate and in turn glucose 6-phosphate which increases sucrose synthesis via SPS activity.

The effect of gibberellic acid on selected physiological process of wax apple

Leaf chlorophyll content provides valuable information about physiological status of plants. It has been reported that GA_3 increased the leaf area and chlorophyll content in apple leaves (Tables 4.2 & 4.3). GA_3 treatments significantly increased the leaf chlorophyll content in wax apple leaves. These results were found to be in agreement with those of Stefanov *et al.* (1998) who observed that application of gibberellins increased the chlorophyll and protein content in the leaves of maize. Ilias *et al.* (2007) reported that chlorophyll fluorescence are linearly correlated with the functionality of PSII, where Fo and Fm are the chlorophyll fluorescence yields corresponding to open and closed PSII reaction centre. GA_3 treatments produced the significant effects on chlorophyll fluorescence and quantum yield or photosynthetic yield of wax apple leaves (Table 4.2). Georgia, *et al.* (2010) also suggested that application of GA_3 increased the maximum quantum yield of primary photochemistry (Fv/Fm) and the ratio of Fv/F0 in *Capsicum annum* L.

Protein content was determined from the selective leaves of treated branches. From the results, it can be seen that gibberellin treatment had a significant effect on soluble protein in leaf. Lu *et al.* (2010) reported similar results in rapeseed and stated that in stress condition gibberellin could increase soluble protein content in leaves. Phenylalanine ammonia-lyase (PAL) is one of the key enzymes in controlling anthocyanin biosynthesis from phenylalanine (Tucker, 1993). McGlasson *et al.* (1978) suggested that gibberellin (GA_3) have an important incidence on the biosynthesis of the anthocyanins. Results showed that application of GA_3 increased the activity of PAL enzyme in wax apple fruits that was statistically difference from the control. These results were found to be in consonance with that of Terasa *et al.* (1998) who reported that GA_3 increased the PAL activity in strawberry plant. Higher concentration of gibberellin showed the negative effects on PAL activity, probably as a result of decrease in the respiratory activity and a delay in anthocyanin synthesis and chlorophyll degradation. From the results, it can be seen that PAL activity (cinnamic acid yield) increased with the incubation time (Fig. 4.54). Similar findings reported by Yueming *et al.* (2003) who reported that PAL activity increase versus time in strawberry fruits.

It has been reported that PAL can be induced by a wide range of factors including wounding, temperature, lights and chemicals and can be induced at any fruit developmental stages (Tucker, 1993). Along with GA_3 treatments, different fruit developmental stages had a significant effect on PAL activity in wax apple fruits. These findings are in agreement with those obtained by Sujitra *et al.* (2005). They reported that PAL activity decreased at early stage and increased at the final stage of fruit maturity in mangosteen. It also be stated that PAL activity increased with the incubation time. Shigeki

et al. (1981) also reported similar findings and stated that PAL activity (cinnamic acid yield) increased with the incubation time in *Rhodotorula glutinis.*

The GA_3 treated wax apple fruit produced more anthocyanin compared to control (Fig. 4.57). This was statistically significant between the treatments and control. These results concur with the findings of Roussos *et al.* (2009) who observed that anthocyanin content in strawberry fruit increased significantly when the plants were treated with GA_3 hormone. Fruit pigmentation is one of the significant aspects of fruit quality and in most of the fruits, the accumulation of anthocyanin is developmentally regulated (Li *et al.,* 2001). Results showed that in early fruit developmental stages anthocyanin accumulation is very low until three weeks after anthesis, after that accumulation started to increase until full ripen stage of wax apple. These results were found to be in agreement with that of Cheng *et al.* (1991) who observed that in early stages of fruit development chlorophyll and phenolic content is high but anthocyanin level is very low. They also stated that anthocyanin accumulation stated to increase at full maturity stages until fruit ripen condition. Tucker (1993) observed that PAL activity high only in the red parts of the apple skin and concluded that PAL activity was closely related to the formation of anthocyanin. A strong correlation between the PAL activity and anthocyanin accumulation was found among the GA_3 treated wax apple fruits. These results are in agreement with the results of Sujitra *et al.* (2005) who suggested that PAL controlled the rate of anthocyanin synthesis in mangostene.

It has been argued that photosynthesis is probably the most important metabolic process on earth and is certainly the most important process to understand in order to maximize plant productivity (Dean, 1994). Loss of chlorophyll in leaves of alstroemines flower is strongly delayed by GA_3 and enhanced the photosynthetic activity (Jordi *et al.,*

1993). Ashraf *et al.* (2002) reported that application of GA_3 increased the photosynthesis in salt-stressed wheat, which was a major factor for greater drymatter production. A stimulatory effect of applied GA_3 on photosynthesis was also observed by Yuan and Xu (2001) in broad bean and soybean. There few contradictory results about GA_3 effects on photosynthesis. Application of GA_3 reduces the photosynthetic rate but promoted the growth (Dijkstra *et al.,* 1990). From this study, it can be seen that GA_3 treatments had a significant effects on photosynthesis of wax apple (Fig. 4.60). These results are found to be agreement with the results of Huber and Sankhla, (1973). They also reported that gibberellin treatments enhanced the activity of ribulose diphosphate carboxylase in leaves. Growth regulators auxin and cytokinin also stimulated the rate of photosynthesis (Treharne and Stoddart, 1970).

Tamas *et al.* (1972) reported that IAA increased photosynthesis of chloroplast through enhancing photophosphorylation. Furthermore, Hoad *et al.* (1977) reported that a change in GA_3 and cytokinin level in grape fruit was observed in response to fruit removal and ultimately the rate of photosynthesis was altered.

Stomatal conductance regulates the photosynthesis and transpiration, which are exchange processes, inside and outside, in which CO_2 and water molecules are transported, respectively. Liang *et al. (*1999) reported that stomatal movement controls mesophyll cells water, CO_2 exchange, and water use efficiency. It has been observed that long-term treatment of GA_3 increased stomatal conductance and photosynthetic CO_2 uptake rate in leaves of bean (Marcelle *et al.* 1974). From the results of this study, it was observed that GA_3 treatments had a stimulatory effect on stomatal conductivity of wax apple leaves. Björkman (1981) reported that GA_3 induced the improvement in photosynthetic carbon

fixation, thus seems to be the only cause of the increase in photosynthesis. It has also been stated that application of GA_3 had a significant effect on transpiration rate of leaves.

It has been shown in our study that GA_3 treatments increased the SPS activity of wax apple plants. Hubbard *et al.* (1989) also found positive relationships between SPS activity and sucrose accumulation in melon. PGRs treatments may also increased the invertage activity, the increase invertage activity suggests for sucrose synthesis and vice versa due to increased photosynthetic product in treated leaves. The increased SPS activity could raise not only sucrose level but also glucose and fructose levels in leaves and fruits of wax apple. It is suggested from this study that, PGRs treated fruits accumulated the high percentage of sugar, polyphenolic compound and antioxidant substances in fruits, thus increase its taste, flavour as well as quality.

RubisCoase is a key enzyme controlling photosynthetic carbon fixation of plants and it is believed that the quantity of activated RubisCoase is closely related to the rate of photosynthetic carbon assimilation (Björkman, 1981). He also stated that there was a good correlation between the light-saturated leaf photosynthetic rate and the activity of RubisCoase. Seemann and Berry (1982) reported that the increase in photosynthetic rate after GA_3 short term treatment can be attributed to the enhancement of Rubisco content and activity. They also stated that GA_3 treatments increased the synthesis of the Rubisco protein in leaves.

CHAPTER SIX

CONCLUSIONS

In the preceding chapters, substantial results on the effects of plant growth regulators, horticultural techniques and growth regulating chemicals for improving growth, development and quality in the wax apple fruit have been established. This study reinforces the use of these selective horticultural techniques to develop the wax apple fruit industry in tropical countries. It has shown that selected horticultural techniques could be used for improving the growth, yield and the nutritional status of the wax apple fruit in tropical climate. It is hoped that this study will be a catalyst to further research on tree physiology and fruit production.

6.2 Characteristics affected by horticultural techniques

The effects of gibberellin, synthetic auxin, hydrogen peroxide and girdling on wax apple fruit growth, development and quality were performed during 2008-2011 under field conditions. Based on the findings of this study, the following conclusions can be made:

6.2. 1. The effect of GA_3, 2, 4-D and NAA on fruit growth and development

Significant enhancement of fruit growth and development were recorded in *S. samarangense* in response to plant growth regulators application. With GA_3, these improvements included increased fruit set and reduced fruit drop (4.1.2), enhanced fruit growth (4.1.3), color development (4.1.4) and increased average fruit weight and yield (4.1.5). Leaf size, width and photosynthetic quantum yield (4.1.6), chlorophyll content (4.1.7) and soluble protein (4.1.8) were also significantly affected by GA_3 treatments. Similarly 2, 4-D treatments significantly reduced the bud drop and increased fruit set

(4.2.1), in addition to increasing yield and fruit weight by reducing premature fruit drop (4.2.2). Chlorophyll fluorescence and photosynthetic quantum yield (4.2.5) and stomatal conductance (4.2.6) increased at lower 2, 4-D concentration (5 or 10 mg/L). The results also showed that bud number, reduction of bud and fruit drop, and fruit set were significantly affected by NAA treatments (4.3.1). Faster fruit growth (4.3.3) and the greatest improvement in fruit color and appearance were observed at 10 mg/L NAA treatment. Increased stomatal conductance, quantum yield and fruit dry matter were observed in treated plant (4.3.6). A high correlation between stomatal conductance and yield was observed (4.3.7). All the growth regulators treatments posted faster fruit growth, color development and yield, possibly due to an increment in carbohydrate content in the developing fruits after growth regulator application.

6.2.2 The effect of GA_3, 2, 4-D and NAA on fruit quality

GA_3 treatment significantly increased fruit juice (4.1.9), potassium and total soluble solids (TSS) (4.1.1), total sugar (4.1.12) and glucose, fructose and sucrose (4.1.14) content of wax apple. The lowest amount of titrable acidity and the highest sugar acid ratio (4.1.15) and vit-C content (4.1.16) were also recorded in treated fruit. It was also observed that GA_3 treated fruits contained the highest phenol, flavonoid, carotenoid and anthocyanin content (4.1.13). Significant variations were recorded with regard to TSS (4.2.7), anthocyanin (4.2.8) and total sugar content (4.2.9) in 2, 4-D-treated fruits. The highest TSS (4.3.8), total sugar (4.3.9), potassium content (4.3.10) and antioxidant activity (4.3.12) were observed in the NAA-treated fruits. The results also showed that soluble protein (4.3.14), phenol and flavonoid (4.3.11) and anthocyanin content (4.3.15) of wax apple increased significantly with NAA treatments. A high correlation between peel color and TSS, flavonoid content and antioxidant activity (4.117), phenolic content and antioxidant

activity (4.3.12) and anthocyanin content and PAL activity (4.3.15) were observed in 50 mg/L GA_3, and 10 mg/L NAA treated fruits.

6.2.3 The effect of girdling on fruit growth and development

C-shaped girdling was observed to have a significant effect on inflorescence development and fruit retention (4.4.2) and bud number (4.4.1), flower and fruit number (4.4.8). Fruit set of girdled branches increased significantly (4.4.3), possibly due to accumulation of carbohydrate in the upper part of girdling. Girdling treatments (C-shaped and I-50 %) reduced the bud and fruit drop (4.4.4), enhanced faster fruit growth and development (C and V-shaped) (4.4.5), thus, finally increased the fruit size, length and diameter ratio considerably (4.4.6). Chlorophyll content (4.4.10), chlorophyll fluorescence and quantum yield (4.4.11) increased notably in C- and V- shaped girdled branches. The results showed that girdling treatments had a significant effects on average fruit weight, fruit dry matter content and yield of wax apple fruits (4.4.7). The highest fruit color development was observed in C and V-shaped girdling treatments.

6.2.4 The effect of girdling on fruit quality improvement

Fruit juice, an important quality factor, significantly increased in girdling treatments (except 100%) employed in this study (4.4.12). Fruit biomass (fresh) of wax apple fruit was also affected by C and V-shaped girdling treatment (4.4.12). The highest total soluble solids (TSS) content was recorded in C-shaped girdled fruit. Remarkable change was seen in the potassium content of girdled fruit juice (4.4.13). In this study, the C- and V-shaped girdling significantly increased total sugar content in wax apple fruits in all three seasons studied (4.4.14). Total phenol, flavonoid contents and antioxidant level of wax apple fruit

were also significantly affected by girdling treatment (4.4.15). There was a high correlation between total flavonoid content and antioxidant activity in C-shaped girdle fruit (4.4.16).

6.2.5 The effect of H_2O_2 on fruit growth and development

From the results of this study, it was observed that the tested concentrations of hydrogen peroxide, particularly 5 and 20 mM H_2O_2, can improve the growth and development as well as the nutritional status of the wax apple fruit (*Syzygium samarangense*). Treatment with 20 mM H_2O_2 improved fruit set and reduced bud drop (4.5.3), enhanced fruit growth (4.5.4), and increased total number of fruits, yield and dry matter content in fruits (4.5.6). The exogenous application of H_2O_2 also increased its endogenous levels (4.5.1). Additionally, the application of 5 mM of H_2O_2 gave promising results with respect to leaf net photosynthesis, stomatal conductance and transpiration (4.5.2). Furthermore, higher leaf chlorophyll, soluble protein and dry matter content were also recorded in H_2O_2 treatments (4.5.1). Fruit color development was also significantly improved by hydrogen peroxide treatment (4.5.5).

6.2.6 The effect of H_2O_2 on fruit quality

With regards to fruit quality, fruit juice and biomass content were significantly affected by H_2O_2 treatment (4.5.6). Total soluble solids (4.5.7), total sugar, glucose, fructose and sucrose content in H_2O_2-treated wax apple fruit increased considerably in all the seasons studied (4.5.8). The results showed that, 20 mM H_2O_2 treatment enhanced K^+ Content (4.5.9), phenol and flavonoid content in the fruits (4.5.10). In addition, antioxidant activity in the fruits was significantly increased with H_2O_2 treatment (4.5.11). The highest PAL activity, anthocyanin content (4.5.14) and carotenoid content (4.5.13) were recorded in 20 mM H_2O_2-treated fruits. The results also showed that H_2O_2-treated fruits exhibited

positive correlations between peel color and TSS (4.5.5) and between phenol and flavonoid content and antioxidant activity (4.5.12).

6.2.7 The effect of GA_3 and H_2O_2 on photosynthesis and PAL and SPS activity

In this study, it was observed that both GA_3 and H_2O_2 provided the best results with regard to effect on fruit growth, development and quality. It was found that both increased PAL activity an enzyme in anthocyanin synthesis, which probably explains how it affected the color development and flavonoid and phenolic content seen. Net photosynthesis, stomatal conductance and transpiration in wax apple plants was also significantly affected by GA_3 treatment (4.6.4) and H_2O_2 (4.5.2). It was also observed that sucrose phosphate synthase (SPS) activity increased with GA_3 application. SPS activity was high in GA_3 treated leaves, implying that net photosynthesis as well as the productivity of treated plants increased significantly after treatment, as SPS activity has been shown to cometate with photosynthesis in these studies.

6.2.8 Correlations among fruit growth, development and quality parameters studied

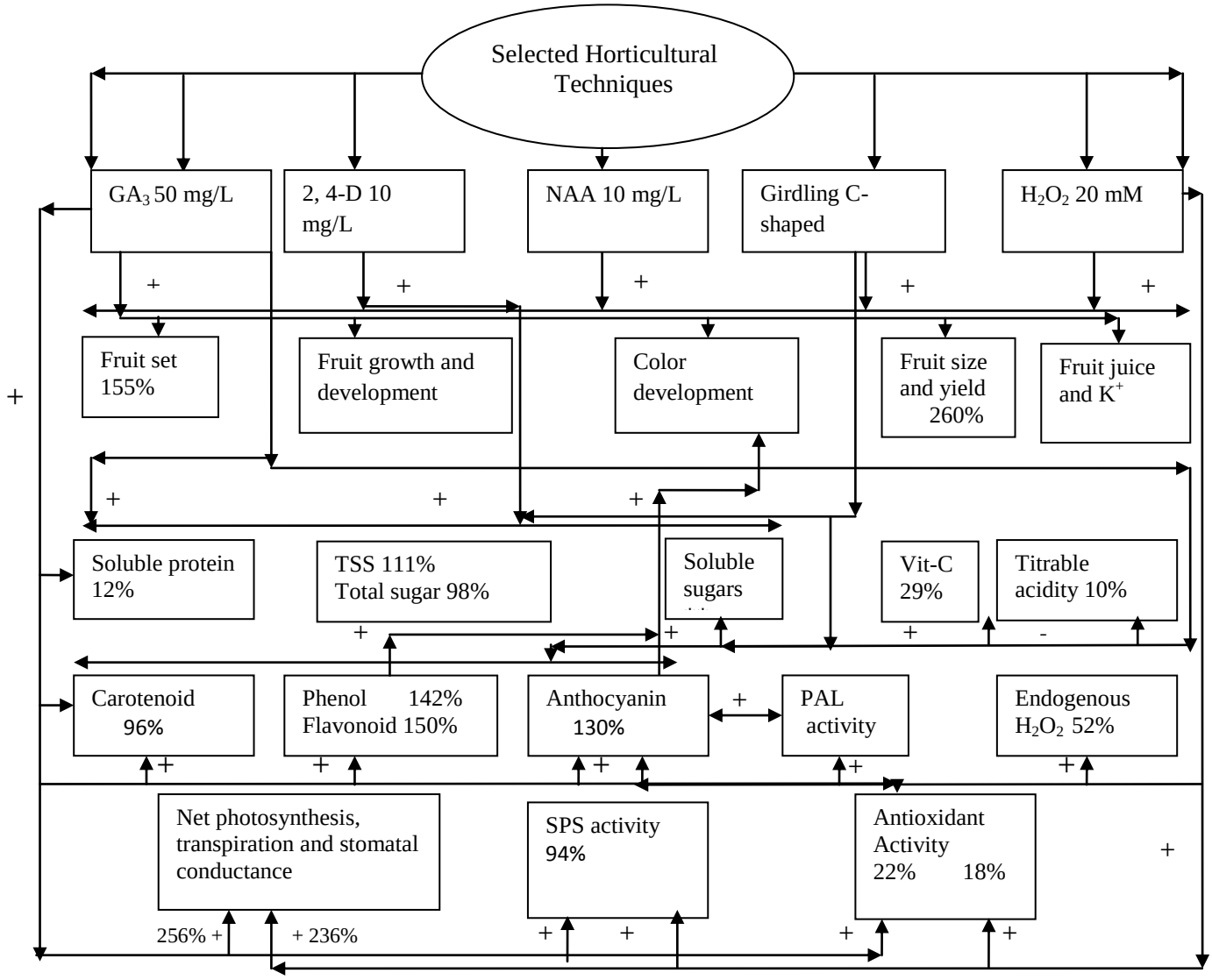


Fig. 6.1. Interaction among the parameters studied

From this study, it can be concluded that the tested concentrations of GA_3, NAA, 2, 4-D and H_2O_2 used and the different types of girdling particularly the C- and V-shaped girdling can improve the growth, development and quality as well as the nutritional status of the wax apple fruit (*Syzygium samarangense* var. *jambu madu*) under field conditions.

Based on the conclusions drawn, the following are the answers for the research questions posted earlier in the thesis (p-7)

(i) The application of GA_3, 2, 4-D, NAA, girdling and H_2O_2 can promote the growth and development of *S. samarangense* fruits.

(ii) GA_3, NAA, 2, 4-D, H_2O_2 application and girdling can affect positively the quality of *S. samarangense* fruits

(iii) From this study, it can be seen that the plant growth regulators (GA_3, 2, 4-D, NAA), growth promoting chemical (H_2O_2) and girdling treatments enhanced the inflorescence development, increased the number of flowers, fruit set and retention, reduced the bud and fruit dropping and promote the fruit growth development as well as yield of wax apple fruits.

6.2.9 Future study

This study has generated an enormous amount of raw data, which are presented in this thesis. It has been shown that application of plant growth regulators and hydrogen peroxide enhanced fruit growth, development and quality of wax apple fruits. The results also showed that growth regulators and hydrogen peroxide treatments increased peel color, anthocyanin and flavonoid content. Furthermore, net photosynthesis, sucrose phosphate synthase and phenylalanine ammonia lyase activities were increased with gibberellic acid and hydrogen peroxide application. It will be interesting to see the effects of gibberellic acid and hydrogen peroxide on rbcA, SPS and PAL gene expressions and to correlate these with the increased photosynthetic rate, sugar content and peel color observed.

PUBLICATIONS

The results presented in the thesis have been published or submitted for publication in the following articles:

Journals: (Published and Accepted for Publication)

1. Moneruzzaman, K. M., Boyce, A.N., and Normaniza, O. 2012. The Influence of Hydrogen Peroxide on the Growth, Development and Quality of Wax Apple *(Syzygium samarangense,* var. *jambu madu)* Fruits, *Plant Physiol. Biochem.* 53:101-110 ***(ISI)***

2. Moneruzzaman, K. M., Boyce, A.N., Normaniza, O. and ABM Sharif Hossain, 2012**.** Physiochemical and phytochemical properties of wax apple (*Syzygium samarangense* [Blume] Merrill & L.M. Perry) as affected by growth regulator application under field conditions, *The Scientific World J.* volume 2012, Article ID 728613, doi:10.1100/2012/728613 ***(ISI)***

3. **Khandaker, M. M.,** Hossain, A. B. M. S., Osman, N. and Boyce, A. N. 2011. Application of girdling for improved fruit retention, yield and fruit quality in *Syzygium samaragense* under field conditions. *Int. J. Agr. Biol*. 13 (1) 18-24. **(*ISI*)**

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