

EFFECT OF PLANT GROWTH REGULATORS ON VEGETATIVE AND REPRODUCTIVE GROWTH IN STRAWBERRY (*FRAGARIA XANANASSA*)

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Abstract: Strawberry (*Fragaria spp.*) belongs to the genus *Fragaria* and Rosaceae family (Staudt, 1989) is native to Europe, Asia, and some others to North and South America, and has 20 recognized species. The cultivated strawberry is a hybrid between two species, *Fragaria chilensis* and *Fragaria virginiana*. The botanical name of the commonly cultivated strawberry is *Fragaria × ananassa*. The strawberry is an attractive, luscious, tasty, aggregate, nutritious fruit. Strawberry is commercially grown in temperate regions, but there are varieties, that can be cultivated in subtropical climate. Plant growth regulators or phytohormones are organic substances produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts. Control of gene expression has been demonstrated for the phytohormones at both transcriptional and translational levels. The paper reviews the influence of various phytohormones on strawberry growth, development and fruit yield. Plant growth regulators were found to be very effective in increasing the vegetative growth, flowering and yield of berry fruits in temperate, tropical as well as subtropical regions. In most of the studies, a high concentration of gibberellic acid increased vegetative growth and runner production in strawberries whereas Cycocel, a growth retardant was very effective in improving fruit quality. Naphthalene acetic acid is an auxin, which is very effective in controlling and directing a number of plant metabolic processes. Effects of ethylene and 1-MCP treatments on strawberry fruit quality have been analysed at the commercial ripening stage.

Keywords: Phytohormone, GA₃, NAA, CCC, Cytokinin, Ethylene, Strawberry

INTRODUCTION

Strawberry (*Fragaria × ananassa* Duch.) is one of the most delicious and nutritious soft fruits in the world (Singh *et al.* 2007). It is an herbaceous perennial and short-day plant cultivated in more than 75 countries. The juicy flesh of strawberry is used in the production of alcoholic beverages and fruit wines. A change in the chemical and physical properties of strawberry fruits and products is chiefly related to the processing operations (Hendawi *et al.* 2013). The modern cultivated strawberry is a hybrid crop evolved by crossing two species, *Fragaria chilensis* and *Fragaria virginiana*. Strawberry is a member of the Rosaceae family with an octoploid chromosome number of 2n= 56 (Vishal *et al.* 2016). Among the fruit crops, it gives quick returns in the shortest possible time with very high returns per unit area on the capital investment. Nutritionally, strawberry is a low-calorie carbohydrate fruit but a rich source of vitamin A (60 IU/100g of edible portion), vitamin C (30-120 mg/100g of edible portion), fibre and also has high pectin content (0.55%) available in the form of calcium pectate. Water is a major constituent (90%) of strawberry fruit. Ellagic acid is a naturally occurring plant phenol that has been found to inhibit cancer disease and asthma through regular consumption (Kumar *et al.* 2015). In India, Maharashtra is a leading state in the production of strawberry fruit. It is also commercially grown in Haryana, Punjab, Uttar Pradesh, Jammu and Kashmir, Uttarakhand and

lower hill of Himachal Pradesh (Singh and Saravanan 2012).

Plant growth regulators are plant hormone enhancers or disrupters that are man-made or naturally derived. Gibberellic acid (GA₃) is a growth regulator which stimulates the effect of long day lengths in short-day plants by improving vegetative development and increasing runner production. Gibberellic acid progressively increased the plant height, canopy spread, leaf area, number of leaves, petiole length and induces stem elongation when applied exogenously to strawberry plants. Gibberellic acid initiates early flowering and thus early fruit development and harvesting occur. It also increases the truss heights, the number of flowering trusses per crown, fruit set percentage, total number of fruits per plant but consequently fruit size and fruit weight decreased (Kasim *et al.*, 2007; Paroussi *et al.*, 2002; Sharma & Singh, 2009). It also enhanced the number of runners in all strawberry varieties by specifically stimulating the stolon forming systems during long days. It is also responsible for increasing the number of runners per crown at higher rates of application. (Hytonen *et al.*, 2009). Gibberellic acid also increases the fruit quality by producing firmer fruit with high ascorbic acid and total soluble sugars, whereas, inducing no significant effect on titratable acidity (Usenik *et al.*, 2005; Sharma & Singh, 2009; Ouzounidou *et al.*, 2010). According to one study, Gibberellic acid induces stem and internodes elongation, seed germination, enzyme production during germination, and fruit setting and growth. The application of gibberellic acid (GA₃) is reported to

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increase leaf size, petiole length, whereas the application of auxins is also known to impart similar effects (Vishal *et al.* 2016).

Chlorocholine Chloride (CCC) is gibberellins biosynthesis inhibitor involved in the inhibition of cyclization of geranylgeranyl pyrophosphate to copalylypyrophosphate. Chlormequat [CCC, Cycocel] chlormequat chloride was discovered during a screening program of quaternary ammonium compound for growth retardant activity. Chlormequat chloride is highly mobile in both xylem and phloem tissue and rapidly absorbed and translocated. It is highly water-soluble and passively absorbed by all plant tissues, allowing it to be effectively applied as a spray or drench. Application of chlormequat chloride to crops results in plants with shorter internodes and thicker, darker green leaves. The chemical control of the plant growth to reduce the size through the use of plant growth regulators is a common practice to make a plant more compact and commercially more acceptable.

Application of Naphthalene acetic acid (NAA) increases fruit size and delays ripening and increases anthocyanin accumulation in strawberry fruits. It also increases the duration of flowering, improves the yield and quality of fruits.

Benzyl adenine as a plant growth regulator, enhances the size and shape of fruits, lateral bud break, and lateral shoot growth, leading to improved branching in fruit trees. Probably benzyl adenine as a cytokinin compound delays the senescence stages of buds and increases the entrance of photosynthetic compounds, hormones and other metabolites to inflorescence buds which are so important for preventing bud abscission and increasing the fruit set.

Effect of GA₃ on growth and yield of strawberry-Effect on vegetative growth-

The plant growth in terms of plant spread (27.72 cm) was noted maximum in the plants which received foliar spraying of 100 mg l-1 GA₃. The leaf with larger leaf lamina (122.75 cm²) and maximum length of petiole (11.50 cm) were also recorded from the same set of plants. This treatment had also found to be the best for producing the highest number of leaves (28.53), crowns (2.93) and runners (3.93) in strawberry. All the above parameters were recorded minimum in control plants. The exogenous application of plant growth regulators failed to influence any significant effect on days taken to 50 per cent flowering in strawberry. However, the plants received 100 mg l-1 GA₃ exhibited earliest flowering with significantly maximum number of flowers in strawberry. A minimum time (15 days) for the initiation of runner and minimum time (24.00 days) for initiation in rooting in runner were found in treatment- GA₃ 75ppm. Whereas, maximum time (31.66 days) for the initiation of runner, maximum time (44.45 days) for initiation in rooting in runner was observed in control. It might be due to the fact that GA₃ perhaps stimulated redistribution of

gibberellins in greater concentration in the crown region which later induced the runner emergence earlier (Palaei *et al.*, 2016). Paroussi *et al.*, (2002) and Sharma and Singh (2009) found that GA₃ increased the total leaf area per plant. GA₃ was found most effective in terms of the vegetative growth of strawberry. The maximum plant height (22.38cm), plant spread (31.10cm), petiole length (11.62cm), leaves (15.10) and runner per plant (4.66) were recorded with GA₃ @ 80 ppm. These results are in close agreement with Singh and Randhawa (1959). GA₃ application to one-year-old strawberry plants promotes vegetative growth and runner production. This may be due to inhibition of the flowering and corresponding increase in epidermal and parenchymatous cell growth (Denis and Bennett, 1969). An increase in height by GA was supported by Khokhar *et al.* (2004) that who found taller plants with a higher dose of GA₃ (75 ppm) over other treatments. An increase in plant height may be due to the fact that GA regulates the growth of the strawberry plant by causing cell elongation and synthesis of endogenous auxin-like substances in the plant system. The maximum number of trifoliate leaves (25.20 at 100 Days after transplanting) was observed in GA₃ 150 ppm. The minimum number of trifoliate leaves per plant was observed in control (20.16 at 100 Days after transplanting). The increase in the number of leaves may be due to the corresponding increase in length of epidermal and parenchyma cells, higher rate of cell division and cell elongation in sub-apical meristems of strawberry shoots which might lead to production of higher number of leaves. The research findings are in line with the results obtained by Uddin *et al.* (2012) and Khalid *et al.* (2013) in strawberry crop.

Effect on reproductive growth

Gibberellic acid induced flowering earlier in strawberry plant as compared to other treatments by minimizing the days required to open first flower. Similar results were shown by many researchers who found that early flowering occurred in strawberry (Paroussi *et al.*, 2002; Sharma & Singh, 2009). Maximum firmness was attained in fruits from plants treated with gibberellic acid (Usenik *et al.*, 2005; Cline & Trought, 2007; Ozkaya & Dundar, 2008; Canli & Orhan, 2009; Khan *et al.*, 2012). The application of gibberellic acid (GA) increased the number of flowers and developed 50 to 66% of the total number of flowers between Nov and Jan, while the control had initiated only 40% (Kirschbaum, 1998). Gibberellic acid application alone, proved to induce the early reproductive growth and a greater number of runners. GA have effect in cell division and cell elongation in strawberry fruit so length of fruit increases linearly but not diameter, hence maximum length:diameter ratio (1.92) was recorded in GA₃ 75ppm. GA₃ at all concentrations produced significantly more flowers as compared to the control. However, the highest number of flowers

(20.54) was observed in plants treated with GA₃ 50 ppm. Enhancement in flowering by GA₃ application is possible due to its effect on hastening flower truss growth when applied at floral initiation stage. Sharma and Singh (1980) also stated that application of GA after fruit bud differentiation helps in hastening the flowering in strawberry. Higher number of flowers (28.00) and fruits per plant (19.08) was recorded in the plants treated with GA₃ 150 ppm and the minimum number of flowers (20.84) and fruits per plant (14.30) were observed in (control). Highest number of flowers and fruits per plant was due to the fact that gibberellins cause the production of large number of flowers with rapid elongation of peduncle, leading to full development of flower buds having all reproductive parts functional thereby accelerates development of differentiated inflorescence, which increases fruit set and number of berries per plant. The research results are in line with the findings of Pathak and Singh (1979) in strawberry crop. The maximum berry set was obtained in GA₃ 75 ppm (46.68) and it was at par with the GA₃ 25 ppm. The increased berry set in GA₃ treated plants might be due to the fact GA₃ induced the production of enzymes attributed to improved fruit set by playing a role in post fertilization stage. In addition, exogenous application of GA₃ shifted the endogenous balance between promoters and inhibitors in favour of fruit forming metabolic process (Sharma and Sharma, 2006). GA₃ 75 ppm recorded highest yield (33.71 t ha⁻¹). The lowest yield of (17.83 t ha⁻¹) was recorded in control. The increase in yield with GA₃ might be due to increase in flower number, better fruit set and maximum number of fruits with maximum weight beside better vegetative growth. The increase in yield with GA₃ might be due to increase in flower number, better fruit set and maximum number of fruits with maximum weight beside better vegetative growth. In fact, the enlargement of strawberry fruit is dependent on the auxin produced by the developing achenes and if the flowers remain un-pollinated, the cells fail to elongate. GA₃ might have helped in elongation of cells in the un-pollinated region of fruit, as well affected the auxin metabolism which might have indirectly helped in fruit enlargement and also higher number which ultimately increase the yield.

Similar increase in strawberry yield following GA₃ application has also been reported by Paroussi *et al.*, 2002.

Effect of GA₃ on yield and fruit quality

Maximum ascorbic acid (63.41 mg/ 100g fruit) and acidity (0.75%) were noticed with GA₃ @ 80 ppm. Thakur *et al.* (1991) and Dwivedi *et al.* (2002). Maximum TSS (10.61°Brix) was recorded in GA₃ 75 ppm. The improved TSS with GA₃ treated plant can be attributed to fact that stress might have caused cell elongation (Syamal *et al.*, 2010). The highest number of berries per plant was recorded in GA₃ 75 ppm (46.68), while control recorded the

lowest (32.00). The marked influence of GA₃ on number of berries may be attributed to its effect on better pollen germination and fruit set (Sharma and Singh, 2008). The maximum yield per plant (299.36 g) was recorded in GA₃ 150 ppm followed by GA₃ 100 ppm and minimum (173.45 g) was recorded in control. This increase in fruit yield per plant in gibberellins treated plants might be due to increased vegetative growth (plant spread, number of crowns and leaves etc.) which enables higher fruit set and fruit weight. The yield attributes on the sink capacity of crop are determined by its vegetative growth throughout the life cycle of plants. Vigorous growth is associated with higher sink capacity of a crop. The higher yield might be also related to formation of more metabolites by large leaves and high rate of photosynthesis. This result is in conformity with the findings of Saima *et al.* (2014) in strawberry crop. In conclusion, results obtained in the present investigation shows that foliar application of GA₃ (150 ppm) has shown better growth and maximum yield ratio in strawberry followed by GA₃ (100 ppm).

Effect of auxin on strawberry

Effect of auxin on vegetative growth

The maximum growth in terms of plant height (18.19 cm) was observed with NAA 400 ppm. Thakur *et al.* (1991) applied NAA at 5, 10 and 20 ppm in the first week of April on strawberry cv. Tioga and observed significant increase in vegetative growth when compared with the control. Adaki and Pekmezci (2011) evaluated the effect of different auxin types and activated charcoal levels on plant growth and development at the acclimatization stage in different strawberry cultivars and reported that activated charcoal 11 usages at the rooting stage had a positive effect on plant growth and development during acclimatization whereas NAA and IBA hormones had a negative effect.

Effect of auxin on reproductive and yield attributes

Zielinski and Garren (1952) recorded 30% increase in fruit size by the spray of 50 ppm of β -NAA made at half-grown stage. The maximum fruit length diameter ratio (1.47 cm) and juice content (94.67%) were recorded with NAA @ 30 ppm. Thakur *et al.* (1991) and Dwivedi *et al.* (2002). The TSS and sugar content of strawberry fruits were the maximum with the foliar application of 125 mg l-1 NAA. The plant received no sprays produced fruit with lowest TSS and sugar content. Fruits with the highest ascorbic acid (Vitamin C) content were produced in plants treated with 125 mg l-1 NAA. Both skin toughness and the hardiness of the underlying flesh determine the firmness of strawberry fruit (Hietaranta and Linna, 1999). In strawberry, the skin toughness is directly linked to hard achene development and auxin is known to regulate the process of achene development and perhaps resulted in hardest fruit in NAA treated plants (Archbold and Dennis, 1984). The application of NAA in strawberry plants

might have increased the concentration of volatile compounds along with hydrolysis of starchy compounds which ultimately raised the TSS level. Paleiet *et al.* (2016) also recorded higher TSS of strawberry fruits with the application 50 ppm NAA. The total sugars content which account for more than 60 per cent of TSS percentage. The higher enzymatic activity like α amylase and invertase with the application of NAA might be responsible for higher total sugar content and non-reducing sugar content of strawberry fruits. Improvement in the ascorbic acid content of strawberry fruits might be due to increase level of metabolites that stimulate the precursor of ascorbic acid biosynthesis in plants which received NAA. Increased level of ascorbic acid with the application of 200 ppm NAA has also been reported in guava (Singh *et al.*, 2017).

Effect of Cycocel on strawberry

Chlormequat chloride is highly mobile in both xylem and phloem tissue and rapidly absorbed and translocated. It is highly water soluble and passively absorbed by all plant tissues, allowing it to be effectively applied as a spray or drench. Application of chlormequat chloride to crops results in plants with shorter internodes and thicker, darker green leaves. The chemical control of the plant growth to reduce the size through the use of plant growth regulators is a common practice to make a plant more compact and commercially more acceptable (Rakesh Kumar *et al.*, 2017).

Ascorbic acid content was increased with Cycocel (CCC) treated strawberry plants (Singh and Phogat, 1983). Foda *et al.* (1979) also found higher berry yield with CCC at 1000 ppm concentration. The increase in berry yield was obviously due to the increased fruit set, higher number of fruits per plant, greater berry size and weight under GA3 at 100 ppm and CCC at 1000 ppm treated plants. The minimum days taken to produce first flower (54.77 days) and fruit bud development (58.11 days) were noticed with Cycocel @ 700 ppm. The Cycocel @ 500 ppm enhanced the number of flowers (22.76), fruits (18.10) per plant and yield (20.44t/ha). The induction of early flowering, fruit bud development and number of flowers per plant with application of Cycocel in the present study are in conformity with Barritt (1975) and Dwivedi *et al.* (2002). The maximum specific gravity (1.16), T.S.S (9.33%) and total sugar (9.06%) were recorded with Cycocel @ 500 ppm. Thakur *et al.* (1991) and Dwivedi *et al.* (2002). Treatment of plants with CCC 500, 1000 and 1500 ppm also gave a higher yield of berries as compared to control. Foda *et al.* (1979) also found higher berry yield with CCC at 1000 ppm concentration.

Effect of Cytokinin on strawberry

Fruit weight (16.69g) was observed better in treatment BA 75ppm and minimum fruit weight (8.32g) was recorded from treatment GA₃75ppm. An

increase in weight of fruit by BA might be due to either

marked increase in a number of leaves/ plants which gave a chance to the tree to carry more flowers and fruits or marked increase in the photosynthetic pigment content which could lead and to increase in photosynthesis, resulting in the greater transfer of assimilates to the fruits and causing an increase in their weight (Abou *et al.*, 2011). The number of fruit/plant (18.97) and fruit yield (225.60g) were found maximum in treatment (GA₃ 25ppm + BA 25ppm). The minimum number of fruit/plant (10.11) and fruit yield (96.00g) observed from treatment GA₃ 75ppm (Ramteke *et al.*, 2015). Application of different growth regulators and nutrients resulted in significant variations in yield per plant between the different treatments.

Effect of ethylene on strawberry

Ethepron treatments did not increase in the length of leaf petioles but rather plants were shorter than in the control treatment. This is due to the fact that Ethepron behaves as growth retardant or at least not growth promoter in some vegetative parameters. Ethepron at 500 ppm showed significantly more leaves as compared to the control and this result was on line with Choma and Himelrick (1982) who also found increase in a number of leaves with the application of ethephon 500 ppm. However, 1000 ppm ethephon showed least number of leaves; this level of ethylene might have reduced both the synthesis and amount of auxin in leaves.

In strawberry, an increase in PAL activity is necessary for the accumulation of anthocyanins during ripening. Manning K detected an increase in anthocyanin content and PAL activity in all fruit after 48 h of incubation at 22 °C, in comparison with the values found in fruit at the initial white stage. Fruit treated with ethephon accumulated more anthocyanin than the corresponding controls, while the opposite was observed in fruit treated with 1-MCP. In grape berries, other fruit considered as non-climacteric, it has been reported that treatments with exogenous ethylene were also able to stimulate anthocyanin accumulation and the expression of genes related to anthocyanin biosynthesis (El keream *et al.*). The increase in anthocyanin content, strawberry fruit ripening is accompanied by a decrease in chlorophyll levels. Degradation of these pigments in fruit incubated for 48 h at 22 °C. Moreover, the decrease in total chlorophyll levels was more pronounced in fruit treated with ethephon with regard to controls, and the opposite situation was observed in 1-MCP-treated fruit.

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