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Plant growth regulators in strawberry: A review

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Abstract
Strawberry (*Fragaria x ananassa* Duch.) is one of the most important delicious fruits of the world. In present days, strawberry cultivation is gaining popularity in different states of India. Some of the important strawberry growing states are Haryana, Mizoram, Meghalaya, Maharashtra, Himachal Pradesh and Kerala. The application of PGR's are increasing day by day in the production of fruit crops, due to its various advantages. Plant growth regulators play an important role in strawberry production by influencing directly or indirectly in various plant processes like seed germination, plant growth and development. PGR's also play a key role in breaking of seed dormancy, to increase the runner's production, to improve flowering and fruit set, to increase fruit size and yield, to improve the fruit quality traits like fruit colour, TSS and acidity and to enhance the shelf life of the strawberry. In this review article a detailed information on use of growth regulators in different stages of plant life cycle were given and also the research work was done in different countries on the use of various plant growth regulators in strawberry production have been incorporated.

Keywords: strawberry, PGR's, flowering and fruit set, fruit quality, shelf life

Introduction
The cultivated octaploid strawberry (*Fragaria x ananassa* Duch.) belongs to family Rosaceae (Saima *et al*., 2014) [52] and has resulted from a cross between two wild strawberries *Fragaria virginiana* and *Fragaria chiloensis* (Ankita Sahu and Chnadel, 2014) [2]. It is one of the most important soft fruits of the world, suitable for cultivation under various agro-climatic conditions (Singh *et al*., 2012) [56]. It is a small juicy fruit that is rich in vitamin C and other minerals (Johnson and Peterson, 1974) [24]. Strawberry being a temperate region crop cultivated in the states of Jammu & Kashmir, Himachal Pradesh and Uttarakhand. Currently, with introduction of day neutral strawberries its cultivation is also expanding to sub-tropical and tropical states like Haryana, Punjab, Uttar Pradesh, Maharashtra, Karnataka and West Bengal. Strawberry is known for its attractive appearance, taste and nutritive value with pleasant aroma. Now, it enjoys a very remunerative market avenue owing to its heavy demand in the food beverage and processing industries (Singh *et al*., 2010) [57]. Strawberry is perishable crop which is exceedingly in demand for its taste, profitability, high yield and good quality. For production at commercial level growth regulators have been used in the past but in present days more attention paid towards pre and post-harvest techniques by using combination of growth regulators with salt (Khalid *et al*., 2013) [33]. The main reasons for low production were, lack of timely available planting materials, short winter period, inappropriate cultural practices, lack of improved varieties and technologies. Although genetic factors and cultural practices controls plant growth, yield and fruit quality in strawberry (Avigdori-Avidov, 1986) [5], strawberry cultivars highly influenced by plant growth regulators (Jamal Uddin *et al*., 2012) [21]. These plant growth regulators may affect the floral induction, flowering, fruit set, fruit growth and development, fruit maturity and ripening, fruit yield and quality, shelf life, seed dormancy *etc.*, This applications growth regulators has been practicing commercially to increase the production and quality in many fruit crops (Nuruzzaman *et al*., 2015) [39]. Plant growth regulators refer to organic compounds other than nutrients which in small quantities promote, inhibit or otherwise modify any plant physiological process. Though there are many growth regulators, the well-established categories of growth regulators are auxins, gibberellins, cytokinins, abscisic acid and ethylene. Hence, in this chapter we have discussed the role of different plat growth regulators in strawberry production.

Role of PGR on propagation
Day neutral strawberries produce flowers and fruit continually during the growing

season and therefore, produce stolons (runners) sparsely (Pritts and Dale, 1989) [46]. In propagation fields, this behavior results in fewer daughter plants than in June-bearing strawberries. Growth regulators can induce runners to form either by stimulating dormant buds to grow or by preventing flower bud initiation (Pritts *et al*., 1986; Reid, 1983) [47, 48]. Gibberellic acid has increased runner production inconsistently (Reid, 1983) [48]. Cytokinins, such as benzyladenine (BA) and tetrahydropyranyl-benzyladenine (PBA) also have shown inconsistent results when applied alone (Kender *et al*., 1971; Pritts *et al*., 1986; Waithaka and Dana, 1978) [31, 47, 62]. When used in combination, cytokinins and GA_3 markedly enhanced stolon formation on the everbearing 'Geneva' and 'Ozark Beauty' (Kender *et al*., 1971; Waithaka and Dana, 1978) [31, 62] and on the June-bearing 'Nyoho', 'Morika-16', and 'Hokowase' (Kahangi *et al*., 1992) [28]. In this regard, Asadi *et al*., 2013 [3] studied the effect of Gibberellins GA_3 (0, 25 and 50 mg/L) in a completely randomized design with 4 replications. Treatments were sprayed on plants after removing the last flower and repeat application once more after a week. GA_3 foliar application at 50 mg/l in Gaviota strawberry plants, increased runner which help us to produce strawberry plant.Increasing the paclobutrazol concentration reduced the number of runners, decreased runner length, and limited biomass partitioned into daughter plants. The optimum concentration of paclobutrazol for strawberries appears to be between 150 and 300 mg/liter (Deyton *et al*., 1991) [11]. In greenhouse and field studies, benzyladenine (BA) and gibberellic acid (GA_3) applied together as a foliar spray increased runner production in day neutral strawberries (Fragaria ×ananassa Duch.) but not when applied separately. Runner production increased linearly with increased BA concentration to 1800 mgL^{-1}. At high dosages, GA_3- treated plants produced elongated internodes that, in the field, led to fewer daughter plants. In Florida, daughter plants derived from plants sprayed with the growth regulators increased yield by up to 10% in fruiting experiments. To induce runnering in the field and greenhouse, a treatment with BA at 1200mgL^{-1} and GA_3 at 300 mgL^{-1} is recommended (Dale *et al*., 1996) [8].

Role of PGR Growth and Development (promotion vs dwarfness)

There are some factors that can affect growth and development of strawberry. Growth regulators are considered one of those most important factors.Strawberry plant, height is determined mainly by the length of the leaf petioles. The results presented in this communication show that increases in length of petiole brought about by the application of gibberellic acid involve increases both in the number and the length of cells in the epidermis (Guttridge and Thompson, 1959) [17]. GA is needed for runner initiation in strawberry, and the inhibition of GA biosynthesis leads to the formation of crown branches.GA plays a role also in the photoperiod-regulated differentiation of axillary buds.GA_3 increases plant height (Paroussia *et al*. 2002) [42] of strawberry (Jamal Uddin *et al*. 2012) [21] The highest plant height in GA_3 treated plants may be due to increase in the cell elongation. El-Shabasi *et al*. (2009) [14] reported that, since plant height depends on the petiole length, this increased plant height may be due to raise in petiole length of strawberry plants. El-Shabas *et al*., 2009 [14] studied the effect of ethrel {ethephon}, GA_3 and uniconazole on strawberry plants and found that application of GA_3 increased the length of plant petiole.

These results are in harmony with those reported by Tehranifar and Battey (1997) [59], who hinted that applying GA_3 caused an increase in vegetative vigor in the absence of chilling. On the other hand, GA_3 treatments showed a gradual increase in petiole length as the level of applied GA_3 increased. The increase of the petiole length of the strawberry following GA_3 treatment was found to involve increases in both the number and the length of cells in epidermis (Guttridge and Thompson, 1959) [17]. This increase in crown length may be due to positive effect of GA_3 on cell division. GA_3 either applied singly or among calcium chloride, radically increased the vegetative growth parameters by increasing plant height, crown diameter, canopy spread, fresh and dry weight of plant and leaves, leaf area, fruit set percentage, number of, runners, trusses, flowers and fruits; as compared to salicylic acid followed by Calcium chloride.Salicylic acid + $CaCl_2$ also improve the fruit quality by significantly increasing ascorbic acid content and Total Soluble Solids while titratable acidity.

Role of PGR's on flowering, fruit set, fruit growth and development, control of fruit drop, parthenocarpy

Many plant growth regulating compounds (auxins, cytokinins and gibberellins) have been used in various crops in order to achieve high flowering, fruit set, larger fruit size, and parthenocarpy (Guardiola and Garcia-Luis, 2000; Stern *et al*., 2007) [15, 58]. Although the efficacy of such product applications is quite easily evaluated based on fruit characteristics.The role of auxin in strawberry fruit development has long been recognized, as it is responsible for the receptacle enlargement and therefore fruit size growth. In strawberry, Nitsch (1950) [37] showed that hormonal compounds produced by the developing seeds were associated with fruit growth.

Flowering

El-Shabasi *et al*., 2009 [14], studied the effect of ethrel {ethephon}, GA_3 and uniconazole on strawberry plants and the results showed that GA_3 application increased plant petiole. GA_3, ethrel and uniconazole increased total carbohydrate percentage in the foliage of strawberry plants. GA_3 at 10 ppm or ethrel at 250 ppm increased the number of flowers and the monthly and total yield. It was verified that although GA 550 ppm, SADH and CCC decreased yield, three applications of IAA 10 ppm or GA 10 ppm have promoted a tendency to increase strawberry yield. It was noted that growth regulators do not alter number of fruit, and GA 550 ppm promotes reduction in fruit mean weight. Asadi *et al*., 2013 [3] documented that number flower on inflorescence and Runner significantly increased with application of GA_3 (0, 25 and 50 mg/L) on plants after removing the least flower and repeat application once more after week.

Different growth chemicals also were found effective for days to flowering from seedling transplanting to flower opening in different strawberry varieties. Days required from bud initiation to flower opening was lowest in GA_3 treated plants. Days required for flowering of strawberry varied and it ranged from 66.1 - 69.0 days (Ahsan *et al*. 2014) [1]. Number of flower bud is one of the key factors for the yield and number of flower bud increased due to the application of GA_3 (Paroussia *et al*. 2002) [42]. Growth regulators significantly influenced the production of flower per plant. The plants treated with GA_3 bear more flowers. This might cause that as the plant bears more flower bud under GA_3 treatment so

flower was more under this condition and this result also same in case of 4-CPA treatment (Nuruzzaman *et al*., 2015) [39].

Fruit set, growth and development

Days to fruiting of strawberry showed significant variation for different growth chemicals used as foliar feeding. Application of 75 ppm GA_3 provided maximum number of fruit in strawberry (Jamal Uddin *et al*. 2012) [21]. GA_3 showed a tendency to increase the number of strawberry fruits (Miranda-Stalder *et al*. 1990) [35]. Total fruit weight varied significantly with the application of different growth chemicals. Maximum fruit weight per plant of strawberry was found in 4-CPA.The total fruit weight was maximum when the plants were sprayed with 4-chlorophenoxy acetic acid (4-CPA). Fresh weight of primary fruit varied significantly with the application of different growth chemicals. Nuruzzaman *et al*. (2015) [39] was also differentiated the strawberry fruit into primary, secondary and tertiary fruit and found significant variation. Foliar feeding of GA_3 @ 75 ppm provided maximum fruit weight (Jamal Uddin *et al*. 2012) [21]. Application of GA_3 may cause excessive elongation of the fleshy receptacle which may reduce the diameter (Nuruzzaman *et al*., 2015) [39].

Parthenocarpy

Auxin produced by fertilised ovules is essential for the growth of strawberry fruits (Mezzetti *et al*., 2002) [34]. An attempt to promote parthenocarpic receptacle development with 4-(indole-3-) butyric acid after the removal of all carpels before pollination was unsuccessful. It was concluded that the active growth substances probably stimulated growth initially in the tissues of the unpollinated carpels, which in turn promoted the development of the receptacle. Beech, 1983 [6] achieved the induction of parthenocarpy in the hermaphrodite strawberry cv Redgauntlet was by applying auxin and auxin analogues in a lanolin emulsion to emasculated, unpollinated flowers. Although applied auxins readily induce parthenocarpy, further endogenous growth regulators are probably required for full fruit development.

Role of PGR's on Fruit maturity and ripening; fruit yield and quality

The effects of growth regulators Succinic acid -2,2 - dimethylhydrazide (SADH) 5000 ppm, (2- Chloroethyl) Trimethyl ammonium chloride (CCC)2000 ppm, gibberellic acid (GA) 550 ppm and 10 pm and indoleacetic acid (IAA) 10 ppm on strawberry cultivar "Monte Alegre" were studied. It was verified that although GA 550 ppm, SADH and CCC decreased yield, three applications of IAA 10 ppm or GA 10 ppm have promoted a tendency to increase strawberry yield. It was noted that growth regulators do not alter number of fruit, and GA 550 ppm promotes reduction in fruit mean weight. GA ppm increased number of fruit on 3rd ad 4th weeks of harvesting, decreasing progressively in subsequent weeks. (Paulo *et al*., 1976) [43].

The plant growth stimulators increased marketable yield and fruit size, while they had no significant impact on fruit juice pH, titratable acidity and total soluble solids concentration. Furthermore, they had no significant effect on fruit organic acid and carbohydrate concentration and on fruit color, although they enhanced total anthocyanin concentration. The antioxidant activity of the fruit juice was slightly higher in the fruits of control treatment, which could be the result of their higher total phenol, o-diphenol, flavonoid and flavanol concentration (Roussos *et al*., 2009) [49]. GA_3 (75 ppm) spray either during mid-November or mid-February or at both times has favourably influenced all vegetative attributes of 'Chandler' strawberry over control. Similarly, fruit set was increased, and production of malformed and button berries was reduced, but albinism remained unaffected. Although individual berry weight was reduced slightly, but fruit number, total as well as marketable yield was increased tremendously over control with no adverse effect on fruit quality parameters. In all, spraying GA_3 both during mid-November and mid-February was much more effective in achieving the desirable results than single application of GA_3 either during mid-November or mid-February (Sharma and Singh., 2009) [55].

Fruit ripening

Foliar feeding of GA_3 reduced the time for fruit ripening. Percentage of brix of strawberry fruits varied significantly with the application of different growth chemicals. Foliar application of 75 ppm GA_3 provided maximum brix in strawberry fruit (Jamal Uddin *et al*. 2012) [21].

Yield

Though Foliar feeding of GA_3 increases the various growth, flowering and fruiting attributes but 4-Chloro phenoxy acetic acid ultimately increases the total yield also degree of brix. So 4-CPA could be used for strawberry production to get higher yield (Nuruzzaman *et al*., 2015) [39]. Application of growth regulators combined with salt was more effective in increasing growth and yield. Among combined sprays of growth regulator and salt, Salicylic acid + Calcium chloride showed significant results in all parameters viz; vegetative growth, yield and fruit quality parameters, than Gibberellic acid + Calcium chloride followed by control. (Khalid *et al*., 2013) [33].

Role of PGRs on Shelf life of fruits

The fruits and vegetables being living tissue, undergo physico-chemical changes resulting in quality deterioration during postharvest handling and storage (Kader, 1992) [27]. The postharvest life of horticultural produce is determined by the rate of respiration, ethylene production and tenderness of the produce (Day, 1990; DeEll, 2006) [9, 10]. Thus, postharvest handling of fruits and vegetables is a major challenge throughout the world (Kader, 1992) [27]. Strawberry is a highly perishable fruit and its postharvest handling is a serious problem throughout the world (Nunes *et al*., 2002) [38]. It is characterized by high rate of water loss (Kader, 1991) [26] and high susceptibility to fungal decay during storage (Ceponis *et al*., 1987) [7]. The rate of respiration, ethylene production and fungal decay can be decreased by application of growth regulators. Thus, application of growth regulators at pre and post-harvest stage is the most common method to extend storage life. The pre-harvest foliar spray of crop with GA_3, NAA, 2,4-D and calcium nitrate extended the shelf-life and helped in developing quality-ripe fruits in terms of retaining high reducing sugars, minimum cumulative physiological loss in weight and higher vitamin C retention (Asrey *et al*., 2004) [4].

Strawberry (*Fragaria X ananassa* Duch.) fruit undergoes extensive softening during ripening due to cell wall disassembly, caused by a coordinated action of cell wall proteins and enzymes including polygalacturonases (PGs). The influence of plant growth regulators on PG activity and expression of the corresponding genes has been scarcely analyzed. In this work, the immuno-detection of PG protein

(FaPG1) during ripening of strawberry cultivars with contrasting firmness is reported. Two proteins were recognized with molecular mass of 45–50 kDa, which accumulate from 25% red stage in the firmer cultivar (Camarosa) and from white stage in the softer (Toyonoka) (Natalia *et al*., 2009) [36] The application of ABA increased slightly the expression of PG transcripts, but did not modify significantly the enzyme activity. The treatment with GA_3 reduced anthocyanin accumulation and PG activity, though did not produce remarkable effects on the expression of FaPG1 and T-PG. Anthocyanins increased after treatment with ethephon (ethylene releasing reagent) or sodium nitroprusside (NO providing reagent). The expression of FaPG1 and T-PG increased considerably in response to ethylene and NO. Western-blot data confirmed the trends observed in the expression analysis in response to ethephon and NO, but PG activity was not modified by any of these treatments. However, the application of 1-MCP reduced PG activity, suggesting that ethylene could play a role in the regulation of polygalacturonase expression during strawberry fruit ripening.

Role of PGR's on Embryogenesis

Strawberry cultivars and selections are highly heterozygous. Therefore, each seed-ling within a population is genetically unique (Scott and Lawrence, 1975) [54]. Strawberry breeders are dependent on this inherent seed-ling-to-seedling variability, for it is among segregating populations that new cultivars are selected. This same variation, however, is also a source of experimental error in studies where individual seedlings are the experimental units. Additionally, seed germination between strawberry cultivars, selections, and crosses is highly variable (Wilson *et al*., 1973; Scott and Draper, 1967; Guttridge and Bright, 1978) [63, 53, 16], which results in the loss of genetically unique seedlings. The ability to generate plants from the nonviable seeds would allow the rescue of potentially valuable genotypes. The highest percent of cultures with somatic embryos was achieved on MS medium supplemented with 1.0 mg/l 2, 4-D, 0.5 mg/l BAP and 50% proline. Darkness was the best condition for incubation with daily light periods over 6 h reducing the frequency of embryogenesis. Regenerated plants (90–95%) were successfully transferred to soil, showed normal morphology and produced fruit four months after planting.

Omar *et al*., 2013 [40], Somatic embryo-like structures (SELS) were produced *in vitro* from leaf disk and petiole explants of two cultivars of strawberry (Fragaria × ananassa Duch.) on Murashige and Skoog medium with different concentrations and combinations of 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP) and sucrose to check the embryonic nature of these structures histologically. A large number of SELS could be regenerated in both cultivars on media with 2 - 4 mg L-1 2, 4-D in combination with 0.5 - 1 mg L-1 BAP and 50 g L-1 sucrose. Histological examination of SELS revealed the absence of a root pole. Therefore these structures cannot be strictly classified as somatic embryos. The SELS formed under the tested culture conditions represent malformed shoot-like and leaf-like structures. The importance of these results for the propagation of strawberries via somatic embryogenesis is discussed.

Role of PGR's on Seed dormancy, germination and seed viability

The *in vitro* germination of the strawberry plant achenes constitutes a necessary stage in the production of sterile seedlings essential to any process in biotechnology as genetic transformation. However, the germination of achenes is often poor. Iyer *et al*., 1970 [19]., were obtained the Germination percentages of 30, 50 and 90 at 30 days with freshly gathered strawberry achenes, cv. Gorella, after 24 hours' steeping in Ethrel [ethephon] at 1,000, 2,500 and 5,000 p.p.m., respectively. The highest concentration induced 45% germination within 10 days. GA and thiourea treatments also gave some improvement in germination.

Role of PGR's on Stress management

Strawberry is considered as a NaCl salinity sensitive species (Saied *et al*., 2005) [50] and it has been shown to reduce leaf number, leaf area, shoot dry weight and number of crowns and low yield (Pirlak and Esitken, 2004) [44]. Strawberry growing and production is an ever-increasing industry around the globe. This plant is considered as a sodium chloride (NaCl) salinity-sensitive species (Saied *et al*., 2005) [50]. There are many reports indicating adverse influence of salinity on overall strawberry plant growth and development (Kaya *et al*., 2003; Keutgen and Keutgen 2003; Pirlak and Esitken 2004; Saied *et al*., 2005; Karlidag *et al*., 2009; Orsini *et al*. 2012) [30, 32, 29, 44, 41, 50].

Tolerance of strawberry can be manipulated and changed by application of SA. Beneficial influences of SA on strawberry plant growth under saline or non saline conditions have been reported by various authors (Kaya *et al*., 2003; Saied *et al*., 2005; Karlidag *et al*., 2009; Jamali *et al*., 2013) [30, 50, 29, 23]. In almost all of previous studies, in regard s to ameliorative impact of SA on salinity stress or other abiotic stresses such as drought, Karlidag *et al*., 2009 [29] found that application of 1.00 mM salicylic acid (SA) 15 days after planting ameliorated the negative effect of salinity on the growth of strawberries by increasing the shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and chlorophyll content in plants. This SA treatment also assisted in increase of Leaf water relative content (LWRC) and almost contents of all nutrients in leaves and roots of strawberry plants and in reduction of electrolyte leakage compared to the control under salt stress. Finally they concluded that, exogenous SA treatments did not completely recover the deleterious effects of salt stress on the growth of strawberry plants, but especially the 1.00 mM SA concentration improved plant tolerance to salinity as compared to the non-treated plants. Based on these findings, the SA treatments may ameliorate the negative effect of salinity on the growth of strawberry.

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