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Influence of plant growth regulators on growth and yield of pome and stone fruits

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Abstract

The 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. The plant growth regulators are used for enhancement of fruit quality and production. The growth regulators are mainly applied through foliar sprays. In apple, preharvest drop is a serious problem and thus to control this problem auxins application is useful. GA₃ increase fruit size, improves fruit firmness, increases vitamin-C, TSS, total sugars. Gibberellic acid and salicylic acid are also reported to increase yield, fruit acidity, reducing sugars, TSS, TSS/acid ratio, fruit firmness and fruit chlorophyll. The growth retardants like paclobutrazol and prohexadione-Ca are useful for controlling growth and improving fruit set. Ethephon is widely used for apple thinning. In this review, we focus on the role of plant growth regulators in pome and stone fruits.

Keywords: Auxins, gibberellins, ethephon, paclobutrazol, pome fruits and stone fruits

Introduction

The 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 (Kumari *et al.*, 2018) [59]. In practical purpose, they are applied directly to plant to alter its life processes or structure in some beneficial way so as to enhance yield, improve quality and facilitate harvesting. Naturally occurring phytohormones, their chemical analogs, hormone-releasing agents, hormone sensitivity altering agents and hormone synthesis inhibitors collectively form plant growth regulators (Hajam *et al.*, 2017) [45]. Plant growth regulators modify growth and development in various ways. Plant growth regulators can be well integrated into orchard production systems. Plant hormones can be grouped into five classes of compounds: auxins, gibberellins, cytokinins, abscisic acid and ethylene. Fruit set represents the very first step of fruit development. In angiosperms, it depends on the successful completion of pollination and a unique double fertilization event where one of the pollen nuclei fertilizes the egg cell, whereas the other fuses with two haploid polar nuclei in the central cell (Raghavan, 2003) [76]. These events set the formation of the seed which eventually controls the cell division and fruit growth in a synchronized manner. Current evidence supports that combined action of three hormones, auxin, gibberellins (GAs), and cytokinin, plays a major role in the regulation of fruit set. The development and use of exogenous plant growth regulators as growth retardants is based on knowledge that endogenous plant hormones play significant role in shoot growth. Daminozide (SADH), chlormequat and paclobutrazol have been found effective in improving fruit set in temperate fruits (Miller, 1989) [67]. The paclobutrazol improves plant water status in apple (Swietlik and Miller 1983) [94], strawberry (Navarro *et al.* 2007) [70], and peach (George and Nissen 1992) [38] under drought conditions.

Fruit quality fulfillment comes to be a paramount concern of the researchers and fruit grower's important goal, particularly regarding optimising size, increasing firmness, TSS and protection against russetting, sunburn and other physiological disorders. The process of adventitious root formation is influenced by a number of internal and external factors.

Among the internal factors, the most important role is ascribed to phytohormones, especially the auxins. It is generally accepted that auxins have a certain role in the rooting initiation [Stefancic *et al.*, 2005] ^[88]. Auxins control growth and development in plants, including lateral root initiation and root gravity response. Many studies have shown that exogenous application of auxins results in increased initiation of lateral roots and that lateral root development is highly dependent on auxin and auxin transport [Chhun *et al.*, 2003] ^[19]. Watanabe *et al.* (2008) ^[97] studied the effect plant growth regulators *viz.*, auxins, cytokinin, gibberellins and gibberellins synthetic inhibitor on flowers of both parthenocarpic (cv. Ohri) and non- parthenocarpic (cv. Fuji) fruits. They found that the auxins and cytokinins are necessary for fruit growth whereas, the gibberellins application before flowering resulted in parthenocarpic fruit production.

Plant hormones are long known to be tightly associated with fruit development and ripening (Klee and Giovannoni, 2011; Seymour *et al.*, 2013) ^[52, 86]. Fruit set represents the first stage of the development after the fertilization event. This is followed by an active cell division and later cell expansion phase; both together contribute to the fruit growth phase. The growth phase causes fruits to attain their maximum size. This is followed by a stage where fruits acquire the prerequisite competence to enter into the final developmental stage, i.e. ripening. Ripening signifies a very important phase change and results in conversion of a less palatable green fruit into a highly palatable, nutritionally rich, and coloured fruit. Most of the beneficial pharmacologically active compounds are accumulated in fruits to higher levels during this phase. Further, fleshy fruits are physiologically classified as climacteric and non-climacteric. Climacteric fruits such as tomato, banana, apple, and avocado show a concomitant increase in respiration and ethylene biosynthesis upon initiation of ripening. Non-climacteric fruits such as citrus, grape, and strawberry lack these two attributes at the onset of ripening (Kumar *et al.*, 2014) ^[88].

Ethylene serves as a key ripening hormone of climacteric fruits and can influence ripening in many non-climacteric fruits (Giovannoni *et al.*, 2010) ^[41]. Treatment with high concentrations of ethylene stimulates respiration and increases the levels of fatty acids (FA) and volatiles and at the same time decreases firmness (Banday, 2006; Harb *et al.*, 2008) ^[7, 46]. In order to increase the yield of monoecious crops, the increase female flowers are prerequisite for the same, Ethrel (250-1000 ppm) and CPPU (20-80 ppm) sprays work to induce female and intersexual flowers in male plants of papaya (Kumar, 1998) ^[57]. Ethephon (Cerone) is absorbed by the plant tissues where it is broken down into naturally occurring compounds: carbon dioxide, phosphate and ethylene, which acts as a plant hormone. Ethylene is known to be involved with both chlorophyll degradation in leaf and with the abscission process (Aharoni *et al.*, 1979) ^[1]. Exogenously applied ethylene mobilized carbohydrates in woody shoots and established its mechanism of action (Eklund and Little, 1998) ^[27].

Role of PGRs in Pome fruits

Auxins

These are primarily growth-promoting substances that contribute to the elongation of shoots, but at high concentrations they can inhibit growth of lateral buds. Auxins are generally produced in apical buds, young leaves, and

developing seeds. In addition to being used as plant growth regulators, auxins can also be herbicides (2,4-D and other phenoxy herbicides). In apple production, NAA and NAD are synthetic auxins that can be used to thin fruit, to inhibit water sprout and sucker growth, and to prevent fruit drop shortly before harvest. Evidence suggests that auxin promotes fruit set and growth, at least partly, by controlling the GA levels (Serrani *et al.*, 2008; Dorsey *et al.*, 2009) ^[82, 24].

In apple, high total carbohydrate concentrations in shoots during winter storage and spring promoted high flower induction, which resulted in high flower numbers and high yields in the following year (Khan *et al.*, 1998) ^[50]. In Delicious apple application of NAA @ 15ppm at 11 mm KFD (king fruit diameter) resulted in maximum amount of small fruit (Black *et al.*, 1995) ^[9]. In Golden Delicious apple the application of NAA @ 20ppm increased the return bloom (Gautam and Jindal, 2003) ^[36]. The effects of auxin group of hormones on rooting and plant development have been discussed in several studies. Alvarez *et al.* [1989] ^[3] analyzed the effectiveness of IAA and IBA in *Maluspumila*. De Klerk *et al.* [1997] ^[53] analyzed the effectiveness of IAA, IBA, and NAA in *Malus*. In apple N-phenyl-phthalamic acid (PPA) at 0.4 kg/ha Nevirol 60 WP was applied at full bloom at extended flowering time increase fruit set (Racsko *et al.*, 2006) ^[74].

Pear cultivars grown in the warm climate of Israel, 'Spadona' and 'Coscia', produced relatively small fruit and to overcome this problem an experiment was conducted for five consecutive years (2001 to 2005). The GA₃ (8.6 mg L⁻¹) application alone at full-bloom, did not increase fruit size but in combination with dichlorophenoxyacetic acid (2,4-D) @ 6 mg L⁻¹ 2,4- and naphthaleneacetic acid (NAA) @ 6 mg L⁻¹ as 0.12% Bolero resulted in an appreciable and significant increase in fruit size without any deformation of the calyx-end. Application of 25 mg L⁻¹ benzyladenine (BA) plus 25 mg L⁻¹ GA₄₊₇ as 0.125% Perlan, applied at full-bloom +14 days, increased fruit size via stimulation of fruit cell division (BA) and fruit cell enlargement (GA), with no negative effect on fruit shape (Stern, 2008) ^[90]. In Pear NAA @ 20ppm and IBA @ 30 ppm at young fruit period enhance fruit growth and improved final fruit size (Chen *et al.*, 2012) ^[18].

Mature loquat trees when sprayed at pea stage of fruits and again one week later with NAA 2,4,5-T or GA₃ each at 10, 20 or 40 ppm, ripened about 10 days earlier than with GA₃ at 10 ppm, gave the best fruit retention (88.5%) and greatest lowest fruit volume (20.6cm³), weight (19.5 g) and pulp content (15.9 g/fruit) and lowest seed total weight (3.6g/fruit). NAA at 40 ppm gave the highest TSS (13.5) and reducing sugars (8.6 mg/100g), and lowest acidity (0.9%) (Chaudhary *et al.* 1990, 1993). The TSS of loquat fruit was increased when it was sprayed with 20 ppm 2,4,5-T as recorded by Chaudhary *et al.* (1992). NAA and NAAM (naphthalene acetamide) applications (25, 50 or 100 ppm) effectively thinned loquat fruits. Optimum level of thinning was obtained with 25 ppm. The effects of thinning on fruit growth varied with cultivar. Fruits on thinned branches developed more rapidly than non-thinned controls in all the cultivars. Thinning had no effect on fruit shape (Eti *et al.* 1990; Kilavuz and Eti 1993) ^[15, 16]. NAA at 20 and 40 ppm significantly increased the ascorbic acid content in loquat fruit. Ascorbic acid in loquat fruit was also enhanced with 40 ppm 2,4,5-T (Chaudhary *et al.*, 1992) ^[17].

Gibberellin

Gibberellins also promote growth. They are produced in very

young leaves, developing seeds, fruit, and roots. Gibberellins cause cell elongation, including shoot growth, and are involved in regulation of dormancy. Commercially, gibberellins have been used to improve fruit size and to prevent russetting. The plant growth regulator Apogee limits biosynthesis of gibberellins and thus inhibits shoot growth. Gibberellins might be crucial in the regulation of fruit set and development, and have been used successfully to induce parthenocarpic fruit development in many fruit trees, such as apple (Bukovac 1963; Watanabe *et al.*, 2008) [13, 97]. Gibberellin biosynthesis inhibitors have received the most attention because of their key role in cell elongation (Luckwill, 1970; Rademacher, 2000) [61, 75].

GA₃ increase fruit size, improves fruit firmness, increases vitamin-C, TSS, total sugars, sweetness index and overall sensory ratings of 'Red Delicious' apples (Hajam, 2017) [45].

In Pear, GA₃ application @11 ppm at blooming stage induced fruit development but led to the production of small fruits due to heavy fruit set (Knight and Browning, 1986) [54]. In Pear GA₄₊₇ or GA₃ @ 10 to 25 ppm at bloom stage improve cropping of pears. In Pear GA₄₊₇ @3% enhance the fruit growth and GA₃ @ 2.7% improved final fruit size when applied at young fruit period (Chen *et al.*, 2012) [18].

Spraying loquat clusters with an aqueous solution of 500 ppm GA₃ or 500 ppm GA₃ + 20 ppm Kinetin greatly stimulated frost-induced seedless fruits to attain the same size as seeded control ones. The GA- treated seedless fruits were more slender but had a thicker pulp than seeded untreated fruits. Application of ppm GA₃ + Kinetin was more effective for enlargement than a single application of ppm GA₃. If sprayed immediately after a frost, the enlargement response of the seedless fruits was significant. Although treated seedless fruits turned yellow earlier, the total soluble solid content in the juice at harvest was significantly lower than that of seeded fruits. No difference in titratable acidity was found between the treated and control fruits (Takagi *et al.*, 1994) [95]. The application of 40 ppm ppm GA₃ increased the reducing and non-reducing sugar in loquat fruit (Chaudhary *et al.*, 1992) [17].

Cytokinins

Cytokinins promote cell division. They are thought to be produced in the roots and by young fruit. Cytokinins are involved in apical dominance, branching, and stimulating bud initiation. Commercially they are used as fruit thinners (Accel and Maxcel). Besides auxin and GA, cytokinin is also known to induce fruit set in several fruit crops (Matsuo *et al.*, 2012) [65]. The endogenous level of cytokinin is directly correlated with the fruit growth, especially in stimulation of cell division. Its external application causes parthenocarpic fruit formation (Gillaspy *et al.*, 1993; Srivastava and Handa, 2005; Mariotti *et al.*, 2011; Matsuo *et al.*, 2012) [40, 87, 63, 65]. The cytokinin plays some role in the fruit maturation (Werner *et al.*, 2003; Bottcher *et al.*, 2011) [98, 11].

In apple NAA @7.5 ppm + Benzyl Adenine @75ppm at Full bloom resulted in Good thinning and good increase in fruit size (Robinson, 2006) [77].

In pear 6-BA @30 ppm at young fruit period improved final fruit size (Chen *et al.*, 2012) [18].

Ethylene

This is the only gaseous plant hormone. Many plant organs synthesize ethylene and it moves readily in the air surrounding the tree. Usually ethylene has an inhibitory effect on plants. It promotes abscission of leaves and fruits, inhibits shoot elongation and favours caliper development, and along with auxin, inhibits lateral bud development. On the other hand it can break dormancy in buds and seeds and causes rapid ripening of apples. In apples, ethylene is involved in the transition of fruit from being physiologically mature to ripe. Once exposed to ethylene their storage life is shortened. Ethepon is a synthetic compound that releases ethylene upon application. Re Tain interferences with ethylene biosynthesis. It allows fruit to hang on trees longer and lengthens storage life (Roper, 2005) [78].

There is an involvement of ethylene in production of aroma in fruits (Flores *et al.*, 2002; Botondi *et al.*, 2003) [33, 10]. Transgenic apple fruits with low endogenous ethylene level exhibit enhanced production of volatiles in the presence of exogenous ethylene (Schaffer *et al.*, 2007) [81]. Ethylene and brassinosteroids (BR) are also believed to play important roles in fruit set (Fu *et al.*, 2008; Serrani *et al.*, 2008; Wang *et al.*, 2009) [34, 82, 96]. Ethepon is widely used for apple thinning (Wertheim, 2000) [99]. Ethepon, as growth retardant is much less useful on bearing trees, because it thins fruit even at rates that have only a small effect on growth. In cv. Red Gold the application of Ethepon @500 ppm resulted in increased thinning (Williams, 1978). In cv. Summer Red apple the application of Ethepon @375 ppm at 20 % flower open increase the thinning and Ethepon @ 625 ppm at 10mm fruit diameter stage also increased the thinning (Meland *et al.*, 2011) [66]. In Golden Delicious apple the application of Ethepon @ 750 ppm increased thinning and the application of NAA @ 10ppm + Carbaryl (0.75%) increased return bloom (Gautam and Jindal, 2003) [36]. The combined spray of NAA 10 ppm and ethephon 150 ppm at full bloom can reduce fruit set up to 34 per cent in apple (Banday, 2006) [7].

Under commercial conditions, Ethrel may be a less practical option because the rate of Ethrel is cultivar-dependent, and there may be increased risk of preharvest drop for some early season cultivars. In addition, rates of Ethrel above 48 oz/acre may trigger fruit drop if the trees are under stress or temperatures during or following application are very high. NAA or Ethrel treatments in the 'on' crop year must follow an effective, aggressive chemical thinning program in order to increase return bloom of apple cultivars that are exhibiting a severe biennial bearing habit (Steven, 2010) [93].

Table 1: Comparison of Summer NAA and Ethrel programs for returns bloom in apple.

	Summer NAA	Ethrel ^z
Timing:	Start program in late June ^y .	Make one application 5-6 weeks after bloom when the thinning window is over.
Frequency:	Four bi-weekly applications.	Usually only one application is needed.
Rates:	5 ppm NAA (<i>Fruitone</i> L) for all varieties.	Rate is variety dependent. 16-24 oz ^z /acre: Gala, Rome, Red Delicious 24-48 oz ^z /acre: Golden Delicious 48-72 oz ^z /acre: Fuji, Cameno
Notes:	Can be included with cover sprays.	Not recommended on early season varieties prone to pre harvest drop eg. Honeycrisp.

z The Ethrel formulation described here contains 240 g a.i. per L. If a 480 g a.i. per L

product is used then the amount used per acre will need to be halved.

y In the Northern Hemisphere. This is approx. 8 weeks after bloom.

x 1 fluid ounce is equivalent to 29.57 ml.

Abscisic acid

Abscisic acid (ABA) is a growth inhibitor. It controls the dormancy of buds and seeds and inhibits shoot growth. ABA may act directly by blocking synthesis of enzymes or it operate indirectly by blocking RNA synthesis thus blocking the formation of enzymes, or it may operate indirectly by blocking RNA synthesis thus blocking the formation of enzymes that in turn form the growth promoters. ABA is produced in mature leaves along with many other plant tissues; it has not been chemically synthesized for commercial use.

The plant hormones/growth regulators abscisic acid (ABA) and polyamines (PAs) are implicated in fruit development, but knowledge of their precise role and mode of action remains sketchy (Gillaspy *et al.*, 1993; Nitsch *et al.*, 2009)^[40, 72]. Evidence suggests that ABA plays an important role as an inducer of ripening along with ethylene (Zhang *et al.*, 2009)^[103]. ABA has also been found to abolish GA-induced changes during fruit set in pea (Garcia-Martinez and Carbonell, 1980)^[35]. ABA has also been implicated in regulation of expansion phase in tomato as fruits of ABA-deficient mutants are smaller in size (Nitsch *et al.*, 2012)^[71]. In the apple (*Malus domestica*) fruitlet, a strong correlation between abscission and ABA levels in the fruit cortex was observed (Eccher *et al.*, 2014)^[26]. During the early phases of the shedding process, major transcriptomic changes and metabolic rearrangements occur within the fruit. A metabolomic study identified isoprene as an early marker of abscission induction. According to the hypothetical model, ABA may transiently cooperate with other hormones and secondary messengers in the generation of an intra-fruit signal, which then leads to the downstream activation of the abscission zone (Eccher *et al.*, 2014)^[26]. In addition, other reports have implicated role of abscisic acid (ABA), salicylic acid (SA), GA, cytokinin, polyamines (Pas), MJ (methyl jasmonate), and nitric oxide (NO) in fruit softening in several fruits crops, such as peach (Martinez-Romero *et al.*, 2000; Bregoli *et al.*, 2002)^[12] and sweet cherry (Kondo *et al.*, 2000)^[56]. ABA is involved in the regulation of flavonoid biosynthesis in highbush blueberry fruits (Zifkin *et al.*, 2012)^[104].

Growth retardants

The growth regulators help in stimulating the roots in cuttings when the endogenous and climatic factors are favourable (Darwesh *et al.*, 2013)^[22]. With regard to treatments with and without PBZ, no significant differences were observed i.e.

they did not contribute towards the root generation process. Thus plant growth retardant has effects on the partition of photo assimilates, on the water status of the cuttings and has been described as a promoter of adventitious roots in many species (Steffens and Wang, 1986)^[89]. Paclobutrazol in addition to stimulating root growth, as also mentioned by Davis *et al.*, (1985)^[23] and Steffens and Wang (1986)^[89] increased rooting potential.

Paclobutrazol is a gibberellin biosynthesis inhibitor that was first reported by Quinlan (1980)^[73] to control growth on apple trees. Prohexadione-Ca (Apogee in the U.S., Regalis in Europe) is a growth retardant registered by BASF that acts by inhibiting gibberellins biosynthesis (Evans, *et al.*, 1999)^[31]. Prohexadione-Ca degrades rapidly, must be applied several times as soon as sufficient leaf tissue emerges, which is usually near petal fall. It can increase fruit set when applied at moderate rates, thus considered as best for aggressive thinning program (Greene, 1999)^[43]. Most of its effects are secondary and generally attributed to increased light penetration due to a reduction in terminal growth. Prohexadione- Ca may control fire blight on shoots by inducing resistance in the tree (Yoder *et al.*, 1999)^[102]. Jasmonates have also been shown to induce aroma production in apples which is expected to be mediated by ethylene (Kondo *et al.*, 2000)^[56]. Besides these hormones, endogenous level of methyl jasmonate (MJ) has been found to increase with the progression of ripening in apple, mangoes, pears and tomatoes (Fan *et al.*, 1998)^[32].

In apple cv. Golden Delicious the application of DNO (375ppm) + NAD (34 ppm) + Ethephon (450 ppm) resulted in higher return bloom, minimum fruit set/ blossom cluster (Williams, 1978).

In pear cv. Bagugosa the paclobutrazol application @10ml/tree at leaf fall stage (second week of February) resulted in reduction in tree height and leaf area (Gupta and Bisht, 2005). In pear the Daminozide @2000ppm resulted in increase fruit set (Costa *et al.*, 2006)^[20]. In pear CPPU @10 ppm at young fruit period enhance fruit growth (Chen *et al.*, 2012)^[18]. Asin and Vilardell (2008)^[5] observed the effect of three different strategies *viz.*, root pruning and Prohexadione-Ca treatments applied separately and in combination of both in 'Blanquilla' Pear the combined treatment produced the least shoot length, increased return bloom in comparison with Prohexadione-Ca which produced similar yields as the control.

Table 2: General functions of different phytohormones with their practical use

Class	Function (s)	Practical uses
Auxins	Shoot elongation	Thin tree fruit, increase rooting and flower formation
Gibberellins	Stimulate cell division and elongation	Increase stalk length, increase flower and fruit size
Cytokinins	Stimulate cell division	Prolong storage life of flowers and vegetables and stimulate bud initiation and root growth
Ethylene generators	Ripening	Induce uniform ripening in fruit and vegetables
Growth inhibitors	Stops growth	Promote flower production by shortening internodes
Growth retardants	Slows growth	Retard the growth

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Role of PGRs in Stone fruits

Auxins

Stefancic *et al.* [2005]^[88] studied the effectiveness of IAA and IBA in *Prunus spp.* Crane (1956)^[21] reported that auxin caused rapid mobilization of soluble carbohydrates into apricot fruit. Stern *et al* (2007)^[91] applied 25-50 ppm 2,4-D,

15 ppm 3,5,6-TPA or 25-40 ppm 2,4-D coupled with 30-50 ppm NAA at the beginning of pit-hardening stage of apricot, when the widest diameter of the fruit was 25 mm. They reported that there was appreciable and significant increase in total yield with application of growth regulators.

In cherry cv. Bing the application of 2,4-D +NAA @ 25+ 30

ppm before pit hardening stage resulted in increase in fruit size and there is no negative effect on fruit quality (Stern *et al.*, 2007)^[92].

Gibberellin

Gibberellins are in the third place with a 17% share among the most commonly used herbal hormones within the natural plant growth regulators. Commercially the most common gibberellin is GA₃, and it is used to increase the length of the plant or to enhance plant yield (Kumlay and Eryigit, 2011)^[60]. Effect of GA₃ on rooting was also analyzed in several studies. The efficiency of GA₃ on *Prunus avium* L. and *Prunus mahaleb* was analyzed by Hepaksoy (2004)^[47]; on *Cydonia oblonga* by Aygun and Dumanoglu (2006)^[6]. Kaur *et al* (2004)^[48] reported that application of GA₃ 20 and 50 ppm, NAA 10 and 20 ppm, 2,4-D 4 and 8 ppm and 2,4,5-T 10 and 20 ppm decreased acid content of fruits of plum cv. Satluj Purple. GeCheng and JiXiang (1999)^[37] sprayed 6-year-old peach (cv. Gangshanzaosheng) and Japanese pear (cv. Huanghua) trees with Guodaduo (a plant growth regulator produced in Chinawhich consists of brassinolide, gibberellic acid and many mineral elements) about 1-5 days after flower drop, 10-15 days later and 25-30 days after the second spray resulted in increased peach yields by 199.76 per cent and Japanese pear yields by 61.57 per cent.

Cytokinins

Mass production of G · N15 rootstock by conventional methods like hardwood and herbaceous cuttings is time-consuming and laborious. Micro-propagation techniques can enhance scale and speed of production of healthy plants [Aka-Kacar *et al.*, 2010]^[2]. Micro-propagation is affected by many factors such as genotype, culture medium [Molassiotis *et al.*, 2003]^[69], and plant growth regulators [Ruzic and Vujovic, 2008]^[79]. One of the most important factors in plant tissue culture especially in proliferation stage is cytokinin hormone. It is well known that cytokinins play multiple roles in the plant development such as promotion of cell division and cell expansion in plant protein synthesis stimulation and the activities of some enzymes. BAP as a synthetic cytokinin in combination with appropriate auxins has been used in micro-propagation of nut fruits [Ruzic and Vujovic, 2008]^[79]. BA and BAP are commonly used as cytokinins in prunus rootstock micropropagation. Moreover TDZ has been reported to be appropriate in *in vitro* proliferation step of some prunus spp. [Durkovic and Misalova, 2008]^[25]. It is worth mentioning that some investigators have reported that TDZ and BA are the cytokinins used the most [Ruzic and Vujovic, 2008]^[79], while Arinaitwe *et al.* [2000]^[4] and Go ralski *et al.* [2005]^[42] reported that kinetin and isopentenyl adenine (2iP) have been used less.

Ethylene

In Plum 1000 ppm IBA + 50 ppm Ethrel application in one year old shoots resulted in maximum percentage of rooting (91.63%), more no. of roots, dry root weight and also developed the largest root (Gill and Daulta, 1996)^[39].

Abscissic acid

ABA appears to be an internal factor inducing dormancy in the buds of at least some temperature zone woody plants. ABA also prevents or delays the germination of seeds. ABA retards the growth of a large variety of plant tissues and organs including leaves, coleoptiles, stems, hypocotyls and roots. It promotes senescence through leaf abscission, degeneration of excised leaves and acceleration of decomposition of chlorophyll (Bisht *et al.*, 2018)^[8]. ABA concentration is very low in unripe fruit, but it increases as a fruit ripens, so it is therefore believed that ABA plays an important role in regulating the rate of fruit ripening (Setha, 2012)^[85].

Moreover, it is found that during fruit ripening, ABA also contributes to other functions, such as ethylene and respiratory metabolism, pigment and color changes, phenolic metabolism and nutritional contents, cell wall metabolism and fruit softening, and sugar and acid metabolism. In climacteric fruit such as apples, the level of ABA increases from maturation to harvest, while in non-climacteric sweet cherries, the level of ABA increases before maturation and thereafter decreases until harvest [Setha *et al.*, 2004; Setha *et al.*, 2005]^[83, 84]. The differing ABA levels suggest that the role of ABA may vary between fruits. In peaches, the ABA solution has been sprayed directly to the fruits at the beginning of maturation [Kobashi *et al.*, 1999]^[55].

Growth retardants

Gibberellin biosynthesis inhibitors have received the most attention because of their key role in cell elongation (Luckwill, 1970; Rademacher, 2000)^[61, 75]. In apricot cv. El-Amar in Egypt, Mohamed *et al* (2006)^[68] evaluated the effect of some summer pruning treatments on old (2 years old or above) branches (thinning branches by removing 1/3 branch number and shortening by topping 1/3 branch length) and/or spraying paclobutrazol (PP333) at 1000 ppm, and their combinations, on 15 July and 15 August. The untreated trees served as the control. The triple-combined treatment (thinning + shortening + PP333) recorded the highest significant values for number of spurs formed on branches (10.17 and 11.64). Yield per tree increased in all the treatments, but the highest yields were obtained from the triple combined treatment on 15 July (7.375 and 8.631 in the two seasons, respectively).

Table 3: Fruit Thinning Chemicals in Pome and Stone fruits (Khalil, *et al.*, 2010)^[49].

Crop	Chemical	Dosage (ppm)	Time of application
Apple	NAA	10-15	Full bloom to 4 weeks after Petal Fall
	2,4-D	2-10	Full bloom to Petal Fall
	2,4,5-T	2-25	Full bloom to Petal Fall
Stone fruits	NAA	10-50	Petal Fall to Pit Hardening
	2,4-D	2-15	Full bloom to Petal Fall
	2,4,5-T	2-25	Full bloom to Petal Fall
	Ethephon	100-500	Petal Fall to Pit Hardening

In plum (*Prunus salicina* cv. Red Rosa), the trees were treated with soil applications of paclobutrazol or uniconazole resulted

in increase in TSS content of the fruits (Lurie *et al.*, 1997)^[62]. In Peach cv. Pratap and Shan-e- Punjab the application of

Ammo Thiosulphate 2% at full bloom stage resulted in increased fruit yield (Saini *et al.*, 2003)^[80].

Eliwa and Ashour (2004)^[28] investigated the influence of paclobutrazol (0, 250, 500, 750, 1000, 1500 ppm) sprayed after three weeks from full bloom, on peach cv. Meit Ghamer. They implicated that spraying paclobutrazol 750 ppm resulted in decreasing the acidity of fruit juice significantly as compared to control. In Peach cv. Early Grande the application of Paclobutrazol @8ml/tree when new growth is just initiated (last week of February) resulted in minimum trunk growth, height and spread (Chanana and Gill, 2007)^[14]. Gibberellic acid and salicylic acid are also reported to increase yield, fruit acidity, reducing sugars, TSS, TSS/ acid ratio, fruit firmness and fruit chlorophyll a and b content peach trees (El- Shazly *et al.*, 2013)^[29].

An experiment was carried out at two locations in South Moravia (Kadov and Slup) during the year 2015. For branching of the plum trees commercial plant growth regulator products Gibb plus, and Globaryll 100 from Globachem, Progerbalin LG from L. Gobbi, Italy were used. For branching of the cherry trees new formulations of plant growth regulators (PGRs) were used beside the commercial products (Gibb plus + 4-CPPU, ethephon + BA + putrescine HCL, TIBA + 4-CPPU, naphthylphtalamic acid + 4-CPPU, TIBA + paclobutrazol, naphthylphtalamic acid + paclobutrazol, ethephon + GA₄₋₇ + putrescine HCL, Progerbalin + 4-CPPU + putrescine HCL, Globaryll + 24-epibrassinolide). The aim of the experiment was to verify the effect of commercial products and new formulations of PGRs on branching of nursery stocks of selected plum and cherry cultivars (plum cultivars: 'Guten von Bry', 'Cacak's Fruitful', 'Cacak's Beauty', 'Stanley', 'Domestic Plum'; cherry cultivars: 'Canada Giant' and 'Kordia'). Each treatment of commercial products was applied to 60 trees of each cultivar. The new formulations were applied to 10 trees. The commercial products and new formulations of PGRs were applied twice during the spring time (with a 12-day interval between applications). The best results were found in 'Guten von Bry' and 'Cacak's Fruitful' cultivars after application of Gibb plus (50% of 'Guten von Bry' trees and 80-90% of 'Cacak's Fruitful' trees were branched). Cherry tree cultivar 'Canada Giant' showed the best results (38% of trees were branched) after application of Globaryll + 24-epibrassinolide mixture. In 'Kordia' the best effect appeared (100% trees were branched) with the application of ethephon + BA + putrescine HCL (Wolf *et al.*, 2018)^[101].

Future Prospectus

The application of plant growth regulators at appropriate time and at recommended stage of a particular crop resulted in good quality fruit production as these help in regulating various physiological processes. The detailed information and understanding of hormone biosynthesis, transport, mechanism of action, rate, time, a method of application etc. are required to predict any specific response in any specific fruit crop. This will not only aid the search for new products, but will be useful in predicting possible secondary effects of potentially market-able compounds in terms of their effects on the environment. Sloppy technique will lead to disappointing results and will waste time and money. Record-keeping is critical. Without good records, it is impossible to keep track from year to year of which rates, material and environmental conditions produced acceptable results on which crop and cultivar. More attention should be required for their mixing

and spraying and these must be applied in recommended dose. New and improved products for better thinning agents and for better storage life growth regulators seem to be most urgently needed for use in fruit trees. Thus, the exogenous application of growth regulators acts as a powerful tool for enhancing the growth, productivity, quality of fruits and also helpful in mitigating the harmful effects of biotic and abiotic stresses in plants.

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