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Nidhi Chauhan
PG Scholars, School of Agriculture, Lovely Professional University, Punjab, India

Jyoti Bharti Sharma
PG Scholars, School of Agriculture, Lovely Professional University, Punjab, India

Kanchan Rana
PG Scholars, School of Agriculture, Lovely Professional University, Punjab, India

Wasif Mir
PG Scholars, School of Agriculture, Lovely Professional University, Punjab, India

Manish Bakshi
Assistant Professor (Fruit Science), School of Agriculture, Lovely Professional University, Punjab, India

Corresponding Author:
Nidhi Chauhan
PG Scholars, School of Agriculture, Lovely Professional University, Punjab, India

Effects of gibberellins and Promalin on the growth and development of fruit crops: A review

Nidhi Chauhan, Jyoti Bharti Sharma, Kanchan Rana, Wasif Mir and Manish Bakshi

Abstract
Gibberellins are the organic compounds which tend to regulate several metabolic processes in the plants. They play an important role in the enhancement of efficiency of fruit crops in terms of growth, quality and yield. GAs are naturally synthesized by the higher plants but in insufficient amounts. Therefore, the exogenous applications of GA at different concentrations and at different stages of growth drastically increase the seed germination, stem elongation, shoot initiation, flower induction, flower inhibition, fruit set, fruit development and modify several other vital processes in the fruit crops. Similarly, Promalin is a mixture of two naturally occurring plant growth regulators: gibberellic acid 4 and 7 (GA_{4+7}), which causes cell enlargement and elongation, and 6-benzyladenine (6-BA) which promotes cell division. Promalin has been reported very effective especially in temperate fruit crops like apple, pear, cherry etc. where it has proven beneficial for increased fruit set.

Keywords: Gibberellins, Promalin, fruit set, flower induction, fruit development

Introduction
Gibberellins are the longest phytohormones among the class of all the plant hormones. The main functions of gibberellins are to increase internodal length of the stem resulting in reduction of dwarf stature of the plant, enhancing seed germination, breaking seed dormancy and inducing flowering and fruit set in plants (Reid *et al*., 1992; Suge, 1985; Karssen *et al*., 1989; Brian, 1959; Prang *et al*., 1997; Jong *et al*., 2009) [54, 69, 31, 5, 50, 29]. GA is synthesized in the growing organs of the higher plants through methyl erythritol phosphate pathway (Sponsel, 1995) [66]. Initially GA was recognized from a fungus called *Gibberella fujikuroi* which is the causal agent of the foolish seedling disease in rice (Tudzynski, 1999; Stowe and Yamaki, 1957) [75, 68]. A Japanese scientist Eiichi Kurosawa (1926) reported that the fungus *Gibberella fujikuroi* releases some chemical which acts as a stimulus for the elongation of stem and shoots in rice (Kalra and Bhatla, 2018; Hedden and Sponsel, 2015) [30, 22]. In 1930s, Teiijro Yabuta and Yusuke Sumiki isolated this compound and gave it the name “gibberellin” (Yabuta and Sumiki 1938) [83]. Later on, in 1950s, GA was isolated from the plants and identified in all the plants and microorganism species especially bacteria and fungi (Rademacher, 1994) [51]. Various enzymes take part in the biosynthesis of GA namely *ent*-copalyl diphosphate synthase (CPS), *ent*-kaurene oxidase (KO), *ent*-kaurene synthase (KS), *ent*-kaurenoic acid oxidase (KAO), GA_{20}-oxidase, GA_2 oxidase, GA_3 oxidase, cytochrome P_{450} monoxygenase and α-ketoglutarate dependent dioxygenase (Grennan, 2006) [18]. The precursor of gibberellins is Geranyl diphosphate (GPS), which is a 20 carbon compound (Schie *et al*., 2007) [58]. GA consists of 2 hydroxyl groups, a carboxyl group and a lactone ring, therefore, these are known as tetracyclic diterpenoid compounds (Tian *et al*., 2017) [73]. More than 125 gibberellins have been isolated from the higher plants, bacteria and fungi out of which GA_1, GA_2, GA_3, GA_4 and GA_7 are widely studied and used all around the world (Hedden and Sponsel, 2015) [22]. In addition to these, Promalin is a synthetic derivative of gibberellins which is the mixture of GA_4 and GA_7 and 6-Benzyladenine. It helps in the elongation and enlargement of fruit cells (cell division), improves the size and shape of fruits and also helps in fruit setting.

Effect of gibberellins in breaking seed dormancy and inducing seed germination in temperate fruit crops
Seed dormancy is a resting stage in which seed is viable but is not able to germinate even if all the environmental conditions are favorable. Seed dormancy is sometimes an undesirable character and possesses many reasons which include the hard seed coat (External factor), presence of immature embryo or higher concentration of Abscisic acid (ABA) in the seed (internal dormancy) etc. Seed dormancy is most commonly found in temperate fruit crops after

the ripening of fruits (Campoy *et al*., 2011) [6]. Scarification is a mechanical method of reducing the seed coat dormancy, whereas internal seed dormancy can be overcome by the stratification (Usberti and Martins, 2007; Gusano *et al*., 2004) [76, 20]. But there are certain limitations of both methods. In mechanical scarification, there are higher chances of embryo damage whereas, stratification is a time intensive process (Rostami and Shasavar, 2009; Webb and Dumbroff, 2011) [56, 80]. Apart from these, treatment of seeds with gibberellins have also been found effective for terminating the seed rest. GA counteracts the effect of ABA and maintains the balance between GA-ABA in the embryo (Skubacz and Golec, 2017) [63]. Stratification takes more time (minimum 2-3 months) to break the dormancy, whereas GA induces the seed germination earlier than it. However, GA is less effective if the seed coat is intact with the seed but when the seed coat is removed, it accelerates the germination process rapidly (Nekrasova, 1960) [42]. Some important temperate fruit crops for which the gibberellin is used for breaking dormancy and enhancing seed germination have been described below.

Apple

For breaking the dormancy in apple (*Malus domestica* B.) seeds, stratification in moist sand at 4-7˚C for 3 months is most commonly practiced. Seed stratification combined with seed treatment with GA gives better results and reduces the chilling requirements (Pipinis *et al*., 2015) [49]. Treatment of apple seeds with GA_3 (500 ppm) for 40 hours resulted in higher germination percent, better seedling growth and saplings survival (Wani *et al*., 2014) [79]. GA in combination with other growth hormones has also reported very effective in enhancing the seed germination. During seed stratification, application of a mixture containing gibberellic acid (GA_3), salysylic acid (SA), jasmonic acid and 6-benzylaminopurine (BAP) in addition with breaking seed dormancy, stimulate the seed germination and seedling growth, enhance the chlorophyll content as well as increase the PSII efficiency to the maximum rate (Gornik *et al*., 2018) [17]. Similarly, combination of GA_3 ($5x10^{-5}$ M), BA ($5x10^{-6}$ M) and ethephon (10 mg/l) has also reported to enhance the germination of embryos, excised from the dormant or partly stratified seeds of apple (Sinska, 1989) [62].

Cherry

Among all the species of cherry, sour cherry (*Prunus cerasus*) requires the longest duration for stratification which is about 90 to 150 days at a temperature range of 33-41° F (Carroll, 2017) [7]. Also, poor seed germination is a major problem particularly in early maturing varieties of sweet cherry which is a major concern for the breeders and nurserymen. Therefore, both chilling requirement and poor germination can be reduced by soaking the cherry seeds (Mazzard and Mahaleb) in 100 ppm gibberellin solution (Pillay *et al*., 1965) [48]. Centinbas and Koyuncu (2006) [8] recorded the higher germination percentage of Mazzard seeds (without seed coat) upto 80% after 120 days of stratification with 500 ppm GA_3.

Peach

About 60 to 100 days are required for the stratification of peach (*Prunus persica*) seeds at a temperature range between 4˚C and 7˚C. GA_3 applied before the seed stratification increases seed germination percentage when the seeds are incubated for at least 35 days at 5˚C (Thomas and Rachmiel, 1986). With the application of GA in a moist medium, the stratification period in Elberta variety has been recorded only 35 days at a temperature near to the freezing temperature (Donoho and Walker, 1957) [12]. Combination of seed soaking in water and seed treatment with 2000 ppm GA_3 has also been found to increase germination rate and seedling growth in peach, plum and apricot (Shah *et al*., 2013) [59].

Almond

The standard time period required for the stratification of almond seeds is about 2 months at 4˚C (Reddy, 2020) [53]. This long duration of time was significantly reduced and higher percentage of seed germination was achieved within 5 days under in vivo and 10 days under in vitro conditions by treating the seeds of wild almond species with GA_3 + H_2O_2 combination (Zeinalabedini *et. al*., 2009) [87].

Effect of gibberellins on flower induction and flower inhibition in fruit crops

GA has the utmost importance in the induction as well as in inhibition of flowering in various fruit crops. As per the demonstration of Lord and Eckard (1987) [35], it was proved that as long as the sepals have not developed, GA prevents the flower formation. Although, the inhibitory action of gibberellins is more evident as compared to flower induction but during vernalization, GA acts as florigen and is highly responsible for inducing the flowering in the fruit crops. Gibberellins induce the flowering mostly in long day plants and have the capacity to replace the environmental conditions which favour the flower induction (King *et al*., 2006) [32]. In most of the fruit crops, the phenomenon of flower induction through GA is not much clear but it largely contributes in prevention or delaying the flowering in various fruit crops.

Citrus

There is a contention about the actual role of gibberellins on floral induction and floral differentiation in citrus. During the expected period of flower induction and differentiation, it was reported that, environmental conditions (Late autumn-early winter decreasing temperature) were the actual reasons behind the flower induction, not GA_3 and on the other hand, several experiments were conducted and reported that GA_3 and GA_{4+7} mixture are responsible for inhibiting the flowering in citrus rather than inducing it (Monselise and Halevy, 1964) [41]. Guardiola *et al*., (1982) [19], reported that, inhibitory action of GA_3 is found significantly high in late summers and early autumns whereas, Monselise and Halevy (1964) [41], reported the maximum flower inhibition during January. Studies indicate that application of GA before flowering also counteracts the floral induction in citrus (Garmendia *et al.*, 2019) [15].

Strawberry

Gibbrellic acid has a potent reported role in growth, flowering and yield in strawberry (*Fragaria* x *ananassa* Duch.). GA_3 application at a concentration of 50 mg/l resulted in early flower emergence, hastened the flowering and number of flower buds in the strawberry cultivars, whereas, the higher concentration of GA_3 (>200 mg/l) resulted in flower inhibition and formation of malformed berries consequently lowered the yield (Paroussi *et al*., 2002) [45].

Apricot

GA_3 @ 100 to 1000 ppm prior to pit hardening stage significantly delays the flowering and inhibits the flower bud as well as vegetative bud development in apricot along with cherry and almond (Bradley and Crane, 1960) [4]. Spraying higher concentration of gibberellic acid (300 mg/l) at the end of September had also been found to inhibit flower opening and fruit set and delayed full bloom upto 10 days in Shahroudi cultivar of apricot (Moghadam and Mokhtarian, 2006) [40].

Effect of gibberellins on fruit set and fruit development

Fruit set

After successful pollination and fertilization, ovary of flower is developed into fruit, resulting in fruit set. Sometimes fruit set also occurs in the absence of pollination and fertilization, consequently give rise to parthenocarpic or seedless fruits. Initially auxins were widely used for fruit setting but now gibberellins are reported to give better results than the auxins. GA successfully replaces the fertilization process in flowers and induces the parthenocarpy in fruit crops.

Guava

Mahmood *et al*., (2016) [37] concluded in their study that, foliar application of 200 ppm concentration of GA resulted in better fruit set of parthenocarpic fruits in guava with improved growth of fruits, high ascorbic acid and better TSS than the β-NOA which significantly failed to produce any parthenocarpic fruit in guava. Similarly, parthenocarpic fruits were also obtained from emasculated guava flowers with the application of 8000 ppm potassium salt of GA mixed with lanolin paste (Shanmugavelu, 1962) [60].

Mango

Non-pollinated mango flowers treated with 500 ppm GA_3 + prohexadiona (0 to 100 ppm) resulted in setting of parthenocarpic nubbins which survived better than the seeded fruits (Ogata *et al*., 2009) [43]. Highest flesh yield of Srisaket cultivar of Kaewmang (*Mangifera indica* L.) with the application of GA_3 @ 1953.25 ppm per hectare has been achieved (Benjawan *et. al*., 2006) [2].

Cranberry

GA application in cranberry (*Vaccinum macrocarpon*) has shown mixed results. Fruit set shows an increment with the application of gibbrellic acid but the similar results are not observed vis-à-vis vegetative growth. Application of GA_3 resulted in a rise in fruit set in cranberries cv. Early Black with application of 300 mg/l GA at 25%, 50%, and 100% blooming but it also resulted in formation of abnormal shoots in the vines of cranberry (Devlin and Demoranville, 1967) [11].

Fruit development

Fruit development refers to the enlargement of the cells resulting in increased fruit size after the fruit has been set successfully on the tree. Application of gibberellins during the developmental stages gives the best outcomes in terms of increased fruit size and yield. Larger and firmer fruits of cherry are obtained with application of gibberellins 20 to 30 days before harvesting (Looney and Lidster, 1980) [34].

Effect of gibberellins on fruit thinning

Fruit trees of temperate fruit crops (mostly pome and stone fruits) bear the flowers heavily which yield the fruits of reduced size with inferior quality thus, resulting in poor marketability. To maintain the adequate number of the fruit producing flowers, hand thinning is most common method but is time and labour intensive. Therefore, gibberellins are reported to successfully replace the hand thinning without damaging the fruit producing flowers. Initially, the effect of GA on the fruit thinning was studied in *Prunus sp.* by Hull and Lewis in 1959. In peach, flower initiation is proved to anticipate by the application of GA during floral induction period (Clanet and Salles, 1976) [9]. Sprays of 1000 ppm GA significantly reduces the flower numbers in apricot, peach, nectarine, plum and prunes and promotes the fruit set and yield (Southwick *et al*., 1995) [65]. Numbers of flowers of Redskin peach variety are reduced with the sprays of 75 ppm GA_3, when applied during flower initiation and just before the leaf fall (Painter and Stembridge, 1972) [44]. Significant depreciation in the flower numbers of sweet cherry was achieved with the application of 100 ppm GA, when applied 43 DAFD (Proebsting and Mills, 1974). Similarly, decreased terminal flower numbers have been found in sweet cherry with 20 ppm GA, when sprayed 19 days before the harvesting (Facteau *et. al*., 1989) [14]. Application of GA (50 ppm) in early June caused the reduction in the flowers of Patterson cultivar of apricot upto 50% (Southwick *et al*., 1997). Inhibition of flower bud formation in one and two years old apple plant (Cox's Orange Pippin) has been achieved with the application of 500 ppm GA and GA_{4+7} whereas, GA_3 showed lesser effect and GA_4 & GA_5 had no effect on the floral buds (Tromp, 1981) [74]. Similarly, Luckwill, (1979) [36], described that the formation of flower buds is diminished with the application of 550 ppm of GA_3 in Golden Delicious apple cultivars whereas, daminozide resulted in increased number of floral buds.

Effect of promalin on fruit crops

Promalin, a synthetic derivative of gibberellin has recorded to promote various growth and developmental mechanisms especially in temperate fruit crops which include, enhancement in fruit size and shape, russeting control, promoting shoot numbers and spurs in fruit trees. In addition to GA_4 and GA_7, promalin also contains one more ingredient i.e. 6-Benzyladenine, by nature which is a synthetic derivative of cytokinin. Cytokinins are subjected to promote cell division in plants while, the gibberellins help in the elongation of cell and growth of the plant organs (Westfall *et al.*, 2013) [81]. Several positive effects of promalin have been found mostly on the pome fruits (apple & pear) in terms of enhanced fruit quality, increment in lateral shoots and branching, reduction in fruit russeting etc.

Control of fruit russeting

Fruit russeting is a serious problem in apples caused by several bacterial species (*Erwinia herbicola, Pseudomonas sp*. etc.). The symptoms of this disease are corkiness of lenticels and formation of tan markings, shaped like rain splashed water droplets on the epidermis favored by cool weather and wet fruits. Fruit russeting can be controlled by application of gibberellins to the infected fruits (Taylor, 1978) [71]. GA_{4+7} doses (62.5, 125 and 250 mg/l) significantly declined the russeting in Golden Delicious cultivar of apple (Eccher and Boffelli, 1981) [13]. Similarly, not only does GA has its effect on fruit russetting, but it also enhances the quality of the fruits by making the fruit skin smoother (Pesteanu, 2012) [46]. Application of promalin (1.25 L/ha) at pink bud stage or at full bloom resulted to increase the fruit size and significantly decreased the russeting in Fuji and Imperial Gala apple cultivars growing in the moderate winter conditions of the southern Brazil (Leite *et al*., 2005) [33].

Effect on branching and shooting

Application of promalin mixed with brown paint on one-year-old wood of sweet cherries near to bud burst stage gave rise to lateral shoots and increased the spur numbers as well as exhibited some localized effect on the plant which led to the emergence of new lateral branches above the area where it was applied (Miller, 1983) [38]. Similar effects were obtained by Jacyna and Lipa (2008) [27] on the two cultivars Regina and Schneider of sweet cherry with the application of 5g/l of promalin mixed with acrylic paint on the beheaded leader, resulted in the induction of new lateral shoots followed by increased flowering and fruiting in the cherry.

Promalin along with PP_{333} @ 250 ppm when applied to pear cv. Gola at petal fall stage has reported to reduce the terminal growth of tree, increase shoot numbers and canopy spread while the PP_{333} treatments enhanced the spur numbers on the tree (Bist, 1989) [3]. Likewise, Gola pear cultivar treated with 250 ppm promalin and 1000 ppm SADH resulted in controlled enormous upright tree growth while promalin alone reported to induce lateral shoots and improved fruit quality when compared with SADH (Rai and Bist, 1991) [52]. Highest branching percentage with the application of promalin @ 1000 ppm from Morettini pear cultivar was found while the highest shoot length was obtained with 750 ppm promalin (Yildirim *et al*., 2010). Three applications of promalin on Gala, Fuji, McIntosh and Empire cultivars of apple induced the lateral branches significantly with acute angles and had least negative impact on the tree (Robinson and Sazo, 2013) [55].

Effect on fruit quality

Two sprays of promalin @ 125 ml/hl and 140 ml/hl on two apple cultivars Golden Delicious and Red Delicious at 80% blossoming resulted in a remarkable increase in the fruit size, improved fruit shape and reduced the number of seeds in the fruit respectively (Icka and Robert, 2009) [24]. Similarly, two sprays of promalin @18 mg/l on Tsugaru cultivar of apples after complete opening of king bloom drastically enhanced the leaf area, leaf weight, fruit weight and decreased the number of seeds/fruit (Youn *et al*., 2001) [86]. Two applications of promalin (100 & 150 ppm) at weekly intervals incremented the fruit weight, length, diameter and fruit shape of the Fuji cultivar of apple (Yildirim *et al*., 2015) [85]. spray of yeast extract at concentration of 200 ppm along with 50 ppm promalin significantly enhanced the fruit set, physical properties (fruit weight, volume, juice content etc.) and chemical properties (juice TSS% and acidity/TSS ratio) in the Jaffa cultivar of orange (Bakry 2007) [1].

Conclusion

Among all the gibberellins, GA_3 has wider applications in order to control different growth and developmental processes in the fruit crops and imparts relatively no phytotoxic effect. However, some amount of endogenous GA naturally occurs in plants but the exogenous applications of gibberellins at various stages of growth modify several phenomena in plants including seed germination, stem elongation, flowering control, fruit set etc.

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