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Impact of plant growth regulators in propagation of fruit crops: A review

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Abstract

Plant growth regulator or phytohormone may be defined as an organic substance produced naturally in plants, controlling growth and other functions at a site remote from its place of production in minimal quantities. Plant growth regulators include auxins, gibberellins, cytokinins, ethylene, growth retardants and growth inhibitors. Apart from it, they also regulate expression of intrinsic genetic potential of plants. Control of genetic expression has been demonstrated for the phytohormones at both transcriptional and translational levels. The use of growth regulators has become an important component of agro-technical procedures for most of the cultivated plants and especially for fruit trees. So far in fruit crops, plant growth regulators were widely used in vegetative propagation *viz.*, cuttings, air layering and invitro propagation *etc.* and also to control fruit drop and to improve the quality of fruit. It is familiar that most of the fruits are single seeded and trees raised through sexual means has long pre-bearing age, to overcome those constrains vegetative propagation is preferred in most of the fruit crops. In this review, we focus on role of plant growth regulators in asexual propagation of fruit crops.

Keywords: Phytohormone, invitro propagation, transcription, translation

1. Introduction

Thimmann proposed the term Phyto hormone as the hormones are synthesized in plants. Plant growth regulators include auxins, gibberellins, cytokinins, ethylene, growth retardants and growth inhibitors. Auxins are the hormones first discovered in plants and later gibberellins and cytokinins were also discovered. During the last 50 years considerable research work has been done in the country on various aspects such as varieties, propagation, irrigation, training and pruning etc. to increase the yield and quality of fruits. The production of poor quality fruits is a matter of common experience. It would be therefore worthwhile to improve the yield and quality of fruit crops by foliar application of plant growth regulators. The plant hormones are the organic chemical compounds, which modify or regulate physiological processes in an appreciable measure in the plant when used in small concentration. They are readily absorbed and move rapidly through the tissues, when applied to different plant parts. These chemicals are specific in their action. Thus the use of plant growth regulators has resulted in some outstanding achievements in several fruit crops with respect to growth, yield and quality. Lawes and Woolley (2001) [13] examined the commercial use of plant growth regulators to regulate fruit development.

Physiological responses that are regulated by plant growth regulators are- Promotion of branching, Increases flower bud formation, Thinning by promotion of fruit/flower abscission, Retards pre-harvest drop, Improve fruit shape, Vegetative growth control, Increase fruit set, Increase fruit colour, Advance fruit ripening, Delay fruit ripening. Apart from all of the above functions, role of growth regulators in propagation is reamarkable.

2. Commercial use of growth regulators in propagation of fruit crops

2.1 Mango

A study was conducted to know the effect of plant growth regulators on shoot tip establishment and growth of mango-rootstock (*Mangifera indica* L.) in vitro. These included the role of growth regulators, basal medium formulations, and genotypes. Shoot growth was better in G medium containing a combination of BA 1.0 mg/l, zeatin 1.0 mg/l, 2iP 2.0 mg/l, IAA 1.0 mg/l and IBA 0.5 mg/l plus CH 300 mg/l, glutamine 300 mg/l and 3 % sucrose than either B-5 basal medium or woody plant medium (WPM). The addition of GA_3 (5–10 mg/l) to the medium did not improve shoot growth. 'Terpentine' gave the best results in shoot tip establishment and elongation compared to other cultivars tested. The highest percentage of proliferated shoots was obtained from explants in May and June in comparison with explants from other months (Yang and Luders, 1992) [24].

2.2 Pomegranate

Sharma *et al.* (2009) [18] studied the effect of PGRs on pomegranate cuttings. Results obtained showed maximum rooting, root number and root length is observed with IBA 500 ppm + Borax 1% both in semi hard and hard wood cuttings. Field survival of the cuttings treated with IBA 500 ppm + Borax 1%, IBA 300 ppm + Borax 2% and IBA 5000 ppm remained maximum. Hard wood cuttings respond better to the hormonal treatment as compared to semi hard-wood cuttings.

Damar *et al.* (2014) [6] studied the effect of growth regulators on survival of pomegranate (*Punica granatum* L.) stem cuttings. Five treatments of growth regulators i.e. Control, 1000 ppm IBA, 2000 ppm IBA, 500 ppm NAA and 1000 ppm NAA were replicated three times in a complete randomized design (CRD Factorial). Results showed that the earliest sprouting of cutting as well as significantly highest percentage of success, number of leaves per shoot, length of root, diameter of root, fresh weight of roots, dry matter percentage of roots and number of roots per cutting, latest sprouting of cutting as well as lowest roots were observed in IBA 2000 ppm, followed by 1000 ppm IBA. In case of different combinations of growth regulators, percentage of success of cutting, number of shoots per cutting, number of leaves per shoot, total number of leaves per cutting, diameter of roots, dry matter percentage of roots, number of roots per cutting, length of root and fresh weight of roots were found significantly superior under treatment combination of 2000 ppm IBA (B2G2).

2.3 Guava

An experiment was conducted to know the effect of growth regulators on the rooting behaviour of soft-wood cuttings of guava varieties namely Baruipur, Sardar and Harijha, in two different seasons (spring and rainy season). The results obtained showed that irrespective of variety and season, all 3 growth regulators *viz.*, indole butyric acid (IBA), indole acetic acid (IAA) and naphthalene acetic acid (NAA), at 3 different concentrations (1500, 2500 and 3500 ppm), significantly increased the rooting of cuttings, number of primary roots and dry weight of roots per cutting. IBA was most effective in this respect, followed by IAA and NAA. Among the different concentrations IBA at 2500 ppm exhibited the best performance (73.3 to 83.3% rooting success, 5.82 to 7.16 primary roots and 101.2 mg to 112.4 mg dry weight of roots) irrespective of variety, while at 3500 ppm it decreased the different rooting parameters. NAA had an almost similar effect. But the response of different rooting parameters to IAA increased with an increase in concentration and attained the highest magnitude at 3500 ppm, irrespective of season and variety. Rooting of cuttings was better in the rainy season than in spring. Among the different varieties Baruipur was most responsive to the treatment with different growth regulators, followed by Sardar and Harijha (Debnath and Maiti, 1990) [8].

An investigation was conducted to find out the "effect of hormones on the rooting of guava (*Psidium guajava* L.) Cv. Allahabad cuttings. The experiment was laid out in Complete Randomized Design. There were two factors that are three different growth regulators and three types of cuttings. Growth regulators for instance, indole butyric acid (IBA), napthalene acetic acid (NAA) and Paclobutrazol each with 1000 ppm in aqueous solution were applied to hardwood, semi hardwood and softwood cuttings. The basal ends of the cuttings were immersed in the desired strength of the growth regulators for 5 min. After treatment the cutting were planted in plastic bags and covered with plastic sheets in order to maintain the humidity in the experimental area. Growth regulator and types of cuttings have non-significantly affected the days to bud sprouting. While sprouting percentage, number of branches, shoot length, root length, root weight, number of roots and survival percentage was significantly affected by various growth regulators and types of cuttings ($p \leq 0.05$). The results had shown that maximum sprouting (71.22 %), more number of branches (3.44), maximum root weight (1.46 g) and better survival (57.22 %) were noted in softwood cuttings treated with paclobutrazol at 1000 ppm solution. Maximum number of roots (59.66) and lengthy shoot (8.24 cm) were recorded in soft wood cuttings treated with IBA at 1000 ppm. Early sprouting (17.68 days) and maximum root length 12.81cm were observed in semi hardwood and softwood cuttings respectively, treated with 1000 ppm NAA (Ullah *et al.*, 2005) [23].

Optimum concentration and combination of IBA and NAA on rooting potential of guava stooled shoots was studied by Lal *et al.* (2007). The treatment IBA (7500 ppm) gave maximum rooting percentage (96.67%), average number of roots per shoots (46.93), average root length (8.45 cm) and survival (75%) after transplanting in the field. The optimum concentration of IBA must have caused the mobilization and utilization of carbohydrates and nitrogen fraction with the presence of cofactors at wounding portion, which helps better root initiation, number of roots per shoots and average root length. The highest survival of rooted stooled shoots may be due to well developed root system, which might cause better absorption of water and mineral nutrients from the soil and ultimately retaining higher percentage of survival in the field.

Gilani *et al.* (2018) [11] investigated the effect of Indolebutyric acid (IBA) hormone in inducing rooting of guava cv. Safeda. One year old healthy branches of guava were selected and wounded by complete removal of 2 inches bark just below the bud for rooting. These wounds were covered with soil media containing different concentrations of IBA hormone, wrapped with plastic and tied at both ends. The air layered plants detached after rooting were shifted to polythene bags and were kept in plastic tunnel to maintain its humidity. Different concentrations of IBA i.e., T_1 (50 ppm), T_2 (100 ppm), T_3 (150 ppm), T_4 (200 ppm) and control (T_0) were applied. According to data analysis layers treated with 150 ppm of IBA (T_3) was successful. Data recorded in control was minimum as compared to other treatments. It was concluded that, the Guava cv. Safeda air layered during monsoon season treated with 150 ppm IBA solution gives best results.

2.4 Grapes

A study was conducted using shoot apical meristems for in vitro regeneration in two grape cultivars -"Soltanin" and "Sahebi". Meristems were taken from 10 cm length shoots. The culture media used in this study was MS, with hormonal supplements consisting of: A (1 mgL-1 BA), B (1.5 mgL-1 BA), C (1 mgL-1 IBA+ 1.5 mgL-1 BA) and D (1 mgL-1 TDZ). Results showed that B and C variants of culture media produced the highest average number of shoots per cultured apex (3.8-5.4). Consequently, the shoots were twice subcultured on a medium variant supplemented with 1 mg/L TDZ+1.5 mg/L GA. The "in vitro" derived shoots were pretreated with 1 mgl^{-1} IBA, and then directly potted, which caused significant enhancement in root number per shoot. The good rooting achieved under "*in vivo*" conditions resulted in the reduction of the time spent for vitroplant acclimatization compared with "in vitro" rooting and, therefore, the time needed for plantlet regeneration was reduced (Aazami, 2010) [1].

2.5 Citrus

The influence of various basal medium and plant growth regulators on the efficient micropropagation of nodal explants from mature trees of alemow, sour orange, and 'Cleopatra' mandarin citrus rootstocks were studied. All three citrus rootstock shoot cultures showed a preference for high-salt media, like Murashige and Skoog or Driver and Kuniyuki Walnut medium. Several combinations of N^6-benzyladenine (BA) and adenine (AD), kinetin (KIN) or gibberellic acid (GA) were tested to optimize the shoot proliferation phase. BA/GA combinations improved the proliferation of all the rootstocks studied, especially alemow. The addition of BA and AD to the culture medium improved shoot proliferation in sour orange and 'Cleopatra' mandarin in the same way as BA and GA. The addition of different combinations of BA/KIN did not result in further improvement of any of the studied variables. The transfer of *in vitro* shoots to rooting media, containing different concentrations of indole butyric acid (IBA) and indole acetic acid (IAA), resulted in regeneration of complete plantlets. Alemow and 'Cleopatra' mandarin shoots rooted well using these plant growth regulators; however, all combinations of IBA and IAA tested resulted in very low rooting percentages in sour orange. To improve rooting in sour orange and 'Cleopatra' mandarin, different combinations of naphthalene acetic acid (NAA) and IBA were tested. All NAA/IBA combinations produced higher rooting percentages than did the IBA/IAA combinations, and in sour orange nearly 100 % of explants developed roots. An efficient and simple protocol for the micropropagation of three citrus rootstocks, alemow, 'Cleopatra' mandarin, and sour orange, by culturing nodes from mature plants, has been established (Tallón *et al.*, 2012) [21].

Fadli *et al*. (2017) [19] conducted a study in order to develop effective methods for rapid clonal multiplication of citrus rootstocks by exogenous application of auxins on rooting and establishment of spring cuttings of two citrumelo accessions (*Citrus paradisi* Macf. x *Poncirus trifoliata* L. Raf). Cuttings were taken from young greenhouse seedlings and exposed for 24 hours to different treatments including three auxin types (indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA)) and two concentrations of each one (100 and 200 ppm). Treated cuttings were then raised along with control cuttings under greenhouse conditions for six weeks. The results taken at the end of this period have shown significant variations in response to auxin treatments regarding survival, rooting and sprouting parameters. These followed similar patterns for the two accessions and were found to be dependant on the nature of the auxin rather than its concentration, the genotype or the interaction of these factors. Among the different combinations tested, IBA at 100 ppm level gave the highest survival (100%), the highest rooting percentage (100%) and was selected as the most suitable for promoting sprouting of citrumelo cuttings. By contrast, the application of NAA gave poor results and exerted a particular inhibitory effect on initiation of Swingle citrumelo roots and sprouts.

2.6 Jackfruit

Mukherjee and Chatterjee (1978) [15] studied the effect of etiolation and growth regulators on air-layering of Jackfruit (*Artocarpus heterophyllus* Lam.). The highest percentage (100.00) of rooting and establishment (91.66) was obtained when the etiolated shoots were treated with 10,000 ppm of IBA, during the month of September. Etiolated shoots also produced significantly more primary roots than non-etiolated shoots after treatment with IBA or NAA or IBA + NAA. Etiolated shoots without the application of growth regulator gave only 12.5 per cent rooting.

Mannan *et al.* (2006) [14] studied the effect of time of the year and growth regulators on in vitro propagation of jackfruit. Shoot tips from fresh sprouts on the trunk of mature jackfruit trees (*Artocarpus heterophyllus* Lam.) were used as explants. The explants were collected at three different times of the year from the same jackfruit plants. The collected explants were cultured on MS medium supplemented with NAA-0.5 mgl^{-1} and fortified with different levels (1.0, 1.5 and 2.0 mgl^{-1}) of BA to study the proliferation and multiplication of shoots from the cultured shoot tips and their subsequent growth and development. The in vitro shoot bud proliferation, multiplication and survivability were found better when the explants were cultured in January. BA at a concentration of 1.5mgl^{-1} was found most suitable for shoot proliferation and multiplication. And all the three concentrations of BA varied significantly. The survivability of the proliferated shoot buds was found better in concentration of BA at 1.0 and 1.5 mgl^{-1}.

2.7 Litchi

An experiment was conducted with two Bio-regulators (IBA and NAA) and there combinations in Randomized Block Design with three replication to know the effect of bio-regulators either alone or in combination on rooting percentage, survival percentage, rooting ability, growth and development of air layers in litchi. In this experiment it was found that IBA 5000 ppm produced maximum rooting layers (90.00 %) in litchi cv. PURBI. IBA 5000 ppm also proved better in survival percentage (86.66 %) fresh weight of roots (4.37 g) was found more in case of IBA 5000 treated layers followed by IBA 5000 ppm + NAA 5000 ppm (4.35 g) and minimum (2.24 g) under control, dry weight of roots was found maximum (1.11 g) by IBA 5000 ppm + NAA 5000 ppm treated layers whereas it was minimum (0.71 g) in untreated layers. The diameter of primary roots was maximum (0.83 mm) in untreated layer and minimum diameter of root (0.57mm) was found in bio-regulators (IBA 5000 ppm) application. IBA 5000 ppm alone or in combination with NAA at higher concentration as IBA 5000 ppm plus NAA 5000 ppm (T9) were found best in various parameters of root formation, root development, quality and growth of layers in the nursery (Das and Prasad, 2014) [7].

2.8 Apple

An experiment was conducted to study the influence of plant growth regulators and culture media on in vitro propagation of three apple (*Malus domestica* Borkh.) rootstocks. In this research, in vitro propagation of three apple rootstocks *viz.*, Azayesh-Esfahan, Morabbaee-Mashhad and M9 were investigated. Shoot proliferation carried out in two basal media (MS and WPM) containing three concentrations of BA (0.5, 1 and 1.5 mgL^{-1}) and also, rooting of microshoots were investigated in two basal media (MS and ½ MS) with three concentrations of IBA (0.5, 1 and 1.5 mgL^{-1}). The results showed that all studied factors including cultivars, media, BAP, IBA concentrations and interaction among them had significant effects on both shoot proliferation and rooting of rootstocks. Regardless of root-stocks and media, the maximum and minimum shoot proliferation rates were obtained in the media containing 1.5 mgL^{-1} and 0.5 mgL^{-1} BA, respectively. The MS medium was more effective on shoot proliferation than WPM medium. The Azayesh-Esfahan and Morabbaee-Mashhad showed the maximum and minimum (4.46 and 3.66 shoots/explants) shoot proliferation values, respectively. However, all rootstocks had the maximum rooting in the ½ MS media containing 1.5 mgL^{-1} IBA. In overall, Azayesh-Esfahan showed the maximum shoot proliferation (5.11 shoots/explants) and rooting (48.33 %) among rootstocks (Ghanbari, 2014) [10].

2.9 Apricot
Suriyapananont (1987) [20] revealed that stem cuttings of Japanese apricot treated with IBA at the concentration of 2000 ppm showed a significant increase in percent rooting and the quality of roots formed, but the quality appeared to decrease at 1500, 2500 and 3000 ppm of IBA, NAA and IAA did not give significant results

Perez-Tornero *et al.* (2000) [16] studied the effect of different cytokinin concentration on the proliferation of apricot cv. 'Canino' shoots. Among the media where optimum proliferation was obtained, healthier and greener shoots were found when a modified WP medium was used. ABA concentration between 0.5 and 0.6 mg l^{-1} produced an optimum number of shoots of a good length to be transferred to further subcultures. IBA and NAA induced rooting in similar percentages and with the same number of roots per shoot. The best rooting percentages (92.8 ± 2.5%) were induced in NAA at 2 mg l^{-1} while the largest number of roots per shoot (5.3 ± 0.5) were obtained after induction of 6 mg l^{-1} of IBA.

2.10 Peach
Chauhan and Maheshwari (1970) [5] conducted an experiment in peach var. Sharbati to know the effects of plant growth regulators on root initiation of stem cuttings of var. Sharbati. Terminal and sub-terminal peach cuttings were treated with different rates of IAA and/or IBA at up to 1000 ppm, planted in July, August and November. Sub-terminal cuttings rooted better than terminal cuttings. Rooting and leaf production were improved by all growth substance treatments at rates of 100 ppm. Best results were obtained with IBA at 50 ppm. The average rooting in the November planting was 40.65 per cent compared with 28.94 per cent and 12.89 per cent in August and July, respectively.

2.11 Walnut
An experiment was conducted in Walnut to commercialize vegetative propagation of the crop. Stooling with the application of plant growth regulators has been found successful in walnuts. In 27 treatment combinations of three levels i.e. 0 ppm, 5,000 ppm and 10,000 ppm each of IAA, IBA and NAA were applied and 14 treatment combinations gave positive results, yet three treatment combinations i.e., 5,000 ppm IAA + 10,000 ppm IBA + 5,000 ppm NAA and 0ppm IAA + 10,000 ppm IBA + 5,000 ppm NAA and 10,000 ppm IAA + 10,000 ppm IBA + 5,000 ppm NAA have given the maximum rooting with a greater number and length of primary, secondary and tertiary roots. The establishment of stools in all the 14 treatments was found cent per cent. The investigation has therefore enabled to screen the effective treatments and perfect a technique for stooling in walnuts (Rashid, 1978) [17].

2.12 Almond
An efficient protocol was established for in vitro shoot multiplication from apical shoot tips derived from mature trees of almond (*Amygdalus communis* L.) cv. Yaltsinki. Explants were cultured on Murashige and Skoog (MS) medium containing various concentrations of 6-benzyladenin (BA) and kinetin (kin) for shoot multiplication. Shoot multiplication was best achieved from explant on MS medium containing 30 gl^{-1} sucrose, 7 gl^{-1} agar and 1.0 mgl^{-1} BA. This amount of BA (1.0 mgl^{-1}) gave the best multiple shoot formation response with an average of 16.10 shoots per explant. In addition, shoots were cultured on the media containing 1.0 mgl^{-1} BA and kin combined with three different auxins (0.25 and 0.5 mgl^{-1} of IAA, IBA and NAA) separately. It was noted that 1.0 mgl^{-1} BA and kinetin combined with NAA had inhibitory effect on new shoot formation and no shoot formation was induced. However, explants cultivated on medium containing 1.0 mgl^{-1} BA and 0.5 mgl^{-1} IAA resulted in 11.25 shoots per explant. The effect of four different sucrose concentrations (20, 30, 40, 50 gl^{-1}) on the multiplication of shoots was also investigated. The best shoot multiplication was obtained in MS media containing 30 gl^{-1} sucrose with an average of 15.40 shoots per explant (Akbas *et al.*, 2009) [2].

2.13 Dragon Fruit
Ayesha and Thippesha (2018) [3] conducted a study on influence of growth regulators on rooting of stem cuttings in Dragon Fruit. The results revealed that number of days taken for root initiation was found early in IBA 7000 ppm (14.54), Percentage of cuttings rooted at 30 and 60 DAP (33.66 and 57.75 %), Length of the longest root at 30, 60 and 90 DAP (4.57, 10.65 and 23.07 cm), Average number of roots per cutting (12.70, 21.74 and 46.68), Average length of the roots per cutting (3.95, 6.32 and 12.41 cm) similar results were reported by Srivastava *et al*., (2005) [19]. Root volume (0.31, 1.81 and 1.97 cc), Root diameter (0.56, 0.76 and 1.47), Fresh weight of the root (0.46, 1.87 and 2.28) and Dry weight of the root (0.25, 0.46 and 0.67) respectively at 30, 60 and 90 DAP. The results revealed that cuttings treated with IBA 7000 ppm gave the best root growth.

2.14 Indigenous Fruit Crops
An effort to prevent the extinction of indigenous fruit crops in Assam, considering their importance in the environment of the rural area and home-life of the community, their large-scale multiplication through cuttings is the need of the hour. Vegetative propagation is particularly important in horticulture because genetic makeup of most fruit cultivars is highly variable, and unique characteristics of such plants are lost forever if these are seed-propagated. In propagation through cuttings, formation of roots is an important stage which is attained only when several influencing factors come into play. Among these, plant growth regulators are known to have a stimulatory effect on rooting of cuttings (Audus, 1965) [4]. So far, among a number of synthetic plants growth substances used for rooting of cuttings, indole butyric acid (IBA) has been found to be (Hartman and Kester, 1993) [12].

An experiment was conducted to study the effect of different concentrations of IBA (250, 500, 1000, 1500 and 2000 ppm) on rooting of cuttings in five indigenous fruit species- Barthekera (*Garcinia pedunculata* Roxb.), Teportenga (*Garcinia xanthochymus* Hk.f), Jalphai (*Eleocarpus floribundus* Bl), Nagatenga (*Rhus semialata* Murr.) and Outenga (*Dillenia indica* Linn.) during March 2011. Among all the five species studied, Outenga registered highest percentage of rooting (38.86), number of primary roots (12.00), survival percentage (40.47) and longest shoots (20.41cm). IBA @ 2000 ppm exhibited highest percentage of rooting (37.13), number of primary roots (9.13), survival percentage (35.69) and longest shoots (17.91cm) by Thejangulie Angami and R.P. Das.

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