

Plant Growth Regulators in Litchi

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1. Introduction

Litchi (*Litchi chinensis* Sonn.) is the most important fruit of Sapindaceae family. It is originated in the Southern region of China and has become widely distributed in the tropic and subtropics. The efficient utilization of plant growth regulators can provide a dramatic change in fruit production. At present, the plant growth regulators commonly used and proved effective on litchi are, paclobutrazol, gibberellin, ethephon, NAA *etc.* These plant growth regulators have the efficacies of growth inhibition, flower induction, fruit setting, ripening and keeping quality of fruits. In India litchi is grown mainly in the states of Bihar, West Bengal and Uttarakhand. It is also grown on limited scale in Tripura, Orissa, Punjab, Himachal Pradesh, Assam and the Nilgiri hills in the south. The problems responsible for low economic potential of litchi cultivation in various litchi growing regions include availability of genuine propagating material, poor fruit set (Sarkar *et al.*, 1984), heavy fruit drop (Singh and Phogat, 1984), fruit cracking (Bhat *et al.*, 1997) and inferior fruit quality (Brahmachari and Rani, 2001). Litchi bears heavy crop in one year and light or no crop in the adjoining year (Pandey and Sharma, 1989). On the basis of above problematic issues of litchi cultivation and production, in this chapter various aspects related use of PGRs in litchi production has been discussed.

2. Role of PGRs on Propagation

As a requirement of faster multiplication rate and availability of planting material, plant propagation methods are of utmost importance in horticultural crops, especially in fruit trees. In some fruit plants, the percentage of success in asexual propagation is extremely low. Litchi can be multiplied sexually but owing to disadvantages of seedling plants, it is chiefly propagated through vegetative means. The absence of easy and reliable clonal propagation method limits large scale cultivation of promising varieties while use of modern techniques like micro propagation has not proved very successful in litchi (Amin *et al.*, 1996). Among the various methods of propagation, marcotting or air layering is the most common and convenient method (Bhambota *et al.*, 1968). Nevertheless, the major bottleneck associated with this method of propagation is varying degree of success of air layering, high mortality of detached layers during establishment in nursery on their own root system. (Bhambota *et al.*, 1968, Menzel, 2001; Singh *et al.*, 2009) also suggested that, air layering with use of indole butyric acid (IBA) is most convenient, common and commercial method of propagation for promoting rooting in litchi. It was hypothesized that by applying proper concentration of plant growth regulators and use of suitable growing medium will help in the production of quality planting material with better root system of air-layers.

The use of plant growth regulators especially indole-3-butyric acid (IBA), naphthalene acetic acid (NAA) *etc.* have been advocated for accelerating rooting in litchi layers (Ram and Majumder, 1983). Chand *et al.* (2014) reported that air layering in litchi in the month of August with the use of IBA @600 ppm concentration can be practiced for obtaining higher rooting and better survivability of air layers of litchi cultivars 'Rose Scented' and 'Calcuttia' under *Tarai* conditions of Uttarakhand. Nanda and Kochhar (1985) reported that application of root promoting substances during layering induces profuse rooting within a short period of time and IBA has been found most effective. However, more information regarding use of growth regulators, ways to overcome higher mortality rate and improving the survival rate of litchi air layers needs to be generated.

In litchi cv. 'Purbi' IBA 5000 ppm produced maximum rooting layers (90.00 %) also proved better in survival percentage (86.66 %) and fresh weight of roots (4.37 g) dry weight of roots was found maximum (1.11 g) by IBA 5000 ppm + NAA 5000 ppm treated layers. Finally it was confirmed that the application of IBA 5000 ppm alone or in combination with NAA at higher concentration as IBA5000 ppm plus NAA 5000 ppm were found best for various parameters of root formation, root development, quality and growth of layers in the nursery (Das and Prasad, 2014).

Chawla *et al.*, (2012) found that application of IBA 5000 ppm treated layers took lesser days to root initiation (25.99 days) and also gave better results in relation to per cent rooting (86%), number of first (27.30) and second (41.20) order roots, total length of first order roots (2.14 m), mean root thickness (1.09 mm), fresh (2.89 g) and dry (1.11 g) weight of roots, fresh (46.29 g) and dry (28.00 g) weight of shoot and root: shoot ratio (0.04) of air layer in comparison to all other treatments. They concluded that application of IBA and NAA in combination (IBA 4500 ppm + NAA 200 ppm) had significant influence on rooting of air layers and was closely followed by IBA 5000 ppm.

Litchi also produced roots successfully from softwood cuttings (Ochse, 1952; Hayes, 1957; Ahmed, 1961) and semi-hardwood cuttings (Galloway, 1922; Paxton *et al.*, 1978). Girdling, growth regulators, misting and bottom heat technique improve the success rate for such cuttings. Abutiati and Nakasone (1972) noted that girdling at the base of the shoot 8 weeks before taking the cuttings increased rooting of 'Brewster' in Hawaii compare with control. Several researchers (Bhandary and Shivashankar, 1970; Abutiati and Nakasone, 1972) found that IBA is more effective than IAA, whereas the application of these hormones were not superior to individual applications. Treated shoots with ethephon @ 200 ppm and IBA @ 2000 ppm gave 85 % rooting of cuttings (Mitra and Bose 1991). However, this method of propagating litchi is not become popular amongst nursery growers in comparison to the air-layering method as it requires special structures like mist chambers for easy rooting and high survival percentage. Moreover, rooting is not that much easy as in case of air-layers that too thick and brittle in nature therefore, later on after transplanting in the field survival percentage is less and plants are prone to uprooting during heavy wind blow due to shallow root system.

3. Role of PGRs on Growth and Development

According to Zhang *et al.*, (2004) application of Forchlorfenuron and SS 3307 could inhibit the germination of winter shoot tip of litchi, decrease growth amount of winter shoot tip, reduce nutrient consumption in tree body and increase spike emerging rate and fruit setting rate of litchi, which lays basis for abundant production of litchi, especially for that with vigorously vegetative growth. Moreover, Forchlorfenuron and SS 3307 agent have obviously promoting effect in increasing spike emerging rate, flower spike length, flower spike width, fruit setting number, spike weight, per plant yield. Gao, *et al.*, (2001) studied the internal hormonal relationships between large and small fruits of two litchi cultivars namely Wanxuan No. 5 and Nuomici. The results from their study concluded that the GA+IAA+CTK/ABA hormones responsible for enlarging fruit size by activating the cell

division in ovary, pericarp and aril. Li *et al.* (1992) reported that spraying ethephon inhibit shoot tip germination for 22-27 days in litchi varieties of 'Baila', 'Heiye' and 'Guiwei' as well as make the leaves quickly ripened, the photosynthesis intensified, the nutrients accumulated and flower bud differentiation promoted. In conclusion, they suggested that ethephon is involved in the inhibition of germination in winter shoots of litchi.

4. Role of PGRs on Seed Dormancy, Germination and Seed Viability

Prasad *et al.*, (1996) conducted seed germination experiment of litchi cultivars namely 'Deshi', 'Kasba', 'Purbi', and 'Early Bedana' at Sabour (Bhagalpur), India. Seeds of each cultivar were treated with 1, 10 and 100 mM of IBA, GA₃ and ethephon. They found that soaking litchi seeds for 1 h in ethephon improved germinability of 'Deshi' at 10 mM and for 'Early Bedana' at 1 and 100 mM. However, 1, 10, or 100 mM ethephon reduced germination in 'Kasba' and in 'Purbi'. The improving effect of IBA on 'Deshi' seed was similar to that of ethephon. In 'Purbi', 10 or 100 mM IBA improved germination. GA₃ at 100 mM increased the germination of all cultivars and finally suggested that GA₃ plays an important role in litchi germination. Sharma and Dhillon (1986) observed that endogenous levels of gibberellins in litchi (*Litchi chinensis* Sonn) seeds decline at maturity stage. Such a decline may be a limiting factor in maintenance of seed viability and/or germination. Das *et al.* (1999) found 100% germination rate of lychee seeds in *in-vitro* on MS liquid medium supplemented with using 20 mg L⁻¹ BAP.

5. Role of PGRs on flowering, Crop Regulation, Fruit Set, Fruit Growth and Development

Kumar *et al.*, (2014) examined that different litchi cultivars show variations in their flowering and bearing habits, may accordingly be classified as regular, irregular, shy bearing *etc.* Litchi bears the flowers mainly staminate, hermaphrodite and pseudo-hermaphrodite. The first flowers to pollen are males, followed by hermaphrodites functioning as females and pseudo-hermaphrodites functioning as males. Kumar *et al.* (2014) mentioned that the two spray of 100 ppm NAA, potassium nitrate (1%) or ethrel (0.05%) during October and November promote flowering and application of paclobutrazol (PP 333) @ 5ml/m canopy diameter at 90 days before the expected date of flowering inhibit the shoot growth and panicle length ultimately increases the fruit set and yield in litchi. Spraying of NAA has been also found to enhance the fruit set, fruit retention and fruit weight. Applications of 100-150 mg/l of the sodium salt of NAA to litchi in Florida in early autumn discouraged vegetative flushes and increased flowering and yields over controls in most years (Ledin, 1953, 1954, 1955; Young 1957). Ethephon also play an important for enhancing the number of flowering

shoots in litchi (Sittichaikasem, 1974; Huang and Weng, 1978; Subhadrabandhu, 1986; Subhadrabandu and Koo-Duang, 1987).

Potjanapimon *et al.*, (2000) found that the cytokinin was low in litchi cv. 'Hong Huay' in Chiang Mai 6-8 weeks prior to flowering and increased at 4 weeks to reach a maximum two weeks prior to flowering. Gibberellins were high at four and three weeks before flowering and decreased two weeks prior to flowering to below detectable levels at flower emergence. The flushing habit of litchi varieties was intimately connected with irregular bearing. Problem is generally due to failure of flower initiation which puts forth vegetative growth prior to panicle emergence and flowering, thus eliminating the crop completely. Observations on young as well as old 'Calcuttia' trees showed that vegetative growth after September was at the expense of fruiting in the following year (Mustard and Lynch, 1959).

Several research workers advocated the use of various growth retardants as an alternate approach in litchi to restrict vegetative growth before panicle emergence Chapman *et al.* (1980). The highest amount of flowering was observed with application of ethephon treatment @ 100 ppm in cultivar 'Hong Huay' (Subhadrabandhu and Duang, 1985). However, Negi *et al.* (2012) obtained early panicle emergence, improved fruit retention and yield in 'Rose Scented' litchi when sprayed with TIBA (1 g/l) at monthly interval from September to December. TIBA is considered a polar auxin transport inhibitor and increases the endogenous cytokinin level in the lateral buds. There is may be a positive relationship between cytokinin level and flower formation which may be due to the positive impact of TIBA. Sing *et al.* (2012) reported that soil drench application of PBZ @7.5 ml proved to be the most effective treatment for suppressing shoot growth, panicle size, male flower percentage fruit drop and sex ratio. While same treatment resulted in increased hermaphrodite flower percentage, fruit set and fruit retention in litchi cv. 'Calcuttia'. At the stage of flower bud differentiation, spraying ethephon could remarkably reduce flower number, make flower bud abscission ahead of time, reduce the nutrition consumption of tree and improve flower quality (Li *et al.*, 1992). Diao (2006) revealed that spray with mixture of 0.05%–0.06% ethephon and 0.05% PBZ has the inhibitory effect on shoot tip growth and promote flowering.

Li and Zhang (1987) examined that before heading stage, timely spraying with suitable concentration of B9 and ethephon could economize nutrients consumed for flower, spike, and thereby improve flower quality. When buds begin to germinate or sprout for 2-3 cm under low temperature and drought conditions, spraying the mixture of B9 and ethephon has a better effect. Treatment of growth regulators including ethephon and paclobutrazol could remarkably change the contents of various endogenous hormones in 'Jizui' litchi trees.

At the early stage of flower bud differentiation, contents of IAA and GA₃ were remarkably reduced while contents of ABA and ZT were found increased level (Tang, 2006). Girdling of branches 3 to 4 cm in diameter with hardened flush in May or foliar application of 0.5 g paclobutrazol + 0.4 g of ethephon l⁻¹ promoted flowering in unproductive litchi trees of cultivar 'Tai So' in Mauritius (Ramburn, 2000). However, related research showed that paclobutrazol treatment was not to a certainly effective in promoting litchi flowering, just effective to the plant with medium flowering proportion (40-60%), to the plant with strong vigour (<30% flowering) this effect was sometimes poor, but to the plant with high flowering proportion (>70 % flowering) it had less effect or even reduced flowering (Menzel and Xiong, 1999).

Chen *et al.*, (1959) reported that gibberellins @ 250 ppm effectively extend the opening of female flowers which helped to get the pollen from male flowers and the same dose encouraged the pollen germination which improved the fruit setting in litchi.

Exogenous CKs could promote flower induction in monocarpic plant as well as polycarpic plant (Bangerth, 2009). In addition, Werner *et al.* (2003) confirmed the essentiality of CKs, using a transgenic CK-deficient *Arabidopsis* plants showing no flowering. Li and Ji, (1984) studied the effect of cytokinin on 'Nuomici' litchi tree outgrown in 1970-80s, and they found that zeatin and several other endogenous cytokinins played important roles in flower bud differentiation stage, once entered into inflorescence primordium formation stage and rachis formation stage, cytokinins content increased gradually and reached the peak at flower organ formation stage (Wu and Huang, 1986). Chen and Ku (1988) reported that foliar application of kinetin @ 200 ppm to 5 month old 'Heiye' litchi stems in the autumn, about 2 weeks after ethephon @ 200 ppm treatment, stimulated bud break 1 month earlier than in control stems. This treatment resulted in 60 % of the stems flowering, and in combination with ethephon raised the flowering rate to 80 % compared with no flowering in the controls. Chen (1991) found that 100 µg kinetin in a 5µl drop of 1 M acetic acid applied weekly to buds from 6 weeks before initiation of generative shoot development, stimulated bud break 1 week early and increased the proportion of generative shoots over non-treated controls. Thus, manually applying exogenous cytokinins or some growth-inhibitory nutrient substances during flower bud differentiation stage, could promote flower bud differentiation. His experiment from another angle proved the hormone balance theory which holds that flower bud formation promoted by growth inhibitor is probably related with the increase of cytokinin/gibberellin ratio. High cytokinin/gibberellin value promotes the generation of large amount of flower bud, while low cytokinin/ gibberellin value leads to the decrease of flower setting capacity.

6. Role of PGRs on Parthenocarpy

Seedless fruit, botanically termed parthenocarpic, do not have any seeds because the ovary is not fertilized. These types of fruit are rare in commercial varieties. One of the fruit on a twin-fruit stalk is sometimes parthenocarpic, although the companion fruit is usually normal or at least aborted-seeded. It is conceivable that plant growth substances are translocated from the actively growing companion fruit to its neighbour to enable it to grow. Artificial induction of shriveled seeds was achieved in 'Huaizhi' litchi by Liang and Qiu (1998) when maleic hydrazide (MH) was applied @ 1000 mg/l about 2 weeks after bloom, when the liquid endosperm was full. The fruit weighed about the same as controls, but had 10% more aril. Stern *et al.* (1997) reported that shriveled seeds could be induced in Floridian by spraying trichloro-phenoxy propionic acid or 2,4,5-TP at 100 mg/l when the fruit weighed 1 g, whereas the rate of seed abortion induced was less than 30%. Wang *et al.* (1997) found seedless fruit in 'Feizixiao' with 2,4-dichloro-phenoxy acetic acid or 2,4-D at restricted by the ability of the cultivar to set fruit parthenocarpically.

7. Role of PGRs on Control of Fruit Drop

The phenomenon of heavy fruit drop is typical to litchi (Menzel, 1984; Joubert, 1986; Galan-Sauco and Menini, 1989) and other fruit trees that produce a very large number of pistil-bearing flowers, such as mango (Singh, 1960) and avocado (Papademetriou, 1976). However, the fruit drop in litchi is often excessive. One of the most effective auxin for its control is 2,4,5-trichloro-phenoxypropionic acid (2,4,5-TP). Stern *et al.* (1995) found 'Mauritius' litchi fruit drop to be reduced and yield significantly increased (42±112%) with 100 ppm 2,4,5-TP applied as Tipimon. Stern and Gazit (1997) also reported that spraying with 50 ppm of 3,5,6-trichloro-2-pyridyl-oxyacetic acid (3,5,6-TPA as Maxim) is as effective (29-550% increase) and unlike Tipimon does not injure the foliage. Stern and Gazit (1999) noted that synthetic auxin 3,5,6-TPA significantly increased fruit retention and yield in 'Kaimana' litchi when applied three weeks after full bloom. This appears to be an effective specific to 3,5,6-TPA which is separate from its effect on litchi fruit-drop reduction, as it does not occur after 2,4,5-TP application (Stern *et al.*, 1995, 1997). Kumar *et al.* (2014) also reported that growth substances like NAA, PCPA (parachlorophenoxy acetic acid), GA, BA and CCC (Cycocel) proved beneficial in minimizing the drop and cracking and enhancing quality of litchi fruits. The minimum fruit drop was recorded with 20 ppm PCPA. Fruit cracking was least with 20 ppm NAA. The maximum fruit weight and highest aril percentage were obtained with 100 ppm GA. Fruits sprayed with 500 ppm CCC had the highest total soluble, total sugars and ascorbic acid contents, but had the least acidity. Fruit set, fruit and aril weights, fruit volume and pulp percentage, total soluble

solid, total sugar content and fruit production significantly increased with NAA and 2, 4-D.

8. Role of PGRs on Fruit Yield and Quality

Singh *et al.*, (2012) noted that soil drenching of PBZ @ 7.5 ml/tree respond better for increasing number of hermaphrodite flowers, fruit set, fruit retention and sugar content whereas PBZ @ 5.0 ml/tree was responsible of increasing fruit breadth and weight. They also found that the foliar application of CCC @ 2000 ppm in mid-September to mid-November resulted in maximum pulp weight, pulp/stone ratio, total soluble solids and minimum acidity

Ethylene is widely applied in litchi production, which can promote fruit ripening, improves anthocyanin levels in peel, and mean-while decreases the chlorophyll content. Suitable concentration of ethephon treated litchi tree generates fruits with remarkably increased soluble solids, sugar and ascorbic acid contents and meanwhile decreased acidity in their pulps (Sadhume *et al.*, 1992). Dixit *et al.*, (2013) reported that GA₃ @ 10 ppm was found effective treatment to increase fruit set, fruit retention and size of fruits. GA₃ @ 20 ppm produce maximum number of fruits/tree. Least fruit cracking was also noted with GA₃ @ 20 ppm treated fruits. Auxin stimulation due to 2,4-D and GA₃ might be the reason for the accumulation of building block at faster rate and better execution of source-sink relation registering higher fruit setting, retention and less cracking (Kumar *et al.*, 2009).

Concentration of abscisic acid (ABA) were found higher in the pericarp, seed and aril of cracked fruits of litchi than normal fruits while the concentrations of gibberellin (GA₃) were higher in the seeds at the critical period of cracking (Sharma and Dhillon, 1986, 1988). Many researchers (Suryanarayana and Das, 1971; Kanwar and Nijjar, 1976; Sharma and Dhillon, 1987; Chandel and Kumar, 1995; Bhat *et al.*, 1997) reported that auxins (2,4-D and NAA) at concentrations (lower than 40 mg/l) reduced cracking. Gibberellin (GA₃) at 10-50 mg/l was partially effective (Suryanarayana and Das, 1971; Sharma and Dhillon, 1987; Sinha *et al.*, 1999). Peng *et al.* (2001) indicated that GA reduced cracking by lowering the activity of cellulose. Shrestha (1981) found that ethephon sprays at 10 mg/l reduced cracking from 12% (control) to 6% in Early Large Red. Stern *et al.* (2001) suggested that synthetic auxins like 2,4,5-TP (as Tipimon) @ 67 ppm and 3,5,6-TPA (as Maxim) @ 50 ppm have very positive effect on yield, fruit size and fruit colour in several litchi cultivars in China.

9. Role of PGRs on Maturity, Ripening and Shelf-Life of Fruits

Even litchi fruit has good demand and vast export potential, yet it is not a major commercial crop, mainly because of its seasonal availability and short shelf-life. Postharvest decay,

pericarp browning and desiccation were identified as major problems restricting expansion of the industry in litchi exporting countries (Sivakumar *et al.*, 2007). The removal of ethylene and/or inhibition of ethylene in stored environments are fundamental in maintaining postharvest quality of climacteric produce (Saltveit, 1999). In recent years, however, there has been a paucity of research on developing new and more efficacious ethylene scrubbing materials. Thus, research activity has drifted away from ethylene removal to preventing the actions of ethylene through using 1-MCP or other ethylene absorbers. The coating of chitosan mixture on the surface of 'Huai' litchi fruits showed better fruit coat colour, low rotten rate, less weight loss and low respiratory rate that prolonged the shelf life of litchi fruits (Wu *et al.*, 2001). Coating with chitosan can hinder the infiltration of oxygen in atmosphere and emit of CO₂ produced by fruit respiration, but it allows the emit of ethylene that ripens fruit, thus inhibits the fungal propagation and postpones the ripeness of fruit, control the rising of POD activity, thus inhibit the yellowing and browning of fruit, ensuring fruit quality (Lin *et al.*, 2005).

Ozone has strong oxidation and germicidal effect to bacteria and mildew. Findings of Lin (1999) indicated that as ozone could kill pathogens on fruit surface and clear up the effect of metabolites, better controlling the putrefactive degree of litchi fruits. Ozone is effective in degrading ethylene, delaying the aging of fruits and vegetables, also it can break the benzene ring and remove off the pesticide residues like organochlorine and organophosphorus (Zhang *et al.*, 2003) and better ensuring food safety. Li *et al.*, (2004) used different concentration of ozone water to treatment litchi fruits after harvest, and found that ozone water had some effect in maintaining and protecting litchi fruit colour and inhibit browning. Combined with other fresh keeping techniques, pretreatment of 1 mg/L ozone water can prolong preservation duration and enhance fruit quality. Yang *et al.*, (2001) reported that ozone water had effective for precooling, colour protecting and rinsing, and it can kill the pathogens carried by the surface of litchi fruit, which to the largest fruit extent reduces the side effect of mechanical damage. Moreover, ozone could narrow the stoma on litchi fruit surface, hugely reduces weight loss.

Different antioxidants and salicylic acid were tested to overcome pericarp browning and to maintain the postharvest quality of the litchi fruits at ambient storage. It was found that 0.5% salicylic acid, 1% isoascorbic acid and 1% *N*-acetyl cysteine performed better over sulphur dioxide (SO₂) fumigation for most of the parameters under study. Application of 0.5% salicylic acid found superior to reduce the pericarp browning, relative leakage rate, and decay percentage. It was effective in reduction of polyphenol oxidase activity and improvement of anthocyanin pigments of the fruit pericarp over other treatments. Total

soluble solid, titratable acidity and ascorbic acid of the litchi fruits were recorded highest with the application of 1% isoascorbic acid followed by 0.5% salicylic acid treatment. Therefore, 0.5% salicylic acid and 1% isoascorbic could be used as an alternative of SO₂ fumigation for quality retention of litchi fruits (Kumar *et al.*, 2013).

Mishra *et al.*, (2012) made an attempt to study the effect of GA₃ and BA on ripening of litchi cultivar 'Rose Scented'. In this attempt, KNO₃ (4%) was sprayed at 1 cm size of panicle in the first week of February. However, other treatments viz. GA₃ (20, 40 ppm) and BA (20, 40 ppm) were applied two weeks before expected date of harvest (on 15th May). They found that KNO₃ (4%) advanced the harvesting date only for 2 days in comparison to control. Whereas GA₃@ 20 and 40 ppm delayed the harvest date for 2 and 5 days, respectively and BA @ 20 ppm and 40 ppm delayed the harvest date for 5-6 days. In all the treated trees, fruit weight was found to be more as compared to control. Higher fruit quality attributes were recorded with GA₃ (40 ppm) followed by GA₃ 20 ppm over other treatments. They also observed least fruit cracking of GA₃ and BA sprayed trees. Tomer *et al.*(2001) noted that spraying of gibberellic acid (50 ppm) and Magic (Uniconazol 0.5 %) during flowering of litchi cultivar 'Mauritius' which affect fruits with a lower sugar and higher acid contents, which may indicate a delay in ripening. Spraying during fruit development with, gibberellic acid (50 ppm) or KNO₃ (4%) had no noticeable or significant effects on fruit ripening and on TSS or organic acids levels.

Litchi is a non-climactic fruit. Yin *et al.*,(2001) studied the changes in ABA and ethylene and their roles in litchi maturation and colouration. Contents of endogenous abscisic acid (ABA) and ethylene in litchi fruit during development were determined and Ag₂S₂O₃ (STS) which inhibits synthesis of ethylene was used to study on fruit maturation and coloration. The content of ABA increased rapidly a week before pericarp colouration. With the occurrence of ethylene climacteric, there was a rapid increase in sugar content and invertase activity. STS delayed fruit coloration and maturation. It was suggested that the increase in ABA might have induced the synthesis of ethylene, which consequently promoted fruit maturation and coloration. Results showed that foliage spraying of STS inhibited fruit maturation and coloration, indicating that ethylene is involved in the regulation of the events. Similar observation was also made by Pal and Mishra (2012).

An experiment was conducted to stagger the harvesting period of litchi in cultivar Rose Scented. In this regard, various kinds of treatments were imposed on 25 years old full bearing litchi tress either at flower initiation or few days before harvest of fruits. KNO₃ (4%) was sprayed at 1 cm size of panicle in the first week of February. However, other treatments

viz. GA (20, 40 ppm) was applied two weeks before expected date of harvest, while shading treatments were given by covering the tree with nylon nets producing 30% and 50% shades respectively, 30 days after fruit set. Shade nets 30% and 50% were most effective in delaying ripening of litchi fruits and delayed the harvest date by 5 and 8-10 days, respectively without deteriorating the fruit quality. GA₃ 20 and 40 ppm delayed the harvest date for 6-7 and 4 days, respectively while KNO₃ (4%) could not play significant role in advancing/delaying the harvest date of litchi. Higher fruit yield and reduced fruit cracking were obtained with shade net (50%) which was remained at par with shade net (30%). Higher fruit chemical quality attributes were recorded in GA₃ sprays over other treatments (Mishra *et al.*, 2014).

Wang *et al.*, (2001) conducted experiment on ten-year-old trees of 'Fezixiao' litchi in Wantian Orchard, Zengcheng, China. A set of three trees with the same date of full bloom (March 25) was chosen for the determination of fruit colour area (%) and the concentrations of endogenous hormones, anthocyanins, chlorophylls and sugars. Another set of three trees with the same full bloom date (April 7) were chosen to test the effect of 6-BA and ethephon. Ten fruit clusters on each tree were dipped in 0.1g L⁻¹ 6-BA or 0.4 g L⁻¹ ethephon on 51 DAFB (Days after full bloom). Control fruits were dipped in water. Result showed that 6-BA delayed the degradation of chlorophyll by inhibiting the activity of chlorophyllase and in the meantime anthocyanin formation. These results indicated that delay in peel coloration be linked with the degradation of chlorophylls, which impeded the formation and masked the appearance of anthocyanin.

The concentrations of ABA in the peel and the aril were increased rapidly around 62 DAFB. And this was followed by an accumulation of sugars and anthocyanin. ABA appears to have a major role in maturation. Application of ethylene @ 400 mg/l advanced colouration in Shahi litchi by 8 days compared with controls (Sharma *et al.*, 1986). Ray and Sharma (1986) reported that spraying with GA₃ at 25-50 mg/l, chlormequat @ 2000 mg/l and daminozide @ 100-2000 mg/l delayed ripening in litchi. Dawson *et al.* (1976) showed a strong relation between fruit growth and ABA in cherry. The delay in colouration by 6-BA was in parallel with ABA in the peel with no obvious changes in ACC (1-aminocyclopropane-1-carboxylic acid). Although ethephon increased ACC and slightly accelerated the loss of chlorophyll, ABA appears to be more closely involved in litchi maturation than ethylene. Stern *et al.* (2001) examined the effect of 2,4,5-TP (as Tipimon) @ 67 ppm and 3,5,6-TPA (as Maxim) @ 50 ppm, or their combination (first with 2,4,5-TP and 4 days later with 3,5,6-TPA) on 'FeiZi Xiao', 'Hei Ye' 'Kaimana' and 'Floridian' litchi cultivars. They sprayed the chemicals when fruitlets diameter at 14 mm and found that in cultivars 'FeiZi Xiao' and 'Hei Ye' the red color of the ripe fruit was enhanced by 3,5,6-TPA.

10. Role of PGRs on Embryogenesis

Embryo development in litchi affects fruit set and eating quality, and is presumably associated with changes in endogenous hormones. Chen and Lu (2000) studied the relationship between embryo development and endogenous hormones in litchi, using normal and aborted ovules of 'Lanzhu'. In normal ovules, the concentrations of Indole acetic acid (IAA), gibberellin1+3 (GA_{1+3}) and abscisic acid (ABA) peaked 7 days after anthesis (DAA), and then declined. The concentrations of IAA and GA_{1+3} increased again during globular to torpedo stage, while cytokinin (CTK) peaked before the globular stage. In aborted ovules, ABA remained at a high concentration throughout, while IAA and GA_{1+3} declined to their lowest level, CTK was lower than in normal ovules during the embryo abortion period. The concentrations of zeatinriboside group (ZRs), dehydrozeatinriboside group (DHZRs) and isopentenyladenosine group (iPAs) varied indicating different roles in embryo development.

One of the first works on somatic embryogenesis in litchi was done by Amin and Razzaque (1995), who managed to induce somatic embryogenesis in the cultures of zygotic embryos of lychee using BA (5 mgL^{-1}) and activated charcoal (1 gL^{-1}). Although about 40% of the in vitro formed embryos matured, no plantlets were obtained. Yu and Chen (1998) reported the development and maintenance of highly embryogenic suspensions and protoplast isolation for several lychee cultivars. In another work, Yu *et al.* (2000) managed to successfully culture lychee protoplasts of the cultivar 'Xiafanzhi' from suspensions, only after embedding them in Ca-alginate beads. Although the successful culture of lychee protoplasts could facilitate their use in lychee breeding, the regeneration frequency was low.

As an alternative to air-layering, micro propagation offers an attractive method for vegetative propagation of lychee. It requires only very small amounts of propagating material and has the potential of providing very large numbers of cloned plants. The exogenous application of plant growth regulators also an important aid to stimulate rooting potential during in-vitro explant culture. Poochua (2005) Studied in-vitro plantlet regeneration of litchi (*Litchi chinensis* cv 'Tai So'). Litchi plantlets were successfully regenerated in-vitro using young leaf explants. They examined that 2, 4-D @ 1.5 mg/L in combination with cytokinin (BAP @ 2.0 mg/L) was more efficient in callus initiation while BAP, in combination with IAA @ 3.0 mg/L was more appropriate for the differentiation of shoots from callus. IBA @ 2.0 mg/L promoted rooting of the shoots. Although this method of propagation has been improved by the use of younger branches, small earth balls and 1, 4-indole-3-butyric acid (IBA), the process is still slow and inefficient.

Fruits that have aborted seed are termed “chicken tongues” and are preferred by consumers (Lake, 1988), since these fruits have a high flesh to seed ratio. In crosses involving plants which have tendency to produce these fruits as the female parent, many of the most valuable progeny are lost prior to harvest. Production of plants by culturing embryos prior to abortion would be expected to yield progeny with high proportion showing the chicken tongue character (Anon, 1991). This technique has the advantage of providing very clean material as well as producing juvenile tissues which usually respond well in culture (Durzan, 1984). In their work on lychee anther culture, Fu and Tang (1983) used opened staminate and hermaphrodite ‘Chenzhi’ and GushanJiaohe’ litchi flowers containing 3-4 mm long anthers with microspores at the optimum late-uninucleate stage of development were utilized. The anthers were removed and plated on induction medium consisting of MS salts and organic components, 2,4-D @ 5-9 μM , 4.6-9 μM kinetin, 3-5 μM NAA, and 16 g/l sucrose. The cultures were incubated at 25°C with a 10h photoperiod. In order to initiate haploid embryo development and maturation, embryogenic cultures have been subcultured on MS supplemented with 2 μM kinetin, 0.5 μM NAA, 500 mg/l casein hydrolysate, 400 mg/l royal jelly, and 16 g/l sucrose. Early heart-stage embryos were transferred to MS with 2 μM kinetin, 3 μM gibberellic acid (GA_3), 500 mg/l casein hydrolysate, 400 mg/l royal jelly, 1.7 g/l glutamine, and 16 g/l sucrose. The haploid embryos matured and germinated. Higher concentrations of 2, 4-D were avoided because of its possible role in chromosomal aberration and suppression of morphogenesis (Murashige, 1974).

11. Role of PGRs on Stress Management

Under stress conditions plant turns ‘on’ its ABA synthesis system to synthesize a mass of ABA. Morrillon *et al.*, (2001) mentioned that under stress conditions the ABA responsive genes will activate which later on act as a stress adaptation mechanism by improving water uptake, decreased the leaf extension rate, by closing stomata etc. Zhou *et al.* (2009) performed low temperature stress and ABA treatment on litchi under the conditions of artificially simulated natural low temperature using artificial climate. ABA can combat the stress condition by reducing transpiration rate, cell membrane integrity and by increasing protein levels and enzymatic activities (Ma *et al.*, 1998). Even stressed by 2°C low temperature, ABA treated litchi also had higher flower formation rate consequently, via enhancing endogenous ABA level. ABA treatment could make stomata closed, transpiration weakened, water potential enhanced to some extent alleviating the damage of low temperature. In addition, ABA treatment changes the balance of ABA/IAA, letting ABA/IAA at high level under low temperature. Paclobutrazol is capable of slowing plant growth, promoting plant tillering and enhancing the ability to anti-lodging, effectively controlling the morphogenesis of fruit

tree, advancing ripeness stage and increasing stress resistance of crops. At the same time, paclobutrazol is efficient in inhibiting bacteria and fungi, effectively preventing the diseases of various crops, which shows broad application prospect in agriculture and horticulture. In general, the polyamines also play a vital role for combating the adverse effect of stress condition.

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