1. Introduction

Aonla (*Emblica officinalis* Gaertn), the Indian gooseberry has emerged as one of the important minor fruits of India owing to its nutritive, medicinal values and suitability for processing of diversified value added products (Pathak, 2003). It is the rich source of vitamin ‘C’ (500-600 mg/ 100 gm) among fruits and ranks second after Barbados cherry (*Malpighia glabra* L.) (Asengo, 1953). It has oxidation of which is retarded by the presence of leucoanthocanins in the fruit itself. Fruits are rich source of minerals like iron, calcium and phosphorous and dietary fiber. The demand for aonla fruits has been progressively increased due to extensively utilized by processing industry for making trifla and chyavanprash. Other preserve products are, pickle, candy, jelly, jam and squash. Fruits can also be dried and powered to be used subsequently in the preparation of inks, hair dye and hair oils (Tiwari *et al.*, 2008). It posses diverse medicinal (useful in haemorrhages, diarrhoea, dysentery, anaemia, jaundice, dyspepsia and cough) and industrial (Trifla and chavanprash) uses. Besides fruits, leaves, bark and even seeds are being used for various purposes.

The use of PGRs in modern fruit culture is well established, more particularly in aonla industrially where its application has been used for breaking seed dormancy to rooting of cuttings and micro-propagation. In addition to this, PGRs have been advantageously used for improving flowering, fruit setting, shelf-life and reducing fruit drop in aonla.
The major areas where PGRs are used in aonla industry are:

- Plant propagation especially breaking seed dormancy and enhanced seedling growth
- Micro-propagation and embryogenesis
- Improvement in flowering and fruit set
- Fruit drop control
- Improvement of fruit yield and quality
- Extending the shelf life of fruits

Plant growth regulators in very minute quantities can induce above mentioned responses dramatically. The response to PGR’s however varies with the cultivar, age of the plant, light, temperature, availability of mineral nutrients, vigour of the plant and its endogenous hormonal content. Use of PGRs on above mentioned responses in aonla production is mentioned below:

2. Use of PGRs in Propagation

2.1. Propagation by seed and use of PGRs

In recent years asexual methods of propagation such as wedge grafting and patch budding have been found to be quite successful and their use has been advocated. However, even for grafting and budding seedlings have to be raised. Seedling trees bear fruits of variable size and quality but such trees are generally long-lived. For raising rootstocks, seeds are used to raise seedlings on which the desired variety or scion are grafted. Most of the fruit crop seeds as is the case of aonla also, germinate poorly and unevenly and require more time for seedling emergence. The dormancy in seeds might be due to hard seed coat, impermeability to water and gases, physiological immaturity of embryo, deficiency of some endogenous growth promoters or excess of endogenous growth inhibitors. Freshly harvested seeds of aonla do not germinate even if exposed to favourable conditions of germination owing to seed dormancy (Srimathi et al., 2000).

The fruit of aonla is fleshy and drupaceous and the seeds are found within the hardened endocarp of the fruit known as stone. The seeds do not germinate easily owing to seed coat related dormancy (Mawalagedera et al., 2014) and the germination rate is also very poor, necessitating pretreatments before sowing, however, the seeds can remain viable for a longer period under natural conditions (Pushpakumara et al., 2007). It has been observed that the concentration of auxin increased in the fruit with the onset of dormancy and decreased to a low level prior to dormancy break and exposure to GA3 500 ppm for 24hrs could have
counter downed the auxin concentration. Besides, the impermeable layer in seed coat allowed water and oxygen to enter the seed and permitted the embryo to overcome the mechanical restriction of surrounding tissue by providing uniform germination when immersed in tap water for 24hrs.

Different methods like water soaking, scarification and PGR treatments are used for breaking dormancy in such seeds to improve germination (Kumar et al., 2012). The some plant growth regulators have been helpful in germination of aonla seeds by increasing water uptake and exerting an effect on membrane permeability. These results indicate that use of plant growth regulators might have helped to break the embryo dormancy and induction of synthesis of alpha amylase and other hydrolytic enzymes (Looney, 1983). The exogenous application of Gibberlic acid antagonizes the ill effect of inhibitors and increases endogenous gibberellin like substances. GA\(_3\) helps in the synthesis of enzymes and one of them is \(\alpha\)-amylase which converts the starch into simple sugars during the process of germination. These sugars provide energy that is required for various metabolic and physiological processes associated with germination.

Pre-seed germination treatments to overcome dormancy, different treatments viz., tap water for 24 hours, thiourea (2%) for 24 hours, GA\(_3\) 500 ppm for 12 hours, GA\(_3\) 500 ppm for 24 hours, stratification at 5°C for 10 days, acid (conc. HCl) scarification for 30 seconds were imposed on aonla seeds. Bhujbal (1975) reported highest germination per cent (92.50%) with minimum period when dried stones of aonla were treated with 500 ppm GA\(_3\).

Dhankhar and Singh (1996) studied the percentage of germination and subsequent growth parameters of aonla. Days to germination were least with 250 ppm GA\(_3\), 250 or 500 ppm thiourea, and distilled water. GA\(_3\) at 250 ppm gave the highest percentage germination (75.98 and 64.14% in the laboratory and pot study, respectively). Significant effects over other treatments on plumule and radicle length at 35 days after sowing (laboratory study), and on seedling height (28.84 cm), seedling girth (0.90 cm), and seedling FW and DW (1.205 and 0.260 g) at 75 days after sowing in the pot with GA\(_3\) at 250 ppm. Maximum number of roots at 35 days after sowing was attained with thiourea at 750 ppm.

Pawshe et al., (1997) observed the effect of pre germination seed treatments on the germination and vigour of aonla seeds. Treatments included gibberellic acid (GA\(_3\) at 50 and 100 ppm, soaking in water for 24 hr and hot water soaking at 60 degrees C for 5 minutes. GA\(_3\) at 50 and 100 ppm increased the percentage seed germination. The tallest plants were obtained following seed treatment with 100 ppm GA\(_3\) and soaking for 24 hr.
Wagh et al., (1998) observed the germination of seeds of *phyllanthus emblica* soaked for 12 hr in 100-400 ppm gibberellic acid (GA3) or water, or not soaked (controls). Treatment with 400 ppm GA3 resulted in the highest percentage of germination (87.25%). Seedling development (plant height, number of leaves/plant and root development) was also best with seed treatment with 400 ppm GA3.

Gholap et al., (2000) also observed better germination and seedling growth with GA3 200 ppm in aonla. Such response is obvious as GA enhances cell elongation, so the radical can push through the endosperm and seed coat that restrict its growth (Hartman and Kester, 1979).

Rajamanickam and Anbu (2001) evaluated the effect of biofertilizers, chemicals and growth regulators on germination and seedling growth of aonla. Treatment of fresh seeds of aonla with Azospirillum + Phosphobacteria+0.5% KNO3 for 8 hr recorded the highest germination percentage (52.08%), and one-year-old seeds treated with Azospirillum+Phosphobacteria+200 ppm GA3 for 8 hr showed higher germination (49.17%), which was significantly superior than all other treatments. The same treatment was found to induce higher shoot length, root length, dry matter production and vigour index. In the case of time taken to reach buddable thickness, fresh seeds treated with Azospirillum+Phosphobacteria+0.5% KNO3 for 8 hr took lesser time compared to other treatments. One–year-old seeds treated with Azospirillum+Phosphobacteria + 200ppm GA3 for 8 hr took lower days compared to other treatments.

Rajamanickam et al.,(2002) studied the seed germination of aonla cv. BSR-1. Earliness in seed germination and germination percentage of Aonla recorded in fresh (6.30 days and 69.33%) and 1-year-old seeds (6.64 days and 46.00%) soaked in 0.5% KNO3 for 8 hours and in 200 ppm GA3 for 8 hours, respectively.

Chemical treatment of seed exposed to GA3 500 ppm for 24hous resulted better germination than other treatments. Imbibitions percent increased in treated seeds upto 90 % in contrast to 70% in non-treated seeds (control). Treatment of seeds with GA3 500 ppm and thiourea were effective in breaking seed coat dormancy (Laishram and Sahoo, 2015).

Chiranjeevi et al., (2017) evaluated the influence of growth regulators on germination, seedling growth and vigour attributes of aonla. The growth regulators like GA3 and NAA were used as treatments at different concentrations for 12 hours. The seeds pre-soaked with GA3 200 ppm solution recorded the earliest germination (8.33 days), highest germination.
percentage (88.88%), faster rate of germination (1.07), maximum seedling height (28.47 cm), seedling stem girth (1.26 cm), seedling biomass (2.28 g) and highest vigour index of seedlings (202.6) compare to other treatments.

2.2. Use of PGRs in Micropropagation

Micro-propagation is the technique of *in vitro* multiplication of large number of plants from its any part (leaves, seeds, nodes etc). The concept of totipotency is the basis for micropropagation. Micro-propagation is used for the production and multiplication of large number of novel plants, which are genetically similar and disease free. Usually, *in vitro* propagation is carried out in two ways: direct and indirect. Callus production from explant followed by induction of shoots and roots is one method, while direct shooting on the axillary explant like nodes, shoot tips followed by rooting is another method. Micro-propagation is the application of tissue culture for efficient clonal plant production has been used commercially since the 1960s and is possibly the oldest example of commercial Biotechnology (Rai, 2010). The technique of *in vitro* propagation is applied with the objective of enhancing the rate of multiplication. Through tissue culture over a million plants can be grown from small piece of plant tissue. Plants can be multiplied throughout the year irrespective of the season. Using this method stock of germplasm can be maintained for many years. It is a matter of the fact that through *in vitro* methods pathogen free plants can be raised and maintained economically. However, there are few reports on the micropropagation of aonla (Mishra *et al.*, 1999, Verma and Kant, 1996 and Goyal and Bhadauria, 2008). In micro-propagation studies, PGRs are used in addition to the supply of other salts, mineral, vitamins and sugars to get the desirable response to achieve the set of objectives. Auxins are a class of PGRs which cause cell elongation, apical dominance and root initiation. The most frequently used auxins are 2, 4-D, NAA, IAA and IBA of which IAA occurs naturally in plants. NAA and 2, 4-D are the most effective auxins to initiate callus. Cytokinins are phytohormones which promote cell division, proliferation of tissues. The most widely used cytokinins are kinetin, BAP and 2-IP. Auxins or cytokinins alone cannot show organogenesis through cell division or callus *in vitro*. The relative concentration of auxin and cytokinin decides the plant morphogenesis *in vitro* (Skoog and Miller, 1957).

Verma and Kant (1996) cultured nodal segment explants of aonla on modified MS medium supplemented with BA (3-5 mg/l) and NAA (0.5 mg/l) during February-April and August-October. The bud break observed in only 8-10 per cent of explants. Leaching of phenolic compounds resulted in the loss of almost 90 per cent in cultures. The regenerated shoots were elongated on hormone free medium and subsequently rooted on half strength MS medium supplemented with IBA (3.0 mg/l) and sucrose (1.5%). Again Kant *et al.*,
(1999) reported 3–4 multiple shoots from nodal explants from mature tree on MS medium supplemented with BAP (5 mg/l) and IAA (0.5 mg/l) along with antioxidants. These shoots were elongated on hormone free MS medium. Rooting of the shoots was achieved on half strength MS medium fortified with IBA (2 mg/l) and sucrose (1.5%).

Micropropagation studies in aonla were carried out from nodal explants by Mishra et al., (1999) to develop the protocol for mass multiplication of true-to-type plants. The better shoot proliferation (33–37.5%) on modified MS medium supplemented with kinetin 0.4 mg/l + GA 1.0 mg/l than in WPM. Higher GA3 concentration (3 mg/l) caused complete defoliation and dropping of determinate shoots. Regenerated shoots from 3 week old cultures in MS media supplemented with growth regulators and antioxidants failed to produce roots.

Kadam et al., (2006) undertook a study to come out with optimal culture conditions for high frequency plant regeneration from nodal segment along with axillary bud and shoot tip explants of the aonla genotype Krishna. Seeds of the genotype Krishna were raised in vivo in sterilized soil rite and supplemented with MS basal liquid medium. Shoot tip and Nodal segment explants were collected from these seedlings and cultured aseptically in MS as well as WPM medium fortified with growth regulators viz., BAP, NAA, GA3 and TDZ. The nodal segment explants showed better response than shoot tip for micropropagation of aonla. The maximum number of multiple shoots per culture (13.67) were recorded by nodal segments cultured in MS + BAP 0.25 mg/l +NAA 0.1 mg/l + GA3 2 mg/l. However, normal rhizogenesis could not be achieved with supplement of IBA and NAA to half MS media and abnormal root induction was noticed which failed to establish during subsequent hardening.

3. Use of PGRs on Fruit Retention and Fruit Yield

In recent past, a set of factors including feasibility of commercial aonla cultivation in marginal lands, availability of improved varieties and huge possibilities for the value-addition of fruits have enabled rapid coverage of vast area under aonla cultivation in many parts of India (Pathak, 2003). However, fruit bearing in aonla trees often suffer from heavy fruit drop which significantly lowers down the yield. Fruit drop often causes poor fruit set in majority of the cultivars and may result in substantial crop loss in susceptible ones. Among the major causes noted for fruit drop are self-incompatibility, inadequate pollination, nutritional deficiency, water stress, insect-pest and disease infestations and hormonal imbalances (Allemullah and Ram, 1990 and Singh et al., 2008). Amongst the reasons, suboptimal biosynthesis and poor translocation of phytohormones may be a major cause of fruit drop (Ram and Rao, 1981).
Good fruit set in aonla is prevented by adverse weather which hinders pollen production, pollination and fertilization and also due to low level of auxin. The auxin from the pollen grain and pollen tube might be responsible for the early stage of fruit growth. However, small amount of pollen in the grain may not carry enough auxin to account for early fruit development. The growing pollen tube may secrete auxin which helps in fruit growth (Muir, 1942). In case of aonla, sufficient auxin may not be available during fruit growth which resulted in fruit drop and low yield. To check the pre-mature fruit drop in aonla and to improve the yield and fruit quality, various investigations were carried out with the application of plant growth regulators.

Results of two years of investigation undertaken by Ghosh et al. (2009) revealed that spray of NAA at 10 ppm was the best to increase fruit retention, followed by NAA 20 ppm, Vermiwash and Borax, which consequently resulted in the highest fruit yield of 54.9, 52.0, 46.8 and 36.2 kg/plant, respectively, against 13.8 kg in the control. Beneficial effect of NAA application in reducing fruit drop may be explained from the fact that it maintains the ongoing physiological and biochemical process of inhibition of abscission (Tomaszewksa and Tomaszewska, 1970). Fruit weight was the maximum with 0.5% ZnSO4 spray, followed by NAA 10 ppm. Fruit quality with regard to TSS, total sugar and ascorbic acid content was better in all the treated fruits compared to control.

Singh and Singh (2015) also reported that application of PGRs on bearing trees of aonla cultivar Narendra Aonla-6 significantly increased fruit retention. The minimum fruit drop was recorded with the use of NAA (15 ppm) + Thiourea (0.1%) followed by 2,4-D (10 ppm) + Thiourea (0.1%) and GA3 (50 ppm) + Thiourea (0.1%). The maximum increase in the size, weight and volume of fruits and the maximum fruit yield were noted on trees sprayed with NAA (15 ppm) + Thiourea (0.1%). The improvement in fruit quality parameters (TSS, ascorbic acid, sugars and acidity) was highest with the application of GA3 (50 ppm) + Thiourea (0.1%) which showed non-significant difference with NAA (15 ppm) + Thiourea (0.1%). Results revealed that foliar application of NAA (15 ppm) + Thiourea (0.1%) or GA3 (50 ppm) + Thiourea (0.1%), twice during mid-May and mid-July, may effectively overcome the problem of fruit drop leading to higher yield of quality fruits.

4. Role of PGRs in Improving Fruit Quality

Patel (2017) observed the total soluble solids was found significantly higher in treatment with NAA 40 mg/l + GA3 50 mg/l. Significantly the minimum total soluble solids was found in treatment Control (Water spray). However, The total soluble sugars was found significantly
higher in treatment NAA 40 mg/l + GA₃ 50 mg/l, which was followed by NAA 40 mg/l, NAA 20 mg/l + GA₃ 25 mg/l, and NAA 20 mg.

Patel (2017) concluded from the experiment that the plant growth regulators are known to increase in total soluble solid in treated fruits which might be due to rapid transformation of complex carbohydrates into soluble sugars and also fast mobilization of metabolites from source to sink. The growth substances like GA₃ which promote quick metabolic transformation of starch and pectin into soluble sugars and rapid mobilization of photosynthetic metabolites and minerals from other parts of the plant. Similarly NAA increase the TSS which might be due to synthesis of auxin in plant, it increase the physiological activities leading to increased TSS in fruits, while total sugars increase might be due to activation of enzymes which affect the physiological processes, which in turn hydrolyzed the starch and helps in metabolic activity during the change in available starch into sugar and soluble solid content. NAA had shown significant increase in the total sugar of aonla fruits and this might be due to synthesis of auxin in plant, it increased the physiological activities. Content of acidity can also be increased by applying plant growth regulator that might be due to catalytic influence of growth regulators on its biosynthesis from its precursor glucose-6-phosphates throughout the development of fruits which is thought to be precursor of vitamin- C. The application of gibberellic acid, NAA may have favorably influenced the metabolic activities possibly due to their increased endogenous level which increased the ascorbic acid of aonla fruit. The shelf life of aonla fruits can be increased that might due to antagonistic effect of GA3 which inhibit ethylene production and delayed the conversion of starch to sugar (Patel, 2017).

5. Role of PGRs on Shelf Life of Aonla

Due to perishable nature of aonla fruits, it becomes difficult to store or transport aonla over a long distance. Shelf life of aonla can be extended by checking respiration, transpiration and microbial infection. The different plant growth regulators exhibited significant influence on physiological loss in weight of aonla fruits. Among the different treatments, i.e. NAA 40 mg/l+ GA3 50 mg/l at pin head and pea stage was found significantly superior over the rest of the treatments and lowest physiological loss in weight after 12th and 24th day storage period for aonla fruit. Similar trend was also recorded after 24th day storage period. The reduction of weight loss in the fruits treated with GA3 might be due to its anti-senescence action. The plant growth regulators and boron treatment resulted in to decrease in the tissue permeability and thereby reduces the rate of water loss. The reduced PLW in fruits during storage is mainly due to reduced rate of transpiration and respiration.
An experiment was conducted during 2001-2002 to see the effect of different post-harvest treatments on storability of Indian gooseberry (*Emblica officinalis* Gaertn) during storage at ambient temperature. Increase in physiological loss in weight, spoilage percentage, total soluble solids, total sugar and reducing sugar and decrease in acidity, ascorbic acid with advancement of storage period were general phenomena in all the treatments. Fruits treated with calcium nitrate 1.5 % + perforated polyethylene bag and GA₃ 100 ppm + perforated polyethylene bag recorded the least physiological loss in weight (2.12-16.00 and 2.15-16.34%) and spoilage loss (2.40-15.00% and 2.50-15.60 %) and exhibited 11 days of storage life. The same treatments also showed lowest respiratory activity (72.10-82.00 mg CO₂/kg/hr and 72.00-82.10 mg CO₂/kg/hr), on the last day of storage (day 13) (Singh *et al*, 2005). Fruits were dipped in GA for 10 minutes.

References


