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Growth Regulator I Propagation Od Passion Fruit - An Analysis

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Introduction:

Passion fruit (*Passiflora edulis* Sims) is an important fruit crop grown in the world, having economic value owing to its unique flavour and aroma in addition to its high nutritional and medicinal properties. It belongs to the family Passifloraceae. It is a native of Brazil and is grown mostly in tropical and sub-tropical parts of the world. The passion fruit is becoming one of the most important fruit crops in the North eastern states of India (Chadha, 2002). Passion fruit is a perennial, vigorous climbing, woody vine with medium to large leaves (8-30 cm long) with toothed margins and usually bearing glands on the petioles (Bailey, 1949; Purseglove, 1974). The fruits are smooth, ovoid and purple/yellow on ripening. The rind is bordered by white pith and small black seeds are present inside, which are surrounded separately by a soft slightly acidic, fragrant and juicy orange-yellow pulp.

As a result of its rapid production rates in comparison with other fruits and its demand in the market, both for fresh consumption and industrial processing, passion fruit is of great interest to fruit producers (Madureira *et al.*, 2012). Even though there is increased awareness of the crop and its production aspects, information related to its propagation is limited. Passion fruit is generally propagated through seeds and cuttings but can also be propagated by grafting or air layers. Seeds are the commercially propagating material and are widely used as compared to vegetative propagation. For increased productivity, a good and healthy planting material along with proper management practices is essential. Passion fruit seeds may show considerable dormancy and seed treatments have been reported to promote germination (Obroucheva, 1999). The use of growth regulators has also been reported to improve the rooting ability of cuttings. However, there exists a lot of contradiction with regards to optimum concentration of growth regulator treatments. Use of appropriate growth regulators in accordance with crop requirement has made a breakthrough in propagation of passion fruit both sexually as well as asexually.

Propagation by Seed:

Propagation of passion fruit is done commercially through seeds. Seeds of passion fruit are recalcitrant in nature and lose viability very fast. Germination decreases with increased period of storage. Germination is variable, depending on the species and even within cultivars of the same species (Andrade and Jasper, 2013; Lone *et al*., 2014). Cleaned and stored seeds have a lower and slower rate of germination as compared to freshly extracted seeds and thus, there is a need to sow seeds as soon as possible after harvesting (Thompson, 1979). The seeds have hard coats with a semi-permeable inner layer. They absorb water easily but contain chemical inhibitors that are difficult to leach possibly due to low permeability of the testa membrane located in the seed coat (Delanoy *et al*., 2006). Embryos that are excised germinate rapidly, thus it appears that *Passiflora* spp. have exogenous dormancy which could be a combination of both mechanical and chemical dormancy (Baskin *et al*., 2000).

Seed dormancy is a strategy that allows seeds to avoid germination under conditions that are unfavourable for seedling establishment and plant survival (Finch-Savage and Leubner-Metzger, 2006). Seed dormancy is divided into two major categories: exogenous and endogenous. Exogenous dormancy is caused by factors outside of the seed's embryo, such as the seed coat and it is classified into three areas: a) Physical dormancy caused by a seed coat impermeable to oxygen and/or water, b) mechanical dormancy caused by a seed covering that does not allow the embryo to expand, and c) chemical dormancy which is related to the inhibitors within the seed coat. Endogenous dormancy occurs due to metabolic inhibition and immature embryos, and it is divided into physiological, morphological and morpho-physiological dormancy (Cardoso, 2009). Seeds of some species which have both exogenous and endogenous dormancy need treatments to overcome the impermeable covering first and then for endogenous dormancy (Bewley and Black, 1994; Leadem, 1997). It is worth mentioning that a single species can exhibit different forms and levels of dormancy.

Since the seeds exhibit slow and less germination, pre-germination treatments may enhance the germination potential of passion fruit seeds. Plant growth regulators are commonly used to overcome physiological dormancy. Gibberellic acid (GA_3) is one of these compounds and it is applied to the outside of seeds to stimulate germination (Shu *et al*., 2016). Treatments with GA_3 have been suggested to tackle physiological dormancy of several species including *P. cincinnata* Mast. (Zucareli *et al*., 2009; Moura *et. al*, 2018), *P. lingularis* (Cadorin *et al.*, 2017), *P. caerulea* (Hossel *et al*., 2018) and *P. edulis flavicarpa* (Lima *et al*., 2009; Cardenas *et al*., 2013). Different doses of this plant growth regulator have been used, as seed dormancy is linked to the genotype (Santos *et al*., 2015). In addition, seedling emergence is primarily a function of the moisture and temperature (Savage *et al.*, 1994). Temperature affects both seed germination percentage and germination rate (Kotowski, 1926). Germination rate is invariably low at low temperature but increases gradually as the temperature rises (Koller, 1972). Availability of moisture in the soil also plays an important role to initiate germination of the seeds sown. Early seed germination begins with the imbibition of water by the seed and thus water stress can reduce the germination (Doneen and Macgillivray, 1943).

- Conegliano *et al.* (2000) observed that seed subjected to extraction method showed higher percentage and emergence rate after pre-soaking in substrate moistened with a solution of 300mg/l GA_3.
- Ferreira *et al.* (2001) studied the germination of *P.alata*, which was subjected to different soaking times and concentration of gibberellic acid and found that higher concentrations are beneficial in faster emergence of seeds of these species.
- Delanoya *et al.* (2006) recorded the germination of *Passiflora mollissima* , *P.tricuspis & P. nov* and recommended removing the basal point in combination with pre-soaking seeds for 48 hrs in GA_3 50ppm for P. *mollissima*, pre-soaking seeds for 24 hrs in 400 ppm GA_3 and removal of the basal point in combination with pre-soaking seeds for 48 hours in 50 ppm GA_3 for *P. nov*.
- Gurung *et al*., (2014) studied on effect of chemicals and growth regulators on germination, vigour and growth of passion fruit (*P.edulis*). The seeds were given different treatments for breaking the dormancy:
  - **a.** Water soaking for 24 hours.
  - **b.** GA_3 250 ppm for 10 mins.
  - **c.** GA_3 500 ppm for 10 mins.

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d. Vermiwash for 24 hours.

e. Thiourea (1%) for 24 hours.

f. Cow urine (1:1) for 24 hours.

g. Cow slurry for 24 hours.

h. Control.

Germination percentage was found to be the highest in thiourea treated seeds (84%) followed by those treated with GA_3 500 ppm (74%) and minimum in control. The increased germination % in thiourea treated seeds might be due to strong neutralizing effect of thiourea on inhibitors present in seed or might be due to increased cytokinin activity of thiourea in overcoming the seed coat inhibiting effect While the increased germination % in GA3treated seeds might be attributed to the fact that GA_3 helps in breaking seed dormancy resulting in early and enhanced seed germination.

The seedling height which was taken after 90 DAS was found to be maximum in those seeds treated with GA_3 500 ppm (20.9 cm) which may be attributed to the reason that endogenous levels of GA_3 synthesized by passion fruit seedling might not be sufficient and external application of GA_3 might have boosted growth by increasing cell multiplication and cell elongation, resulting in better plant growth.

The shoot length was highest in seeds treated with GA_3 500 ppm (20.91 cm) followed by cow urine (18.22 cm) and GA_3 250 ppm (17.95 cm) respectively. The root length was also significantly higher in GA_3 500 ppm treated seeds (10.76 cm). Thus, use of growth regulators is recommended as an approach for propagation of passion fruit.

Imliwabang and Alila (2017) carried out an experiment on the germination of passion fruit with different pre-sowing seed treatments. Seeds of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener.) stored for four years and one-week-old seeds of purple type (*Passiflora edulis* Sims.) were used to evaluate the influence of different pre-sowing treatments on the germination and survival percentage of seedlings. The pre-germinationtreatments included soaking for one hour in GA3 100 ppm, thiourea 100ppm, apical tip clipping+GA3 100 ppm, apical tip clipping+thiourea 100 ppm and control (distilled water). The two passion fruit types showed no significant difference in their germination percentage. In general, the percentage germination (49.91) was found better in older seeds of yellow type while the fresh seeds of purple passion fruit showed slightly lower percentage (48.16). The poor germination in fresh seeds of passion fruit may be due to the presence of ascorbic acid and other growth factors which inhibit germination in most seeds. Low germination value in purple passion fruit could be due to genetic properties of this species or incomplete seed development in the plant or incomplete processing of seeds which requires further studies. Use of growth regulators increased the germination percentage and reduced the mean germination time in both types. The highest germination (90.0%) and survival percentage (89.58%) were recorded by clipping of apical tip and soaking in GA3 at 100 ppm in purple type. The greatest survival percentage (62.41%) was recorded from purple type while yellow passion fruit recorded 57.26%. Yellow passion fruit seeds retained its viability when hermetically sealed and stored under low temperature condition (7-10^{o}C). The germination %, attributes of germination and initial plant growth were enhanced by these pre-sowing treatments.

Rezazadeh and Stafne (2018) conducted an experiment to evaluate four different pre-germination treatments on enhancing germination potential in seven *Passiflora* spp. They are- a) Scarification with sand paper. b) Scarification and pre-soaking for 24 hours in 100 ppm $GA_{3.}$ c) Scarification and fermentation for 7 days in 10% sucrose solution and d) Pre-soaking in distilled water for 3 days. *Passiflora laurifolia* L. and *P. edulis f. edulis* showed maximal germination percentage with stratification plus fermentation. The highest germination was obtained for *P. maliformis* L. In stratification plus $GA_{3.}$ *P. tripartia* var. *mollissima* showed highest germination percentage when soaked in water or scarified plus GA_3. Germination was observed in *P. incarnata* and *P. quadrangularis* only in response to soaking in water and stratification plus fermentation treatments. Scarification alone resulted in the best germination percentage in *P. Lingularis* while it was highest for *P. incarnata* when pre-soaked in water.

Propagation by Cutting:

As passion fruit orchards are established with seedlings raised from seed, there is high heterozygosity and variability in this species. Vegetative propagation has advantages in the maintenance of good agronomic characteristics by encouraging the production and multiplication of true to type quality planting materials. Rooting of cutting depends on various factors such as type of cutting, pre-treatment of cutting, environmental factors, etc., which influence the regeneration ability of cuttings. Plant growth regulators are found to have a stimulatory effect on the rooting of cuttings (Audus, 1954). It has been confirmed many times that auxin, natural or artificially applied, is a requirement for initiation of adventitious roots on stems (Gautheret, 1969). So far, a number of synthetic plant growth substances were used for rooting of cuttings, but only Indole butyric acid (IBA) has been found superior overall (Kester *et al.*,1997). Different parts of the plant were shown by Thimann (1934) to contain different concentrations of endogenous growth hormones. The tips of both the shoot and root were observed to have high hormone concentration which decreased towards the proximal end. Different plant species of the same genera tend to show different hormone concentration requirements for optimum rooting (Gil-Albert and Boix, 1978).

Thimba *et al., (1985)* studied the effect of IBA on the rooting of passion fruit cuttings. Four concentrations of IBA (0, 1000, 3000 and 8000 ppm) were applied to the cuttings. They reported that the best rooting was observed in those cuttings which were treated with 3000 ppm IBA, where the no. of roots and root dry weight were highest. The rooting also increased with time in the other IBA treatments.

Meletti et. al., (2007) evaluated the influence of season of year, the presence of leaves and IBA on the rooting of sweet passion flower (*Passiflora alata* Curtis) cuttings. Treatments included IBA (0, 1000, 2000, 3000 ppm) and with and without half leaves. The treatment 3000 ppm IBA showed better results, independently of the type of cuttings. Cutting without leaves showed higher percentage of rooting (80.9%) and half-leaved cuttings showed good results for root, dry weight and longest root length.

Vaz et al., (2009) studied on the rooting potential of five wild species of passionfruit i.e., *Passiflora setacea, P. coccinea, P. amethystina, P. edulis, P. edulis x P. setacea* and *P. coccinea x P. setacea* under green house conditions using different doses of IBA (0, 250, 500, 750 and 1000 ppm). Base of the cuttings were immersed (approx. 10cm) for 1 min in IBA solutions and planted in vermiculite substrate. Among the wild species, *P.amethystina* species presented better development than other species, showing rooting potential with 88.67 % of cuttings with roots and sprouts while *P. setacea* showed a slow response to rooting when compared to other species. The use of IBA was efficient as the doses were increased, providing a good development for cuttings of the studied species.

Gurung (2010) reported that NAA 250 ppm was found to be significantly better w.r.t. rooting percentage, sprouting percentage, number of leaves, number of roots, etc. but in case of CMS, NAA 500 ppm was better to obtain maximum callus formation.

Parse *et al.,* (2018) studied on the effect of chemicals on success of cuttings in passion fruit (*Passiflora edulis* Sims). 1-2 cm of the lower portion of the cuttings were treated with different concentrations of chemicals by quick dip method for 3-5 seconds and allowed to dry for 5 mins. The cuttings were treated with IBA (500, 750 and 1000 ppm), NAA (500, 750 and 1000ppm). They observed that cuttings treated with IBA 1000 ppm showed the highest fresh weight of shoot while it was minimum in control. This might be attributed to the increased plasticity of the cell wall due to auxin which in turn increases the permeability of cell for moisture and nutrients resulting in enlargement of cell causing more growth of plant parts. Maximum length of root and fresh weight of root was observed in the cuttings treated with IBA 750 ppm and minimum in control. Superiority of the root length and diameter could be due to the initiation of lateral and adventitious roots because of the effect of auxin. The better response in rooting might be due to the cumulative effect of auxins and the time of propagation in relation to environmental factors.

Joseph and Sobhana (2020) evaluated the effect of different concentrations of NAA and number of nodes on the rooting of cuttings and survival percentage. The experiment consisted of 20 treatments with two factors, *viz.*NAA concentration (200ppm, 400ppm, 600 ppm ,800 ppm and control) and number of nodes (one node, two node, three node and four node) following quick dip method. It was observed that four noded cuttings had maximum survival percentage and higher root and shoot parameters, while there improved survival percentage and enhanced root and shoot characteristics with increasing NAA concentration. The high success in treatment (4 noded + 800 ppm NAA) maybe due to favourable interaction effect between the no. of nodes in the cuttings and NAA concentration. Thus, four noded cuttings dipped in 80 ppm NAA can be used effectively for large scale multiplication of passion fruit plants for commercial cultivation thereby ensuring true to type plants.

Micropropagation:

It is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. Due to the problem of systemic viruses in passion fruit, propagation through tissue culture may offer a fast and efficient method of producing clean virus-free planting material. *Passiflora edulis* is self pollinated and genetically fairly homogenous and since poty viruses, which include passion fruit woodiness virus (PWV) is rarely transmitted through seed, a good selection of seed from healthy producing vines can eliminate this virus. Such seed can be germinated *in vitro* to produce a fairly virus-free explants source (Vos, 2000). It has been reported that for each species, the certain combination of PGR is needed to achieve the maximum efficiency of *in vitro* propagation. In passion fruit, the half strength medium is used for root induction. It has been reported that low salt concentrations in the half strength medium induce rooting of the shoots in several plant species. (Rai *et al*., 2010)

Lee *et al*., (2017) carried out an experiment on the effect of plant growth regulators and antioxidants on *in vitro* plant regeneration and callus induction from leaf explants of purple passion fruit. Young leaf explants taken from central part of leaves, which contained mid rib vein were cut into discs (approx. 12-13 diameter) and were cultured in Murashige and Skoog (MS) medium containing different growth regulators and antioxidant additives to induce the shoot organogenesis. After 8 weeks, the highest embryonic callus formation was obtained in MS medium supplemented with 1mgL^{-1} BAP and 2mgL^{-1} 2,4-D, furthermore, the shoot development via organogenesis was also observed. Auxins and cytokinins are the main PGR involved in the regulation of plant cell differentiation and their ratio is very critical for the specification of cell identity during early stages of morphogenesis. They also observed that shoots were most vigorously regenerated and elongated with a regeneration rate of 75.7% in the medium supplemented with 1mgL^{-1} BAP and 1 mgL^{-1} GA_3. In addition, most shoots rooted successfully in half strength medium with 1 mgL^{-1} IAA and more than 90% of plantlets survived after 4 month acclimatization period.

Khas, *et al.*, (2020) studied on the optimization of in vitro propagation of purple passion fruit. Explants were taken from 2 year old plants grown under the greenhouse. For proliferation stage, nodal segments were cultured in media supplemented with various combinations of different plant growth regulator including: BA, TDZ, GA_3 and IBA. For rooting, shoots with about 1.5 cm long originating from explants were removed and cultured in half strength MS medium containing different concentrations of auxin based plant growth regulators including: IAA, IBA and NAA. The observed that for proliferation stage, the best plant growth regulator combination was 8.9µM BA + 2.9µM GA_3 + 0.5 µM IBA, resulting in maximum shoot proliferation, number of shoots per explants and shoot length, while half strength MS medium supplemented with 5.4 µM NAA or 8.9µM IBA was the most effective treatment for rooting the shoots.

Conclusion:

Passion fruit production is constrained by the lack of suitable varieties, technical knowledge on crop cultivation, pests and diseases and relatively higher capital investments. Passion fruit having a unique and excellent flavour and aroma has not reached the majority of the people even within the country due to lack of publicity. In addition, growers are apprehensive to expand the area under its cultivation without assured quality planting material and market for their produce. As there are different requirements for propagation in different species of passion fruit, proper understanding of the species is important to get the best results. There is a need for more research on propagation aspects considering the great number of species and inter-specific hybrids. Understanding the dormancy characteristics that inhibit seed germination potential may further improve germination potential, reduce propagation cost and facilitate cultivation of passion fruit and thus further research is needed to determine dormancy types present in the various species and individual best treatment to overcome them. There are a number of factors which affect the propagation of passion fruit such as type of cutting, method of storage of seeds, environmental factors, etc., however with the use of required concentration of growth regulators and proper treatments; there is immense potential for production of quality planting material for the growers in a large scale.

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