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Importance of micropropagation in pineapple for disease free plantlets and rapid multiplication

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Abstract
Pineapple (*Ananas comosus*) is a delicious tropical fruit with a superior flavor and high nutritive value. It is one of the most important commercial fruit crops in the world. Pineapple is propagated by vegetative means from basal, stem slip, and crown suckers with varying degrees of success. The traditional methods of propagation would take 8 years to obtain enough planting materials from one mother plant to plant just only a half hectare. Plant tissue culture methods have been successfully applied to pineapple and it is an efficient method for rapid *in vitro* clonal propagation. Micro-propagation of pineapple plants also has many advantages, such as, it allows rapid increase of selected varieties, overcomes serious diseases causing considerable production losses of pineapple at the time of planting material preparation disease free plants and increase the multiplication rate of elite genotypes.

Keywords: Disease free**,** sucker**,** explant

Introduction
Ananas comosus, commonly referred to as pineapple, is a herbaceous perennial of the Bromeliaceae family. There are 56 genera of pineapple which include 2921 species [1]. Mature fruit contains sugar, a protein digesting enzyme bromelain, citric acid, malic acid, vitamin A and B [2]. It can be used as supplementary nutritional fruit for good health with an excellent source of vitamins and minerals and contains considerable calcium, potassium, fiber, and vitamin C. Pineapple is the third most important tropical fruit in the world after banana and citrus [3, 4]. Thus, for its medicinal content pineapple exhibits increasing demand worldwide, over the years. The global trade is around 50% as fresh fruit, 30% as canned product and 20% as juice concentrate. World trade on fresh pineapple has shown 100% increase during the last one decade. Even though India is the sixth largest producer of pineapple in the world with a share of about 8% in production, its share in the world market is negligible. The main pineapple producers are Brazil, Thailand, Philippines, Costa Rica, China, India and Indonesia. The different Asian countries and the countries around the Indian Ocean are importing about two lakh tonnes of pineapple in a year, mostly coming from distant countries. The leading exporters are Costa Rica, Belgium, Cote d' lvoire, Philippines, Ghana, Netherlands, USA and France. Major importers are USA, Belgium, France, Italy, Germany, Japan and United Kingdom. India ranked sixth with a share of about 8% of the world production of pineapples. The total area under pineapple cultivation in India is 89000 hectares with a production of about 1415000 t and productivity 15.9 t/ha during 2010-11. India exports pineapple mainly to Nepal, Maldives, United Arab Emirates, Saudi Arabia, Kazakhstan, Oman, Bahrain, Bangladesh, Zambia, Pakistan and Qatar. 'Kew' and 'Mauritius' are the two varieties of pineapple grown in India. It is grown in Karnataka, Meghalaya, West Bengal, Kerala, Assam, Manipur, Tripura, Arunachal Pradesh, Mizoram, and Nagaland. It is also cultivated on limited areas in the coastal belt of Tamil Nadu, Goa and Orissa. Though Assam has the largest area under pineapple West Bengal is the largest producer. Karnataka, West Bengal and Bihar are the three states reporting high productivity. MD2 or Dinar pineapple developed through hybridisation by Del Monte scientists in Costa Rica is the most popular variety in the international market because of its colour, flavour, shape, life span and ripeness being superior to other varieties [5]. Overall, Indian productivity of 15.9 t/ha poorly compares with the world average of 22.58 t/ha [5, 6]. Pineapple holds third rank in world tropical fruit production only preceded by banana and citrus [7].

Vegetative propagation of pineapple from suckers and crowns is often restricted by the limited availability of propagules in some cultivars such as Smooth Cayenne. There are also reports on Natural regeneration is one of the long lasting agronomic problems in smooth cayenne pineapple cultivation causing considerable losses [7, 8]. In order to overcome these limitations,

cell or tissue culture methods can be a good alternative for producing large quantities of disease-free propagules[9]. Plant propagation through *in vitro* methods is mainly aimed to produce plants of superior qualities, free from microorganisms and with high multiplication rates. A number of reports dealing with this aspect of tissue culture has been published for many species such as *Asplenium nidus* [10], *Petunia hybrida* [11], *Ananas comosus* [12, 13], *Lilium regale* [14] and *Platycerium coronarium* [15]. There is also another important point to consider when dealing with *in vitro* work is the changes that occur when cells are transferred from *in vivo* environment to *in vitro* environment. The changes in cellular parameters such as ploidy level, Mitotic Index (MI), mean cell and nuclear areas can be associated with regeneration potentials [16]. Moreira *et al*. (2016) [17] also reported the first efficient *in vitro* regeneration of *Ananas erectifolius* via indirect organogenesis. Leaf segments (leaf base, middle, and apex) excised from 3-or 5-week-old *in vitro* plantlets were cultured on 1/4 strength MS medium supplemented with different concentrations and combinations of plant growth regulators.

Traditional plantation materials for pineapple

Pineapple is propagated vegetatively by slips, suckers, crowns and hapas [18]. The average production of conventional material is 4-5 propagules per year, which takes considerable time to produce adequate planting material [19]. The following types of propagating materials are commonly used to establish a crop of pineapples:

Ratoon Suckers: These arise from buds below ground level. These suckers are the most difficult to plant because of their large size. It takes an average of 15-18 months to harvest fruit from a ratoon sucker and their fruits tend to mature unevenly. Ratoon suckers, however give the highest yields [18].

Side Shoots or Suckers

These are leafy branches arising from buds in the leaf axils and are therefore produced above the ground. Up to three side shoots may be produced on each plant but they are not produced in some varieties. They reach a length of 35-40cm when mature, but are suitable for planting when 30-35cm long. When left on the plant, side shoots produce a ratoon crop [18].

Slips: These are borne on the peduncle just below or on the base of the fruit. The size and number (0-10) produced varies according to the health of the plant. The best slip material is from plants with no more than three slips. The average length of suckers from the Smooth Cayenne is 26 cm weighing 285-450g, but those which weigh 350-450g are the best for planting. Fruits from slips, take an average of 20 months from planting to harvest and they tend to ripen unevenly [18].

Crowns

They are located at the top of the fruits. Normally, only one crown is produced. At maturity, the crowns become dormant. Crowns tend to produce a more uniform crop. It takes an average of 22-24 months from planting to harvest. Crowns are not commonly used by farmers as planting material because the pineapples are sold with the crowns attached and commercial supplies are usually not available [20].

Plantlets Generated from Butts

Planting material may be obtained from the stem material of the harvested plant or 'mother plant' by promoting the formation of plantlets from vegetative buds within the leaf axils of the stem. This is done by placing the cleaned stem material called the butt or stump on a specially prepared raised propagating bed. The propagating bed should contain friable soil which has been cleared of weeds and other vegetation and worked into a fine tilth. Firstly, the leaves, roots and peduncle from the mother plant are removed to obtain the bare stem material. The butts are then placed horizontally on the surface of the propagating bed and covered with soil or preferably, river sand. After about 3-4 weeks, shoots emerge from vegetative buds within the butts and eventually develop into young plantlets which can be used as planting material as soon as they are 3 cm long. Large number of propagules is required as 60,000-80,000 planting material are needed for a hectare. Thus, traditional method of propagation is not meeting the international market demand. Besides, the importation of the conventional material for direct planting by farmers is also expensive and may not meet quarantine requirement [21]. In most commercial growing areas, many diseases of pineapple represent one of the most perplexing problems of the pineapple production. Thus, *in vitro* multiplication would be a useful alternative method for mass production of plant. Large scale production of planting material can be achieved by using *in vitro* propagation techniques.

Importance of tissue culture in pineapple over traditional vegetative production

Recently, plant tissue culture techniques have expanded their scope and potential on commercial scale. Micropropagation is suitable for rapid and large-scale clonal multiplication of elite germplasm [22]. The technique has been referred to as micro-propagation because the size of the tissue in culture is very minute as compared to conventional vegetative cutting or any other plant part [23].

Since the introduction of tissue culture technique, it has contributed significantly in various fields, such as agriculture, forestry, horticulture and medicinal. Micropropagation techniques are valuable tools for mass clonal propagation of superior clones or varieties. A good micropropagation protocol should provide plantlets with a high quality and multiplication rate at a low cost[24]. Successful plant regeneration from small pieces of plant tissues and organs, either through micropropagation or somatic embryogenesis indicates that almost any plant can respond to tissue culture/*in vitro* protocols. The first pineapple micropropagation protocol was described in 1960 [25]. Afterwards, a protocol for multiple proliferation was established from lateral buds [26]. Several other explant sources have been tested successfully in several regenerative protocols [27, 28]. An alternate pineapple micropropagation protocol with etiolated shoots was established resulting in high multiplication rates [29]. Other protocols based on organogenesis were described [28, 30] using different explants such as lateral buds, crown apical dome and ovules. A micropropagation protocol superior to the previous one should be enhanced to be used widely. There are several work has been carried out to improve the previous pineapple micro-propagation protocol [31, 32] through manipulating both explant size and medium composition, as well as acclimation substrates and plantlet size, aiming for the mass clonal micro-propagation of plantlets free of fusariosis. In addition to yielding abundant planting material of uniform age, micro propagated plants have been known to mature and fruit all at one time. The micropropagation protocol has now become fairly standardized for some important cultivars of pineapple and with minor changes can be applied to newly derived ones. Since all the plantlets are developed from one initial culture,

most of them should flower and set fruit at the same time when transferred to the field [33, 34, 35].

Conclusion

It has comparative advantage over the traditional methods, as it leads to the production of large number of disease free uniform planting materials in a relatively shorter period independent of the season. Perhaps the use of micro propagated (tissue cultured) pineapple plants as planting material would help to minimize the problem of 'natural flowering' which is sporadic and rampant in pineapple cultivation.

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