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Advancements in horticultural crops using shoot tip culture

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Abstract
Haberlandt envisioned plant tissue culture over a century ago and laid the framework to produce plant cells, tissues, and organs in culture. Plant tissue cultures began as a research tool, focusing on attempts to culture and analyse the development of small, single-celled organisms. Isolated cells and plant tissue segments in the 1980s, at the height of the plant tissue culture era. In a relatively short period of time, a slew of commercial laboratories popped up all around the world to cash in on the trend. Micro propagation's potential for large production of clonal plants in the horticulture business. Plant tissue culture uses now extend well beyond clonal propagation. The variety of Somatic embryogenesis, somatic hybridization, and virus production have all become commonplace technologies. Bioreactors for bulk propagation, as well as their removal. Perhaps the most important aspect of the importance of these tissue culture technologies is not so much in their application to mass clonal multiplication as it is in their development. More than in their position as the foundation for plant improvement research and applications, molecular genetics as well as being a basic research tool, it is used in biology and bioprocessing.

Keywords: Shoot tip culture, micropropagation, hybridization, tissue, clonal

Introduction
Meristem and shoot tip culture have been used at a considerably larger scale among the current tissue culture techniques of agricultural and horticultural promise, principally due to their application in different fields such as rapid regeneration. Virus eradication, clonal proliferation of vegetatively produced crop plants and the conservation of both vegetatively and seed-propagated crops' germplasm (Kartha, 1981; 1986; George and Sherrington, 1984; Hussey, 1986) [6, 3], and (Kartha, 1981; 1986; George and Sherrington, 1984; Hussey, 1986) [6, 3]. Lately, in the creation of genetic transformation procedures for Crop enhancement by gene transfer (Ulian, 1988; Gould *et al*., 1991) [4]. The shoot apical meristem, which is characterised by a dome of totipotent cells at the tip of the shoot, is the hub of activity for many developmental programmes in a higher plant's life. The apical meristem goes through different vegetative and reproductive phases during its post-embryonic development. In the vegetative phase, there are many ridges of progressively increasing size right below the apical meristem that indicate newly differentiated leaf primordia. Apical meristems in their juvenile vegetative phase are thought to be the best explants to use for meristem culture. When we come across the phrases meristem culture and meristem tip culture in literature to describe the explants used for viral elimination, we are frequently confused. In actuality, meristem culture refers to true meristematic dome culture, whereas meristem tip culture includes meristematic dome culture as well as two or three rudimentary leaf primordia. However, meristem tips are frequently utilised as explants to obtain virus-free plants in practise (George and Sherrington, 1984) [3]. Shoot tip explants are often utilised for *in vitro* propagation and are differentiated from meristem tips chiefly by their size. Meristem tips are a fraction of the size of shoot tips (0.1 to 0.5 mm) (0.5 to 5.0 mm). However, these measurements are subjective, and the exact size of the explant employed in culture is dependent on the plant species and the research goal.
The first successful culture of Nasturtium (*Tropaeolum majus*) meristem tips and generation of rooted plants marked the beginning of meristem culture (Ball, 1946). Plant pathologists have been interested in meristem tip culture since then because of its potential for producing virus-free plants. The discovery by Limaset and Cornuet (1949) that viruses are not evenly distributed in plants and are frequently absent or undetectable in the apical meristem was significant in this context. This observation inspired Morel and Martin (1952) to experiment with Dahlias, hypothesising that virus-free plants could be obtained by growing isolated apical meristems.

The notion was then proven by utilising meristem tip culture to produce virus-free plants of six potato varieties (Morel and Martin, 1955) [9]. The technique has now become a regular procedure for virus removal in a significant number of commercial crop species (with various modifications and enhancements). When Morel (1960) succeeded in inducing multiple protocorm development in Cymbidium orchid, he added yet another chapter to the history of meristem tip culture, which revolutionised the use of this technique as a tool for efficient and rapid clonal multiplication. The discovery of cytokinin and the development of superior tissue culture medium (Murashige and Skoog, 1962) gave the meristem tip culture technique even more impetus, to the point where it is currently regarded as a commercially viable technology for mass propagation of a variety of crop plants (Debergh and Zimmerman, 1991) [1]. The use of meristem tip culture in germplasm storage has been expanded thanks to developments in cryobiology (Kartha, 1985a) [6]. Meristem tip culture has been used as a technique for gene transfer in higher plants, thanks to recent advances in molecular biology and gene editing (Ulian, 1988; Gould *et al*., 1991) [4].

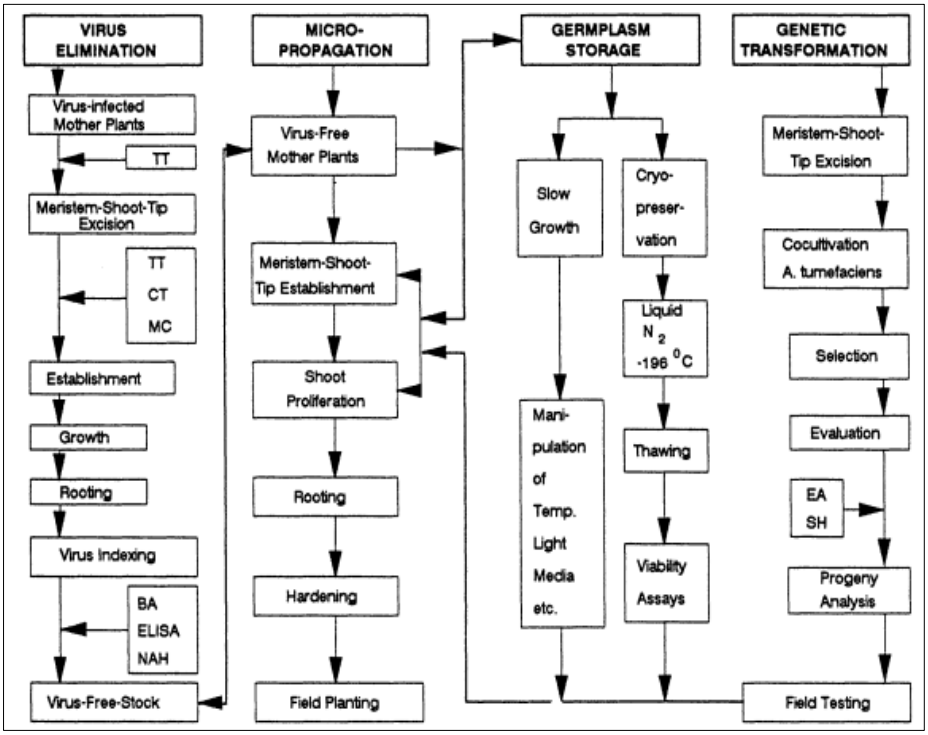

Fig 1: A schematic representation of methodology used for various applications of meristem and shoot tip culture

A schematic representation of methodology used for various applications of meristem and shoot tip culture. Abbreviations: 1T =Thermotherapy; CT = Chemotherapy; MC = Merisd term culture; BA = Bioassay; NAH = Nucleic Acid Hybridization; EA = Enzyme Assay; SH =Southern Hybridization.

Meristem culture used for eliminating viruses in potato

A method in which apex or axillary growth tips (0.1–0.3 mm) are removed and allowed to develop into seedlings on artificial nutritive medium under controlled circumstances is known as meristem culture. The shoot tip culture for viral deletion is based on the idea that many viruses are unable to infect the apical/axillary meristem of a developing plant, and that if a tiny (0.1–0.3 mm) portion of meristematic tissue is grown, a virus-free plant can be created. Although the procedure is primarily employed to eliminate viruses, it frequently also has the added benefit of eradicating other pathogens such as mycoplasmas, bacterial, and fungus. The greater the meristem, the higher its possibilities of surviving *in vitro*, whereas the smaller the meristem, the better its chances of being virus-free. PLRV is a simple virus to eradicate since it only affects the phloem bundle. PVA and PVY are also easy to get rid of since they can't get inside the meristematic cone. Contact viruses such as PVM, PVX, and PVS, on the other hand, have varying degrees of difficulty in being eradicated from the plant system. PVY and PYA have been shown to be able to be removed from around 70% of plants produced from meristems; however, PYX has never had a success rate more than 10%. However, viruses may be eliminated from plants treated using just meristem culture, and mericlones that have previously tested negative for potato viruses stay negative in tissue culture after 2 years of subculturing. Plant rejuvenation from meristematic tissues takes 4–8 months, depending on the size of the meristem and the donor plant's genotype. Furthermore, the percentage of virus-free plants produced from regenerated meristems varies depending on the virus. As a result, the meristem culture method is frequently coupled with thermotherapy and/or chemotherapy to increase the proportion of virus-free mericlones.

As in case of potatoes, meristem culture in combination with thermotherapy has been found to be effective. Tissue Culture for Quality and Recent Advances in Virus Elimination Potato seed is extensively used to eradicate viruses. In this combination method, infected source plants are incubated in a growth chamber for 2-6 weeks at 35-37 °C under 30-50 mol m2 s1 light intensity. The meristems are removed and grown on nutritional media for growth and proliferation after thermotherapy. Because the potato spindle tuber viroid (PSTVd) demands high temperatures for reproduction and accumulation in plant tissues, thermotherapy is unsuccessful for its eradication. Instead, cold therapy (5-10 °C) is used,

followed by meristem excision and culture. Thermotherapy-meristem culturing is a time-consuming method with a low effectiveness for viral eradication (25-40%). Furthermore, virus susceptibility to heat treatment varies greatly, and heat-stable strains of potatoes viruses have been identified. As a result, chemotherapy, which involves treating plants/explants with antiviral drugs like ribavirin or acyclovir, has been utilised to eradicate viruses, either alone or in conjunction with Meristem culture and/or heat treatment.

For virus removal from potato plants, another approach called as electrotherapy is used. Electrotherapy is the process of removing viruses from diseased plants by shocking the explants with electricity. PVX-infected potato plant shoot cuttings have been demonstrated to yield virus-free plants when subjected to 5, 10, or 15 mA for 5-10 minutes followed by rapid culture of the shoot tips *in vitro*.

In vitro propagation of two triploid hybrids of watermelon through shoot tip culture

Watermelon shoot tips were used to create an *in vitro* propagation technique for two triploid hybrids. Shoot tip explants were used to test five benzyladenine (BA) concentrations. Murashige and Skoog medium (MS) containing test concentrations of benzyladenine (2.22, 4.44, 10, 24.61, and 44.4 M) was used to culture shoot tips from 6 and 15-20 days that had been aseptically germinated**.** A high cytokinin (BA) level stimulated the formation of axillary buds from resected shoot tips. The multiplication rate of axillary shoots generated from triploid watermelon shoot tips ranged from 2 to 5.6 plants, depending on benzylad-enine content and genotype. The data obtained revealed that there was a variation in the rate of regeneration. Shoots were excised and extended without hormones in MS media. In MS media with 0.1 M A-naphthalene acetic acid, the elongated shoots were rooted (NAA). With an 80% survival rate, rooted plants were effectively acclimatised and eventually hardened-off to green-house conditions before being placed in soil.

Plant regeneration from shoot tip of seedless watermelon

Two triploid watermelon hybrids were examined in order to achieve results for triploid watermelon *in vitro* propagation. The maximum axillary shoot development and multiplication rate was seen in MS medium containing 4.44, 10 and 24.61 M BA. The multiplication rate in this study ranged from 2 to 5.6, and the proportion of rooted micro-shoots ranged from 63.3 to 90%, according to the findings. The development of roots is inhibited by a higher quantity of BA**.** Watermelon plants were propagated by micropropagation of shoot tips (Anghel and Rosu 1985), who found that a 1 cm shoot tip of triploid genotypes produced two axillary shoots every 21 days when cultured on MS medium containing 1 M BA. Rooted plants from the experiment were successfully acclimatised and gradually hardened-off to green-house conditions, and then established in soil with an 80 percent survival rate. This regeneration technology could be applied in the development of transgenic watermelon plants as well as the micro-propagation of seedless watermelon elite hybrids using shoot tip culture.

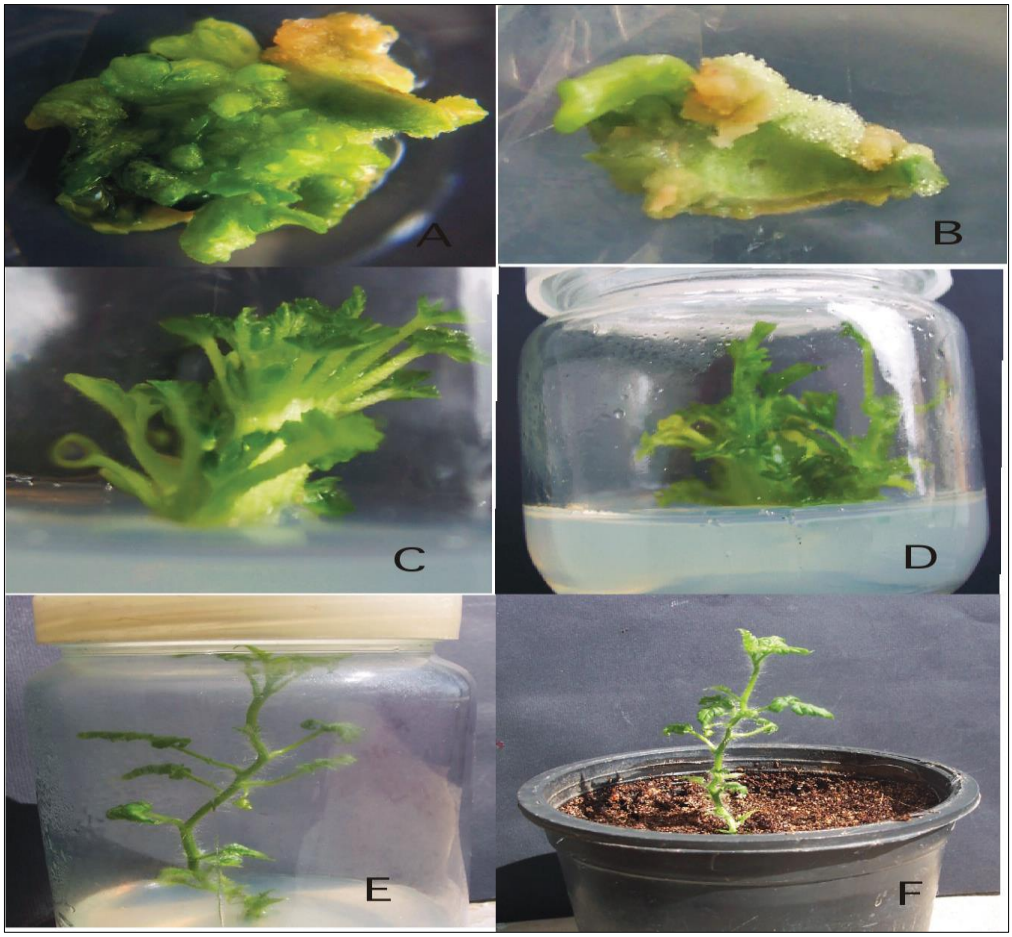

Fig 2: Regeneration of shoots from the cotyledon explants and shoot tip culture of triploid watermelon A and B: adventitious shoot initiation and regeneration from cotyledon explants; C and D: axillary shoot formation from shoot tips; E: elongated shoots cultured on rooting medium; F: acclimatized triploid watermelon plant.

Cryopreservation techniques applied for cryotherapy of shoot tips

Cryotherapy of shoot tips is a new cryopreservation-based treatment for disease eradication. Cryopreservation is the storage of biological material at extremely low temperatures, usually liquid nitrogen (2196C), and is thought to be an ideal method for storing plant germplasm for lengthy periods of time. Plant diseases such as viruses, phytoplasmas, and bacteria are removed from shoot tips by cryotherapy, which involves short exposing them to liquid nitrogen. Because viruses and obligatory vasculature-limited microorganisms are distributed unevenly in shoot tips, infected cells can be killed by cryo-treatment and healthy shoots can be regenerated from pathogen-free meristematic cells. To improve virus elimination, thermotherapy followed by cryotherapy of shoot tips can be employed. Cryotherapy of shoot tips is a simple procedure. It enables for the treatment of huge numbers of samples and produces pathogen-free regenerants in a high frequency. The difficulties associated with tiny meristem excision and regeneration are mainly avoided. Cryotherapy has been used to remove serious infections in banana (Musa spp.), Citrus spp., grapevine (Vitis vinifera), Prunus spp., raspberry (Rubusidaeus), potato (Solanum tuberosum), and sweet potato (Ipomoea batatas). Nine viruses (banana streak virus, cucumber mosaic virus, grapevine virus A, plum pox virus, potato leaf roll virus, potato virus Y, raspberry bushy dwarf virus, sweet potato feathery mottle virus, sweet potato chlorotic stunt virus, and sweet potato chlorotic stunt virus), sweet potato little leaf phytoplasma, and the Huanglongbing bacterium that causes citrus greening are among the pathogens. Cryopreservation methods for a wide range of plant species, including agricultural and horticultural crops and decorative plants, have been devised and can be utilised as is or modified for cryotherapy.

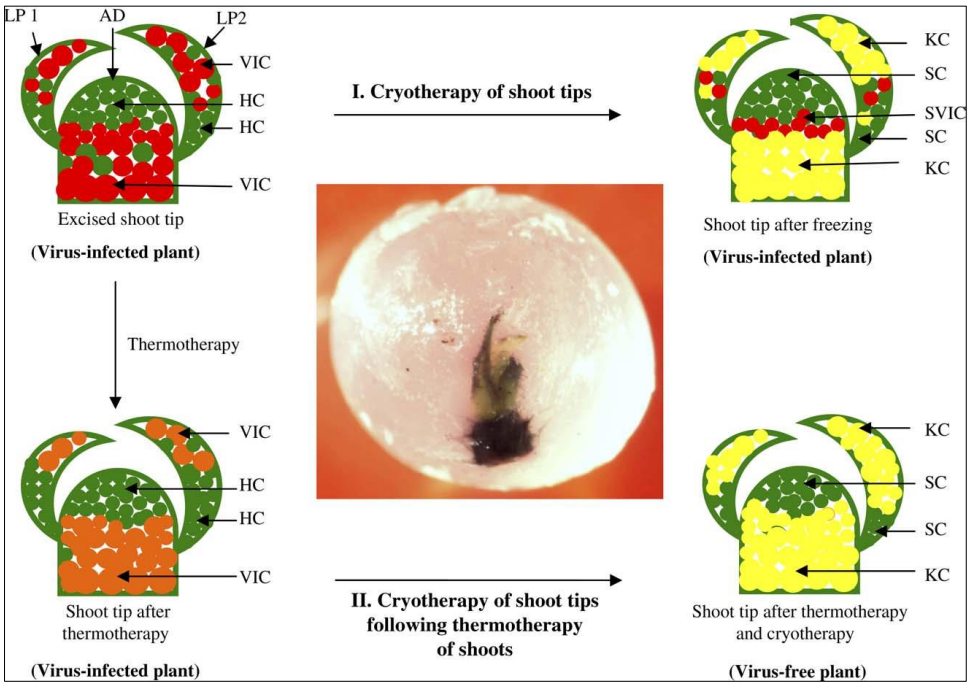

Fig 3: Cryopreservation techniques applied for cryotherapy of shoot tips

Virus free propagation technology for garlic using shoot tip culture

The focus of this research was to come up with a virus-free propagation method for cultivating Yulin garlic. As expiants, shoot tips from Yulin garlic plants with 1-3 leaf primordia were cultivated in MS or B5 basic media with various hormone doses (1.0-2.0 mg/L 6-BA, 0.1-0.5 mg/L NAA). The results showed that B5+1.5 mg/L 6-BA+0.5 mg/L NAA was the best medium for inducing adventitious buds, with a rate of 96 percent adventitious buds induction. B5+0.5 mg/L 6-BA+0.05 mg/L NAA was the best medium for bud multiplication, with a multiplication multiple of 4.5 times. When the shoot tip was 0.5-0.6 mm in diameter and had 1-2 leaf primordia, the virus-free rate of the shoot tip was above 90%, according to the ELISA data.

Development of technology for production of virus free plantlets of sweet potato using the shoot tip culture

The goal of this research was to use shoot tip culture to create virus-free sweet potato plantlets. The sweet potato variety Hongguniang-2 was used to test the effects of different concentrations and combinations of 6-BA, NAA, and GA3, as well as the size of the shoot tip and soaking at different temperatures for varied periods, on virus-free shoot tip culture. The virus was found in regenerated plantlet clones using the indicator plant approach. The results revealed that diverse combinations of 6-BA, NAA, and GA3 in medium had a substantial impact on the induction of adventitious buds and roots in Hongguniang-2. MS+1.0 mg/L 6-BA+0.01 mg/L NAA+0.1 mg/L GA3 was determined to be the optimal medium for inducing callus and cluster buds. 1/2MS+0.2 mg/L NAA was the best medium for rooting. Furthermore, the average virus-free plantlet generation rate for the 90 plantlet clones evaluated was 96.7 percent.

***In vitro* plantlet regeneration from nodal segments and shoot tips of Capsicum Chinese**

Capsicum chinense Jacq. Cv. Naga King Chili, an extremely spicy chilli cultivar and an important horticultural crop in Nagaland, has been given an *in vitro* regeneration process

(Northeast India). By growing nodal segments in Murashige and Skoog (MS) medium supplemented with 18.16 lM Thidiazuron (TDZ) followed by 35.52 lM 6-benzylaminopurine, a maximum number of shoots (13 0.70) was produced with a bud-forming capacity (BFC) index of 10.8. (BAP). Multiple shoot (10 0.37) (BFC 8.3) was also induced in MS medium fortified with either 18.16 lM TDZ or 35.52 lM BAP using shoot tips as explants. In MS medium with 5.70 lM indole-3-acetic acid, elongated shoots rooted best (IAA). Rooted plantlets were hardened in plastic cups with a potting mixture of a 1:1 mix of soil and cow dung manure for 2–3 weeks before being transplanted to clay pots. In comparison to the parent plant, the regenerated plants showed no differences in morphology or growth. The current study provides a straightforward and promising methodology for C. chinense *in vitro* plantlet regeneration from nodal segments and shoot tips, which is roughly twofold greater than what has previously been reported. For root induction in this species, IAA may be preferable than NAA. This procedure can be used to conserve and propagate individual genotypes of this chilli species on a wide scale.

Fig 4: *In vitro* plantlet regeneration from nodal segments and shoot tips of Capsicum Chinese

In vitro regeneration in *Capsicum Chinese* a Shoot induction from nodal explants in MS +18.16 µM TDZ after 5 weeks b Shoot induction from shoot tip explants in MS +18.16 µM TDZ after 5 weeks Elongated plantlets of regenerated shoots. D Rooting of *in vitro* regenerated shoots after 3 weeks of culture e Regenerated hardened plantlets. F Regenerated plantlet transferred to earthen pot (*Bar* 4 cm). g Regenerated plantlets bearing healthy fruit

***In vitro* flowering from shoot tip explants of cucumbers**

In vitro flowering is an alternative breeding strategy for creating hybrid Cucumis spp. since it overcomes interspecific incompatibility constraints. The current study outlines an effective strategy for inducing multiple shoots and *in vitro* flowering from cucumber shoot tip explants (Cucumis sativus L.). Explants of shoot tips from 7-day-old seedlings were removed and grown on Morishige and Skoog (MS)medium fortified with varying concentrations of 6-benzylaminopurine (BAP; 0.5–2.5 mg/L) alone or in conjunction with 0.5 mg/L kinetin (KIN). On MS medium supplemented with 1.0 mg/L BAP, the highest frequency (93.1%) of multiple shoot formation with the highest number of shoots (15.2 shoots/explant) was achieved. Shoots were cultivated on MS medium supplemented with 0.5 mg/L BAP and various sucrose doses for *in vitro* flowering. About 95% of the *in vitro* shoots that were cultivated bloomed. On MS medium supplemented with 6% sucrose (w/v) and 0.5mg/L after 15 days, BAP. Shoots (>2 cm) were cultivated on for roots. MS medium supplemented with varying in dole concentrations 3-butyric acid (IBA; 0.5–2.5 mg/L) can be used alone or in conjunction with other substances. With a KIN concentration of 0.5 mg/L Among the combinations that were put to the test, IBA (1.5 mg/L) and KIN (0.5 mg/L) supplementation with 7.8 roots/shoot, we were able to achieve maximum rooting rates (95.4%). Plantlets were successfully rooted in plastic cups containing a 1:1 mixture of soil and sand, established in the greenhouse, and then acclimatised in the field. The *in vitro* blooming described in this study may help Cucumis species hybridise more quickly and provides a model system for understanding the physiological principles of flowering.

Effects of Media Composition on Growth and Development of Banana Shoot Tips:

The Inorganic salts, organic chemicals, complicated natural preparations, and inert supporting elements are among the components of plant tissue culture mediums. The use of adequate nutritional medium has proved crucial to the success of plant cell and organ cultures. It has been feasible to develop cultures from nearly any plant part by delivering the appropriate chemicals in the right proportions and forms. The levels of inorganic macronutrients and micronutrients utilised

in most plant tissue culture media are based on those found in the "MS medium" created by for tobacco tissue culture. As a result, no single medium can be recommended as being completely suitable for all plant tissues and organs. The most extensively used plant culture medium is Murashige and Skoog. With minor adjustments to MS media, several media formulations for banana shoot tip culture have been reported. B5, SH (Schenk and Hildebrant), N6 and Linsmaier and Skoog (LS) media are also popular. Murashige and Skoog's MS medium (1962) is a salt composition that provides the required macro and micronutrients. In order for cells or tissues to develop and differentiate, inorganic nutrient concentrations must be tuned so that the medium satisfies the needs of the cells or tissues.

The ions of various sorts, rather than the molecules, are the active agent in the medium. More than one molecule can contribute to the same type of ion, for example, NO3 ions can come from both NH4NO3 and KNO3. Micropropagation of bananas has recently begun in East Africa with the goal of developing better or more suitable growing conditions. However, these attempts have had minimal success. Maerere *et al*. (2003), for example, were successful in (developing a protocol for cv. Bukoba and Uganda) in Tanzania, although the field performance of these varieties has yet to be determined. Depending on the dietary requirements, optimal tissue growth and morphogenesis may differ from plant to plant. Explants derived from various portions of the same plant may have distinct requirements for optimal growth. As a result, establishing a media with the right composition for a certain banana cultivar is critical to ensure the cultivar's effective *in vitro* growth and development.

Inconspicuous endophytic bacteria mimicking latex exudates in shoot-tip cultures of papaya

After comprehensive surface sterilisation, shoot-tip cultures of papaya (*Carica papaya* L.) cv. Surya were established from field-grown plants for use in micropropagation and to examine endophytic bacterial interactions with seemingly clean cultures. During the first *in vitro* passage of one month, 40 percent of the 150 explants cultured showed microbial contamination with visible colony growth on tissue culture medium. Among the 90 cultures that appeared to be clean, 12 percent had cloudiness in the medium at the base of the explant that looked like latex exudates. These 11 cultures were found to have inconspicuously associated endophytic bacteria after indexing, which involves testing the cultures for any covert bacteria by transferring traces of tissue culture medium to enriched bacteriological media, whereas the remaining cultures with clear medium were found to be index-negative. According to 16S rRNA gene sequence homology analysis, each of the 11 cultures revealed a single organism, which included 10 Gram-negative isolates (5 Ralstonia mannitolilytica, 2 *Paenibacillus sp*., 2 *Sphingomonas sp*., 1 *Pantoea sp*.) and 1 Gram-positive isolate (*Lysinibacillus fusiformis*). All endophytes except *Pantoea sp*. remained inconspicuous or non-detectable *in vitro* after inoculation of index-negative papaya cultures with the above organisms on Murashige and Skoog based tissue culture medium at 25 C, and they affected the survival and growth of papaya cultures according to visual assessment. With an increase in incubating temperature (30-37 C) or the addition of host tissue extract, the organisms tended to develop vigorously on tissue culture medium and become expressive. The study found that hazy exudates from the explant, which are common in plant tissue cultures at the start of the culture, could be caused by inconspicuously developing endophytic bacteria, posing a risk to diverse tissue culture applications. Early detection of such cultures using indexing aids in the containment of the problem, the prevention of lateral contamination, and the security of *in vitro* germplasm and culture exchange. Tissue culture system also helped in isolating some novel endophytic bacteria associated with papaya shoot-tips.

Conclusion

In the past 2–3 decades, encouraging progress has been made regarding *in vitro* propagation of guava via organogenesis and somatic embryogenesis by manipulation of growth media and culture conditions as well as by testing a variety of explant sources. However, some of the long-standing problems such as guava wilt disease, short shelf life of fruits, and abiotic stress sensitivity requires urgent attention of researchers. There is need to exploitation of modern tools of biotechnology in improvement of watermelon. An increase in genetic transformation studies aimed at improving visual and growth characteristics of the plants has been hindered by low transformation efficiencies and genotype dependence of protocols. As a result, papaya regeneration studies have once again emerged as an essential complement of transformation studies. Since genetic transformation system for guava is not yet well developed, efforts need to be made to develop an efficient transformation system for papaya. For instance, insertion of genes controlling ethylene biosynthesis could be helpful in increasing shelf life of fruits of papaya. Transformation of genes encoding hydrolytic enzymes such as chitinase and glucanase (which can degrade fungal cell wall) could also be beneficial in development of wilt resistant plant of papaya.

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