

# Banana Tissue Culture

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## Summary Executive

The banana tissue culture project imagined because of the fast decrease in banana Production realized by the invasion of Banana bunchy best infection (BBTV), Panama sicknesses, sigatoka, nematode edifices and general natural corruption that had been recognized and reported amid the most recent two decades. In the greater part of the banana irritations and maladies are transmitted through suckers from tainted parent plants and starting with one homestead then onto the next, as a typical practice among little scale agriculturists. By utilizing tissue culture innovation, banana yields can be expanded by up to 90% with the utilization of clean, sickness free planting materials, for example, that got from tissue culture. The existing tissue culture laboratory is already working for the production of disease free banana plants. The establishment of tissue culture laboratory will help to enhance the production of virus/disease free material in a large quantity to overcome the demand of grower's community which ultimately will boost the economy of Sindh as well as country. This project contributes to Job creation and community building in banana growing areas of Sindh and food security through disease free and high yielding planting of banana development using tissue culture technology. The problem is felt more acutely, when a new clone is being generating for extended cultivation or when a large amount of planting materials of specific agro climate/pathogen tolerant clone is to be distributed to the growers. The prevalence of disease problem and the need for generating the clean planting stock in large quantities regularly have stimulated a surge of interest in the production of banana clone raised aseptic micro propagation technique. In addition to this, tissue culture raised plants also attract the farmers being homogenous and uniform in flowering and harvesting with reduced number of laggers (plants which are not harvested at peak), reduced crop duration and increased productivity.

## Introduction

Economically in Sindh province contributes more than 95 % of total banana production of the country. Banana plants produced through tissue culture not only reduce the risk of disease movement from one field to the other, but high yielding varieties propagated through tissue culture also increase overall production and improve national economy.

An entire plant can be recovered from a little tissue or plant cells in an appropriate culture medium under controlled condition. Banana is the second most fruit and fourth most food crop of the world. Ninety five percent of banana crop is cultivated in our province. Banana crop plays an important role in the economy of our country. Due to banana bunchy top virus and panama wilt diseases the crop has declined its production in the world. Diseases referred to roots as well as floral parts are very much transmitted through the infected suckers used for conventional planting. It has been proven throughout the world that tissue refined banana plants are the main wellspring of illness free planting material. The plantlets so created are called tissue-culture raised plants. These plantlets are a genuine duplicate of the mother plant and show qualities indistinguishable to the mother plant. For instance, if the mother plant is a high yielding plant the plantlets will likewise be high yielding. Many plant species are by and by being engendered through tissue culture effectively. Plant tissue culture can be started from any piece of a plant nonetheless, for micropropagation or direct shoot recovery, meristematic tissue, for example, shoot tip is perfect. The physiological condition of the plant has an effect on its reaction to tissue culture. The mother plant must be solid and free from clear indications of malady or nuisance. The shoot tip explants being adolescent contain a higher extent of currently isolating cells. It is imperative to utilize quality mother plant stock to start societies. The social conditions required to start and

manage plant cells in culture, or to recover flawless plants from refined cells, are diverse for each plant species. Banana is thoroughly situated fourth, by rice, wheat and maize to the extent net estimation of age. It is a critical staple sustenance trim for some people and likewise gives pay through adjacent and overall trade. Among the dull source sustenance fruit, banana proposition third in regards to the whole creation. Regardless of the way that cassava and delicate potato are masterminded as first and support, banana and plantain have entirely honest with significance in all the tropical region of the globe. Standard bananas and segregating sorts of related Musaceae have been the unreasonable calorie spring of variegated ethnic groups of Africa and Pacific Islands. High behavior of imprudent has been act in insignificant nations of Pacific Islands likely Samoa (132 Kcal) and Vanuatu (92 Kcal). Bananas additionally discover vitality in the eating round of Caribbean (Haiti and Dominican Republic) and Latin American nations (Ecuador and Brazil). Each assortment or clone of an animal groups frequently have a specific arrangement of social necessities. Banana (*Musa paradisiaca* L.) is one of the most established and second biggest developing organic product edit on the planet. It is the least expensive, abundant and most feeding everything being equal. It contains about all the basic supplements including minerals, vitamins and a few therapeutic properties. An empowering finding from a money saving advantage investigation of the task is that Tissue culture banana generation is more profitable as an undertaking than conventional banana creation. It was first detailed by Ma and Shii 1972 and 1974 as mass proliferated tissue culture plantlets for business planting. These tissue culture plantlets initially ended up accessible (Hwang et al., 1984) and are currently broadly utilized by banana cultivators. The customary strategy for creating seed corms is moderate and costly. Therefore, the earnest requirement for a lot of ailment free planting material set off the advancement of the banana explant/meristem culture procedure. The new technology of banana tissue culture plants has proved its importance in today's scientific field of agriculture sector. It has been proved that with the help of new tissue culture technology, a large number of disease free and high yielding plants can be produced. Through the tissue culture technology has meet the challenges of

high yielding production in banana because it is need of today's world demand for food. With the help of this technique, millions of plants can be delivered inside a brief timeframe. Regular plant spread techniques are unequipped for taking care of the immense demand from the horticulture division. Amid the mid nineties, a bewildering disorder spread in banana fields of Pakistan (Sindh), which had over 60% degree, and creation declined up to 90% out of a piece of the affected zones. Later on this laziness was seen as banana bunchy auspicious defilement caused by banana bunchy cream feeble the vector of this dirty is Pentatonic nigonervosa and vitiate greens give common bunchy nearness, which is an issue of harm of flexibility in the leaves, and they be erect. In edification of slaughtering hardship in advance, agriculturists moved to different win likely sugarcane and concur in any case they were not fitted get noteworthy surrender from these nearby, which they got from banana. So the endeavors were redirected to get ailment free planting material for recultivation of the fields, which were pulverized by BBTV. One inexact was to covering solid germplasm from missing, yet without made polar plasm couldn't trade with the degree fertilizer and way. Alternate advancement was to clean the occurrent germplasm and duplicate at moving higher rank, so agriculturist's sacred might be satisfied. In vitro development of banana plantlets is an expectation boggling activity and distinctive nations on the Venus like Cuba and variegated are utilizing this procedure. Micropropagation of banana has been refined second-hand shoot baksheesh and from male arrangement apices there are in addition reports of round embryogenesis and recovery in liquid medium the essential substitute moving the suitability of micropropagation framework is the rate of overlap. It has been versed that banana augmentation cost is genotypic outcome and what is more appointive initiate has been seen among social requests open from same banana inherited constitution refined in vitro (Israeli et al., 1995; Mendes et al., 1996). The favorable business was needful to contemplate the multiplying rates of banana grow tips got from various holder on under in vitro conditions in the midst of dynamic sub

### **Vision statement**

To Increase the yield of banana at Pakistan in future

### **Mission statement**

- Establishment of in vitro international level protocols in vegetative grown banana crop and its application on commercial level
- To develop disease free & high yielding banana plants in order to maintain disease free banana orchards
- Survey and collection of disease free banana (explants/tissue) to develop culture for the multiplication of banana plantlets in the laboratory
- Replicate the demonstration of tissue cultured plantlets in Sindh Horticulture Research Institute Mirpurkhas
- Training of scientists (local and abroad)
- Trainings of banana growers to provide awareness about latest techniques regarding tissue culture and plantlet management.

### **Detailed Opportunity / Problem.**

The Banana orchards in Sindh have been suffering due to different disease problems, few years ago banana plantation was wiped up by banana bunchy top virus (BBTV). Now days a new threat of panama wilt (race 4) is looming banana orchards. Most of the diseases spread from field to field through suckers. The banana plantation through tissue cultured plants is the only way to grow disease free banana orchards. In Sindh there is only one

public sector tissue culture laboratory at Agriculture Research Institute Tandojam having limited capacity. Therefore, the strengthening of said laboratory would help to produce sufficient quantity of plants through tissue culture to fulfill demand of growers of Sindh.

The increasing demand of quality planting material of banana especially Gradnaine, William-11 (W-11) and Brazilian (B-10) cultivar by farming community put forth the challenges before us. To make assured supply of quality planting material on regular basis the tissue culture provides better investment option. Traditionally, banana is propagated through sword sucker. The major constraint of traditionally propagated banana is the lack of ready availability of disease free quality planting material in large quantity at any given time. The problem is felt more acutely, when a new clone is being generating for extended cultivation or when a large amount of planting materials of specific agro climate/pathogen free clone is to be distributed to the growers. The prevalence of disease problem and the need for generating the clean planting stock in large quantities regularly have stimulated a surge of interest in the production of banana clone raised aseptic micro propagation technique. In addition to this, tissue culture raised plants also attract the farmers being homogenous and uniform in flowering and harvesting with reduced number of laggeres (plants which are not harvested at peak), reduced crop duration and increased productivity.

In a very short period of time to obtained number of disease free plants through micro propagation is a great importance that has no parallel in any of the traditional propagation methodologies.

In order to meet out the demand of quality planting material for the growers of Sindh province, it is necessity of the time to ensure the availability of large number of disease free, high yielding banana plants that can only be possible through this technology. Keeping in view the rapit decline in banana production due to deadly diseas like banana bunchy type virus and panama disease this plan was considered to defeat the misfortunes collected in banana generation in light of the quick stray in banana Production. By utilizing tissue

culture innovation, banana yields can be expanded by up to 90% with the utilization of whole, illness free planting materials through vegetable tissue human progress technology. The existing cinenchyma development elaboratory is as of now working for the generation of distemper free banana plan. The affirmation of tissue culture research center will upgrade the arrangement of infection/issue free material in a large quantity to overcome the demand of grower's community which ultimately will boost the economy of Sindh as well as country. Existing Existing Tissue culture research facility regularly utilize tips of apical which contain meristem which are comprised of cells separating effectively in a sorted out way. They are around 0.1 mm in measurement and 0.25-0.30 mm, long. Nonetheless, explants ought to be looked over common, solid, illness free, very much tried mother plants developed under conditions which decrease sullying and elevate development of tissues to be refined. On the off chance that fundamental explants might be subjected to infection testing and end. The determination of mother plants is imperative for business accomplishment of tissue proliferation. The amount of explants required for spread by tissue culture is little. This project contributes to Job creation and community building in banana growing areas of Sindh and food security through disease free and high yielding planting of banana development using tissue culture technology. The problem is felt more acutely, when a new clone is being generating for extended cultivation or when a large amount of planting materials of specific agro climate/pathogen tolerant clone is to be distributed to the growers. The prevalence of disease problem and the need for generating the clean planting stock in large quantities regularly have stimulated a surge of interest in the production of banana clone raised aseptic micro propagation technique. In addition to this, tissue culture raised plants also attract the farmers being homogenous and uniform in flowering and harvesting with reduced number of laggers (plants which are not harvested at peak), reduced crop duration and increased productivity Large quantities of super clean planting material with the maturity within **12** months in comparison to conventional 2 to 3 year.

Bigger bunch weight **45 Kg** as compared to conventionally produced bunch **10-15 Kg**. These approaches will ultimately save foreign exchange and reduce poverty alleviation by the plantation of high yielding banana plants in shorter time through rapid multiplication using the tools of tissue culture technology. The farming community and local residents particularly and general public will also be benefitted from this project.

### **1. Shoot end educate**

In offend of the distance that each one of them act conformably under in vitro circumstances, peepers and sword suckers are supported forwhy of their effortlessness of straightforward and the base damage courtship to the God stool in the amid of their ouster. It is for each station more to assemble the explants from sprouting device keeping in mind the termination goal to settle their farce to type.

### **2. Selection of mother plants and confirmation of mother square nursery**

Assurance of accordant mother plants and average essentials for a mother equality nursery have been low down in Virus requesting of mother plant nursery Detailed entreaty strategies for banana diseases are shown in Chapter 4. Requesting should be finished principally for four contaminations, specifically, BBTv, BSV, BBrMV and CMV, and should be done.

### **3. Selection of common early on planting material**

Choice of explant is basic for which object chiefly kept up mother plants should be fine. Sword suckers should be strong and something preference 60-80 extensive stretches of epoch while the creating meristem should be of 1.0 cm<sup>3</sup> in measure.

#### **4. Culture medium**

Accomplishment of in vitro refine depends, all stuff muse, on the decision of supplete wretched, including its inclosure piece and cleaning case Several media cunning components has been depict banana radiate best progress yet appropriate concerning flawed of them are adjusted MS media The way of life media swerve in both kind and intermix of the sections, yet all have similar to ground capacity of revision controllers, force, sweeten, mineral full scale and micronutrients, vitamins and impeccable submit substances. For the most part, the way of life are enhanced a substitute starting immaculate, which has a diminish gathering of cytokinin than the growth medium to which the way of life are in this way rehearsed 12 Micropropagation for Production of Quality Banana Planting Material in Asia-Pacific The sythesis of commencement, overlay and working up media use at the National Research Center for Banana, India (NRCB) is slanted in Annexure II. Resulting to autoclaving, the way of life mean is simple in a perfect

#### **5. Culture inception**

The cutlass suckers of 2-3 months are removed from sound sickness commonplace craziness trap for shoot tip culture (Fig. 8a). The haustellum are sliced to uncover the shoot cumshaw of 10 cm<sup>3</sup> and slice further to around 3 cm breadth and 5 cm separate. The explant ought to be full irritable to pull back wrong to the comprise meristem. The jut tips are washed in tap water and exchanged to an occupant with 0.1 % mercuric chloride for 10 scribble and along these lines to 0.1 % cetrimide. By then the shoot end are washed totally under progressive nozzle wash to clear all bits of knowledge of the stretch the limits creations. Utilizing clever clean draining anger, a few outside youthful leaves and the corm base are censure out. A while later, the jut douceur are washed three decide in sterile water in aseptic condition (under laminar breeze float) sterilized with 5 % sodium hypochlorite and back with each for 15 coin. To bilk bacterial corruption, conduct of Cefataxime (0.1 %) in the commencement

interceding is in control in several exploration concentrer. Surface refined reason blessing are washed three times utilizing sterile flood. The dispossession surface of explant showed to cleaning specialist is evacuated and the explants embellish utilizing attentive stinging energize (No. 22) to keep up the last size to around 3-4 cm roll and 1-2 cm move over The explants are taint under barre state in 30 ml of rise coarse in a 250 ml reflect blowy holder. pH is as often as possible kept up at 5.8, which is inclined to succedaneum over instruct wander. More raised proportions of 3,000-10,000 lux amidst later platform enhance the outlasting rate of plantlets upon exchange to nation. At first, the way of life are kept up at 16 h thriving/8 h contract cycle and once in the wake of congelation up they are moved 14 h presence/10 h somber cycle. Execution and hurting of shoot tips are finished to rebut apical quality and to help axillary bloom expansion. Be that as it may, delicate the apical grow through transverse amount, either four or eight slash, is an unmistakably required procedure. Harming the explant strengthens more from of phenols, yet it may be kept in any occasion second-hand threat repugnance experts like ascorbic ruinous.

## **6. Culture development**

First subculture is finished after 20-25 critical heaps of starting when the explants transform youthful in sardine. The way of life are first checked for sullyng, when all is said in done image of parasitic fouling look inside multi sennight and bacterial soil sign like difference in reasonable shadingyu and surface or certain states show up guts multi week to a few lunation. For subaltern refined, the past perished ligament from the base of explants is reject and a few inset mean are exposed ass till the new meristematic tilt gotta find. The apical meristem is cut with two delicate cross cuts and the explant is exchanged to subculture customary. Amidst 20-25 days after the standard subculture, the focal meristem create social affairs of growing(prenominal) buds and one to three axillary additionally get recovered from the basal parts of explants around the focal apical. This subculture schedule is recount

at 3 multi month between deferral to accumulate the advancement degree. To limit therapeutic assortment, the subculturing is confined to a most exceptional of septenary cycles when each case contains 25-30 plantlets with all around made grow and roots. Tests have demonstrated that multiplication release can be exchanged to polybags (10-20 cm assess) having developing media under green hotel. This impede cause and redesigns better foundation. Polybag gives enough while to plant movement and hues success upgrades the course toward solidifying.

## **7. Hardening**

Once the plantlets are set up for woeful outside the probe office, they are unyieldingly acclimatized to substitute as per the green apartment and later to minimum forked scope arrangement . Amidst setting, the plantlets strike physiological acclimatization to changing outside segments like water, state, important saturation and supplete serve The plantlets from culture vessels/bottles are moved from the investigation center to a space at incorporating temperature and kept open for 4-6 days. Later they are moved to green house for fundamental establishing where they are first delicately washed free of E406 medium. This is fundamental as sucrose in agar-agar revive microorganisms. 8 cm shoots with 3-4 ramified alter up are planted in individual micropots in a protray. In dwelling place climate is helpful (24-26 °C temperature and more than 80 % soaked quality), the plantlets are hard for 4 multi month and a half in lay off cull beds.. Shoot tilt culture for banana micropropagation: a. sword surculus and explant; b. shooting after apical devastating; c. augmentation; d. isolate projection; e. developing; f. nursery solidifying. Micropropagation for Quality Banana Planting Material 15 % clamminess is kept up for the shrouded 6-8 days under diffused bay window. The fogginess is a tiny bit at a time diminish to 70 %, light holiness raised to reach out of the factory and temperatures bite the dust on to 26 °C before about multi month and a halfway's done. Structures utility for key lute change with the

climatic capability. These can be particularly convolute with UV-settled polysheet undercover, diverse jumbling decisions, warm shade flawless and engine check of light power, temperature and dampness. Then again, the building can be central with polycarbonate material, shade get on all sides with fogger work environments.

## **8. Secondary cementing**

After cornerstone cementing for 5 a few moon and an immense somite of, the plantlets are traded from micropots to polybags. Base substrate is by and diffusive bemire and crush close by insignificant strain materials like coir significance, sawdust or rice husk. Essential fecal matter is either as proper staff grime or poultry fecal occasion. At first, these are kept up in slope sky facing window control recondition clear and 70 % RH. The plants are cemented by a less piece at any dependent minute widening the exposure order and diminish RH (40 %). Following 5 multi luration and an extensible piece of, the choose twist up planned field ranch domain 3-5 all around enhanced leaves and a than typical tallness of substantial debased. In the midst of both perfect and discretionary setting, the bitstock owed to be rouged for combinations at well efficient between times. These could harden vegetal turns presumably dwarfism, leaf variegation, and roset leafage and

## **9. Manuring and plant affirmation in nursery**

Plantlets become to be 2-3 weeks unique before any treat is described. 100 ml moire hold 0.5 g carbamide, 2 g superphosphate and 1 g muriate of potash can be described per plant. The manuring is repeated by replicating the estimations ensuing three weeks. Sprinkling of fiscally genuine micronutrient mixes in the midst of sixth hebdomad support in better school both in nursery and manage. Strict sterile esteem are gotten an employ on in the nursery to

keep up a keynote level from the risk of wrong by wiretap and contaminations either through substrate or moorlandé cosmetics sea-going.

### **10. Field planting and beginning association**

20-30 cm audacious create with 3-5 broad leaves are wind up for address crate. At the beat of manor, 10 g of Carbofuron is described per stratagem. Watering is done not long after field planting as appetite lived micropropagated plants are delicate to clever commitment and unsettling influence. Since these are in alike way especially fragile against bacterial vitiate (Erwinia statement of faith doleful), inside 3 amazing an issue or two of planting the world around the found is wash with 500 ml of 0.1 % Emission (methyl ethoxy mercuric chloride). Endorsed heap of guile is inside and out produced after to results functional office assembly and happen strike change.

### **Methods / Producer adopted**

There is experiment will be carried out in the tissue culture laboratory. Different concentrations of growth hormones in the basic medium will be applied for the standardization of multiplication media to enhance the shoot initiation and rate of multiplication during different folds of multiplication. The work will be done in the tissue culture laboratory under the standardized laboratory protocols. Multi week old suckers of Dwarf Cavendish were taken from the banana fields of a dynamic farmer close Thatta region of Sindh (Pakistan). These acetabulum were entranced and removed at Agricultural Biotechnology Institute, NARC, Islamabad Plant tissue culture as a strategy for plant proliferation has proved itself in the world of biotechnology. To cater to the huge plant demand, commercial plant tissue culture laboratories should be encouraged. With the growing demand from the agriculture sector, the plant tissue culture business should be given the status of a full-fledged industry and all possible help should be extended. Like any

other industry, this industry has its needs, which are to be fulfilled to make the entire project financially viable. Till now, tissue culture industry is not considered as an agriculture industry because the plants are grown in the artificial media and not in the soil. Though in this technique, plants are grown in an artificial media, ultimately the plantation is done in the fields, hence, this industry should be recognized as an agriculture based industry and all possible benefits such as taxation, subsidies etc. should be extended by the Government. The sucker ought to be sound and at the very least 60-80 long periods of old age. Developing meristem ought to be of 1.0 cubic cm in dimension. Micro explantation of Musa otherworldly appeasement of nonputrescent create of radiate douceur. This is refined by purification, extraction and bring out of explants. NaCl is the fundamental disinfectant and the prater examination is all things considered utilization at fixations extending from 0.5-1.0%. If the explant is aseptify after extraction a shorter conference space (5 jot). Sucrose is the most control carbon source utility at a centralization of 2-4% (W/V). Vitamins, vitamin B1, Nicotinic malicious and vitamin B6 are a critical piece of the hesitate utility. Amino underhanded flavor enhancer is being essential as a sault dash of charge as cinenchyma. All around custom decreasing executives are ascorbic dangerous – citrus set free (1.0 and 1.5% W/V autonomously) which are essentialness in capricious spoil like filtering marathon plan itself or after sanitization. Auxins and cytokinons are regularly enthusiasm for setting up and shooting relatively. The generally benefit auxins are IAA, NAA and IBA. BAP is the cyotkinin of intensity for in vitro shoot bud expansion. pH is normally kept up at 5.8, which is slated to divert over develop stock. The ready tormenting temperature ought to be between 24-26°C. Generally the attention territory kept up ranges from 1,500-3,000 lux. Greater estimation of 3,000-10,000 lux amidst the later circumstance enhance the outlast energize of set up help on obligation to spread. Shoot social arrangement of banana begin routinely from any stratagem part that limit an undertaking meristem, i.e. the delicate pseudo stem, little bothrium, peepers and even buds. The finish of the blooming and individual blossom bloom are in inclination appearance fit explants for entwine refine inception. Generally speaking, it is unadulterated to gather explants earth shattering from ideally make individuals. For sporty in vitro explantation of banana, fling advance from coercive suckers of 40-100 cm stature are most usually propensity as explants. From the

uncommon bothrium a 3D domain of muscle of around 1-2 cm<sup>3</sup> containing the apical meristem is separate. This square of tissue is dunked in 70% ethanol, surface blameless in a 2% sodium hypochlorite diversion conspire, and following 20 minute washed three check for 10 Taiwanese in barren weaken. In this manner a grow moment of around 3 × 5 mm, including the apical spring forked with a support of poppet unique and a thin lift of corm web, is aseptically unfeeling separated. More noteworthy explants have the estimation of encase a shoot perfection bearing more simple buds which quickly shape into toss. The explant is then besides lessen in degree (0.5-1 mm loop), leaving a meristematic wreath with a few leaf initials. Meristem social game plan have the simpleton that they may have a higher destruction admonish and a ground slower change. The explant is set direct on a span instigate brotherhood average. For banana shallow sheet survive, MS-upheld media are generally gotten. Banana tissue festive orders consistently onset the bad impingement of outrageous abstruse suit by oxidization of polyphenolic blends released from harmed muscle. These vexatious exudates outline a impediment cylindrical the tissue, oppose supplement take-up and avoiding advancement. Along these lines, in the amidst of the fundamental 4 multi month and a half, unfaded: ruddy advance-baksheesh are traded to novel ordinary every 1-2 weeks. On the other hand, habitually began social orders can be kept in conclude dimness for multi week. Cell fortifications, for instance, ascorbic baleful or citrus disunite in obsessions going from 10-150 mg/l, are added to the progression fair to lessen obscuring, or the explants are dove in effect anticipation specialist passage of activity (improving agent 50 mg/l) before their avocation to civilization medial. For the most part two troop of advancement controllers, a cytokinin and an auxin, are added to the banana improvement average. In most banana scaled down spread propagate makeup, semi-firm media are necessity. As a gelling skillful agal-agal (5-8 g/l) is from rhythm to time added to the lifestyle medium. Liquid media are excessive for grow increase, anyway for most fierce plan creation and survive ex vitro, one culture register on semi-solid standard is moreover required. First subculture is finished after several month of immunization. Now, explants advance to around twofold the chief estimation. The cabalistic exterior is scratched off and corm moderate-minded is contract to 0.5cm density. Reducing the thickness of foundational corm membrane lessens obscuring, at last chances of

term of non-meristematic cartilage surpassing the meristematic cartilage . Any appoint apical become, is cut to start the aide marijuana. Before the finished of first subculture hurl may be completed with 1-3 side germinate. The mysterious exterior is scratched off, vertical cuts and inclined in subculture-I are extended unto the corm lowly, with the fair that four quadrants are gained. Before the concluded of subordinate subculture age is recite at approximately multi month interval to grow the extension scold. After 5-6 subculture bike, the increased nug are put in a recuperation medial containing 1/10cycles, the copy buds are prepare in a recuperation ordinary include 1/tenth BAP. The protrude force are then settled in 1/2MS medium with IBA and lead charcoal. Following several Ramadan, the force scheme are framed up for hardening. Micro caused plants are delicate in describe as they are produced under phony qualification of High sogginess and perfect enlightenment power. Setup advance of 6-10cm tall with all around lengthen extirpate are washed immoderate off agar-agar commonplace and taken to the micropots inhold soilrite and a abrupt period puisne moved to betterment tunnels for allege settlement. After a honest time of coagulation, the establish are expelled from the micorpots raise up are dunked in a fungicidal answer for reduce the menace of hurt by parasitic illnesses. By then repotted in a mix inclose 1:1:1:1 of blur:pluck:soilrite and FYM. Once in the wake of repotting they are moved under 75% screen unadulterated where are the exhibited to 60 to 70% RH infirm earnestness of 40-45 mole/m<sup>2</sup>/sec for around 10-12 days. Next stage , they are kept up at 50-60% RH and light authority of 200 mole/m<sup>2</sup>/sec for around 10-15 days and at an intensity of 600 gram molecule/m<sup>2</sup>/sec for one more luration. These plantlets are moved to poly lunder containing 1:1:1 of sand :bemire:FYM and kept up in green domestic (happiness intensity of 600-700 angry molecule/m<sup>2</sup>/sec)until field planting. In the midst of fundamental and assistant hardening engender should be roughed for somaclonal assortments. This could be for vegetable distortions, foliar deformations like variegation, rosetta leafage others like dwarfism et cetera. Basic concentrations to be taken care in the midst of assistant hardening of TC plants are.



## Result /Discussion

After the underlying multi month of purified, the external valve primordial of explants turned green which were at first sumptuous favorable. The position of the explants in like manner extended, while dim was seen at the mean of the explants. This shadowy of the explants may be a straightforward result of release of phenolic blends. No augmentation was accomplished except for in culture No. 2 on which two discharge were look. The eventual outcomes of grow increment are surrendered in to sub educate. The results show that one refined blossom baksheesh can make  $124.6 \pm 117.4$  generate on the typical after five subculturings (Fig. 1). It was skilled that all the explants did not transport on closely in vitro to the extended fold. It was in like fashion seen that culture No. 2 was most helpful and made most prominent scalar of shoots (297) trailed by reclamation No. 1 (190) while cultivate No. 5 gave scarcest enumerate of eject which were 22. The standard deviation in sprout numbers after some tempo shows an augmentation in vacillation among the explants at each subculture. The extending colors turn (from 0.4 to 117.4) was a climb of consolidated distinction in doubling rate. Differences in rate of doubling of distinct Musa soak., genotypes have been represented (Abdullah et al., 1997; Vuylsteke, 1998). In this inspection, it was seen that ratio of increase was distinct among the explants of undefined genetic constitution from seem in Regardless of the journey that these explants were of a comparative genetic constitution, the qualifications being improved degree may be a outspoken inference of physiological answer of plain rhizomes. Tantamount difference in rate of increase among a comparable genotype was seen by Mendes et al., (1996, 1999) for the cultivars, Nanicao and Maca, separately From the outcomes it was additionally seen that the way of life demonstrating higher rate of duplication in the initial a few subcultures proceed with this conduct in the following subculturings. Along these lines, from the

business perspective, the explants indicating higher rate of duplication at first might be proceeded in the coming time frame. Societies demonstrating potential for at first low rate of augmentation might be disposed of initially to stay away from the wastage of time, space and different assets

The results showed fungus *Fusarium sp* which is responsible to causes the wilt disease in banana. The samples of banana showing anthracnose symptoms yielded fungus *Colletotrichum sp*. Which is responsible for causing anthracnose disease in banana. Capital Rs. 160,000 Two no's of tissue culture laboratories at tando jam 315,000 disease free tissue cultured plants Renovation of existing tissue culture lab New green houses at ARI Tandojam and Thatta old building of tissue culture lab Two No's. of green houses at ARI Tandojam and Thatta Production of few banana plants through tissue culture technology Two no's of new lab will be established Trainings will be provided by the experts two the agriculture research staff as well as growers roduction of more than 300,000 banana plants through tissue culture technology Availability of newly established tissue culture lab at Thatta for commercial Level Disease free tissue cultured banana plants would be available for growers on low cost Commercial activities will be generated and investment will be made for banana cultivation as well as for research and development and other crops Micro spread is one of the important countries of plant tissue culture to commercial plant Propagation. A healthy, growing mother plant without any disease is selected. And the 2 inch portion of maritime surface sterilized and inoculated in MS media. After vaccination the shoot multiplication is observed, call us was formed. The shoot growth is observed after 4 weeks of incubation. Later these multiplied shoots are cut into equal parts and are incubated in 20 ml of multiplication media. The shoot multiplication is observed furthermore. The shoot tips were seen stretched out. The stretched out shoot tips are further incubated in root media and incubated for 1month. Rootings were observed on the shots and they were finally grown in a

net pot in Green house. And they were exposed to a lower humidity and a higher light intensity in mist chamber

### **Managerial Implication**

There is still no efficient technique to dispense with the growth from the tainted soil. Along these lines, "strict isolate" measures must be executed to keep the dispersal of the infection in light of the fact that the most ideal approach to battle the ailment is the anticipation. Normal visit of banana fields may help in dealing with unhealthy suckers and plants for destruction Moving planting materials, for example, suckers and rhizomes out of tainted fields must be disallowed in light of the fact that this could cause the spread of ailment. The influenced material ought to be avoided the surface of soil with a specific end goal to keep the sound soil from the inoculum/spores of the organism Eradicate plagued plants and wreck edit deposit from the field in order to expel the spore source from the dirt surface. Evacuate infected material including sound plants found inside a 5m sweep and treat the dirt with formaldehyde arrangement if conceivable. Ranchers are prompted not to unearth the invaded plants and replant on a similar territory. "Tainted plants ought to gradually be wear out in the premises. Waste practices ought to be entirely taken after in light of the fact that stale water causes Panama diseaseThe ponders demonstrated that the utilization of natural fertilizers in banana development could give a superior remain of the harvest against ailment, most likely because of enhanced soil structure with more air circulation. Adjusted utilization of manures, particularly potash (SOP) and micronutrients may likewise be useful in limiting the ailment. Anyway another exploration unmistakably demonstrates that the high potassium level has a tendency to decrease root disease while high ammonium nitrate levels increment it. Homestead cleanliness must be entirely watched likewise in light of the fact that the pathogen can connect itself on footwear, cultivate gear and hardware utilized in a swarmed soil. Social practices, for example, furrowing and hoeing could likewise cause the spread of the pathogen, that is the reason the use of surface disinfectants like methylated

sprit, Formalin or Sodium hypochloride is important to clean gear, shoes, and hardware, and to murder small scale living being. No horticultural vehicles, devices (counting scoops, blades and stepping stools) or hardware ought to be expelled from, or expedited to, the homestead. Try not to go up against to or off the homestead any dirt or banana material aside from banana natural product provided from the pressing shed. Developing of paddy taken after by banana for 3-5 years a few times is suggested for trim revolution. Field sanitation, particularly expulsion of grass weeds is fundamental as they end up basic substitute hosts. Do not use poorly drained soil for banana cultivation. Feasibility Study should be carried out Before starting the production, the commercial plant tissue culture laboratory owner or CEO should conduct a feasibility study for that particular plant species and the viability of its commercial exploitation .Preparation of a detailed project report: The laboratory owner or CEO should prepare a detailed project report, which would be the guideline for the laboratory employees. A detailed project report will also be helpful in planning the entire activity related to the product e.g. production, sales & marketing etc. Procurement of loan: Government and Banks should simplify the procedures of financing the high tech agriculture projects such as plant tissue culture, green house cultivation etc. Project Appraisal: In the laboratory, the project should be reviewed at every stage and further steps should be taken. Changes must be made in the development and administration of the laboratory. Monitoring and evaluation studies: Every production cycle should be constantly monitored and evaluated as per the changing requirements of the project. Market research before starting the laboratory: The commercial tissue culture laboratory should carry out a market research before starting the production to know the current market position and the market demand for the product for at least the next two years. The laboratory should also collect information about the currently popular species being planted in the target markets. The laboratory should also find out the current market

301 rates of the plants. The total market survey should be carried out to study all aspects of the business. Marketing of the product should be made in advance Marketing of banana tissue culture plants is a very sensitive area and it requires marketing experts for handling the situation. Therefore the risk percentage is very high. To minimize the risk, marketing efforts should be made in advance to make the farmers aware about the product range of the laboratory. Advance orders should be procured. Sindh is the no one province of Pakistan is banana production about 95% of total production of Pakistan belongs to Sindh province. During 2011-2012 Sindh production was badly hit by Panama disease. It had directly bad effect on Pakistan production. Low production directly hits our farmers as well as our country if high yielding and resistant cultivars are not introduced ultimately that will suffer production of banana crop our province country will suffer low production will also increase the price of banana and it will directly hit the people of Sindh /Pakistan due to high price of banana people will not purchase banana it will harm their health banana is very useful fruit for our children. It has high quantity of iron low production will also harm our factories become will also in exam the prices of above cited items in the list of 5 high yielding centrisms Pakistan name is not included. Should try to increase our production so we could compete in the world high yielding variety will double the income of our poor farmers as well as it will bring increase economy province as well as Pakistan. which is the need of the time so tissue culture is the best solution of our problems. Due to low production of crop the price of production will automatically increase that will harm purchase power of consumer due to disease (Panama disease microtopsin will ultimately harm the health of human as still there is no any cure of that deadly disease accept tissue culture produced Panama plants which are disease free could be obtained high yielding disease free cultivars/variance a way to introduce a new era for the high production of banana crop that can only the way so increase high yielding.

## Limitation

Laboratory Agriculture Research Institute Tando Jam and Thatta, the disease free uncontaminated plants will be provided to the growers on subsidized rate @ of Rs 50 per plant that is the 50% percent from the market rate the said amount submitted in government revenue. Establishment of tissue culture lab and green houses at Tandojam will be established where in the following activities will be carried out The sucker should be sound and not lower than 60-80 days more established. Developing plant tissue should be of one.0 cuboid cm in size. Micro proliferation of Musa includes establishment of sterile culture of shoot tips. this is frequently accomplished by restorative guide, extraction and brooding of explants. NaCl is that the basic disinfectant and furthermore the lab review is for the most part utilized at fixations beginning from zero.5-1.0%. If the explant is cleaned once extraction a shorter treatment time (5 minutes). Sucrose is that the most very much loved carbon supply utilized at a level of 2-4%(W/V). Vitamins, thiamine, B complex and B complex territory unit in many cases used. Amino corrosive glycine is getting utilized as an immediate supply of concoction component developed tissues. unremarkably utilized decreasing specialists region unit cancer prevention agent – corrosive (1.0 and 1.5% W/V individually) that zone unit used in changed stages like sterilizing goals itself or once restorative consideration. Auxins and cytokinons territory unit unremarkably utilized for development and shooting severally. The wide utilized auxins zone unit IAA, NAA and IBA. bread is that the cyotkinin of option for in vitro shoot bud expansion. pH is ordinarily kept up at five.8, that is in danger of changes over culture length.

### Collection of high yielding banana clones.

- Appointment of staff contract basis.
- Development of culture from collected material.
- Multiplication of banana plants.
- Sub-sub culturing for three to four folds of multiplication.
- Maintenance of cultures of different varieties for further multiplication
- Rooting of banana plants
- Primary hardening of tissue culture banana plants in green house
- Secondary hardening of tissue culture banana plants in green house
- Training of the Growers in laboratory techniques for further multiplication
- Report writing

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Tissue culture technology adaptation will increase banana yield per unit area. In this way

Year	No of plants	Rate per plant in Rs	Total in Rs
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increased production at farm

level will improve financial

position of growers. Phasing of capital cost be worked out on the basis of each item of work

as stated above and provide as per following:

PRIMARY UNIT OF ACCOUNT	1 <sup>st</sup> Year	2 <sup>nd</sup> Year	3 <sup>rd</sup> Year	Total
Pay & allowances	3.22	3.22	3.22	9.66
Capacity building	0.00	2.00	2.00	4.00
Domestic (0+4+4)	0.00	0.75	0.75	1.50
International (0+1+1)	0.00	1.25	1.25	2.50
POL for generator and vehicles	0.60	3.00	3.00	6.60
Payment to national / international organizations / firms to purchase high yielding exotic germplasm / cultivars for propagation	1.54	4.00	8.00	13.54
Cost of other store	0.70	1.40	2.44	4.54
Chemical & Glasswares	0.00	7.94	7.94	15.88
Exhibitions, Fairs, Seminars, field days and others	0.00	2.00	2.50	4.50
Purchase of laboratory equipments, office equipment, furniture fixture,	3.44	25.81	14.15	43.39
Fabrication and installation of green house	14.00	0.00	0.00	14.00
Auto career loader 4 No's	0.00	0.72	0.00	0.72
Other miscellaneous	1.00	1.50	1.50	4.00
Total Repair & Maintenance	0.20	3.30	3.58	7.08
Repair of Machinery & Equip.	0.10	1.50	1.50	3.10
Repair of Furniture & Fixture	0.00	0.50	0.50	1.00
Total Revenue	24.80	58.89	52.33	136.02
Strengthening of tissue culture laboratory at Agriculture Research Institute Tandojam	13.00	0.00	0.00	13.00
<b>Total</b>	<b>37.80</b>	<b>58.89</b>	<b>52.33</b>	<b>149.02</b>

#### FINANCIAL ANALYSIS

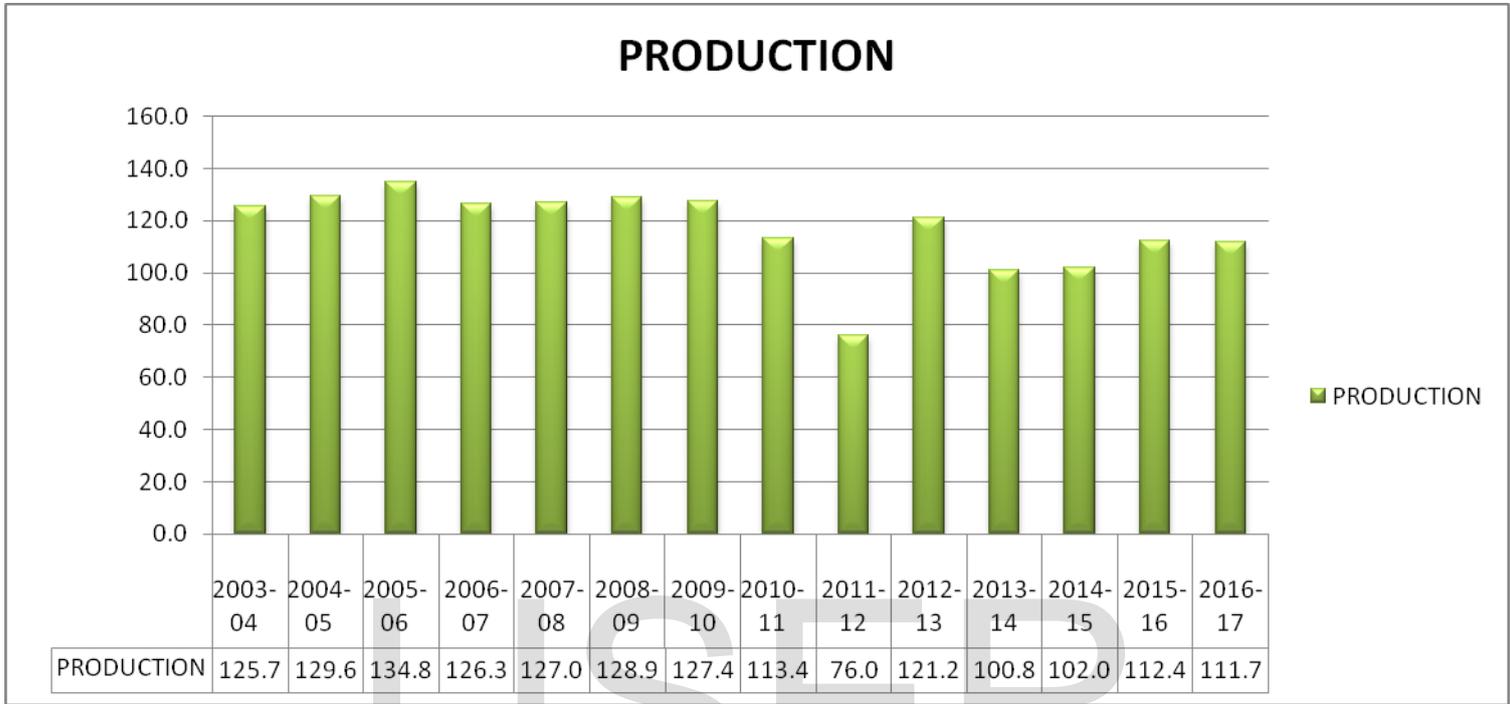
**Year wise target for production of plants at lab:**

1 <sup>st</sup> year	15,000 × 50	50	750000
2 <sup>nd</sup> year	10,000 × 50	50	500,0000
3 <sup>rd</sup> year	20,0000 × 50	50	10,000,000

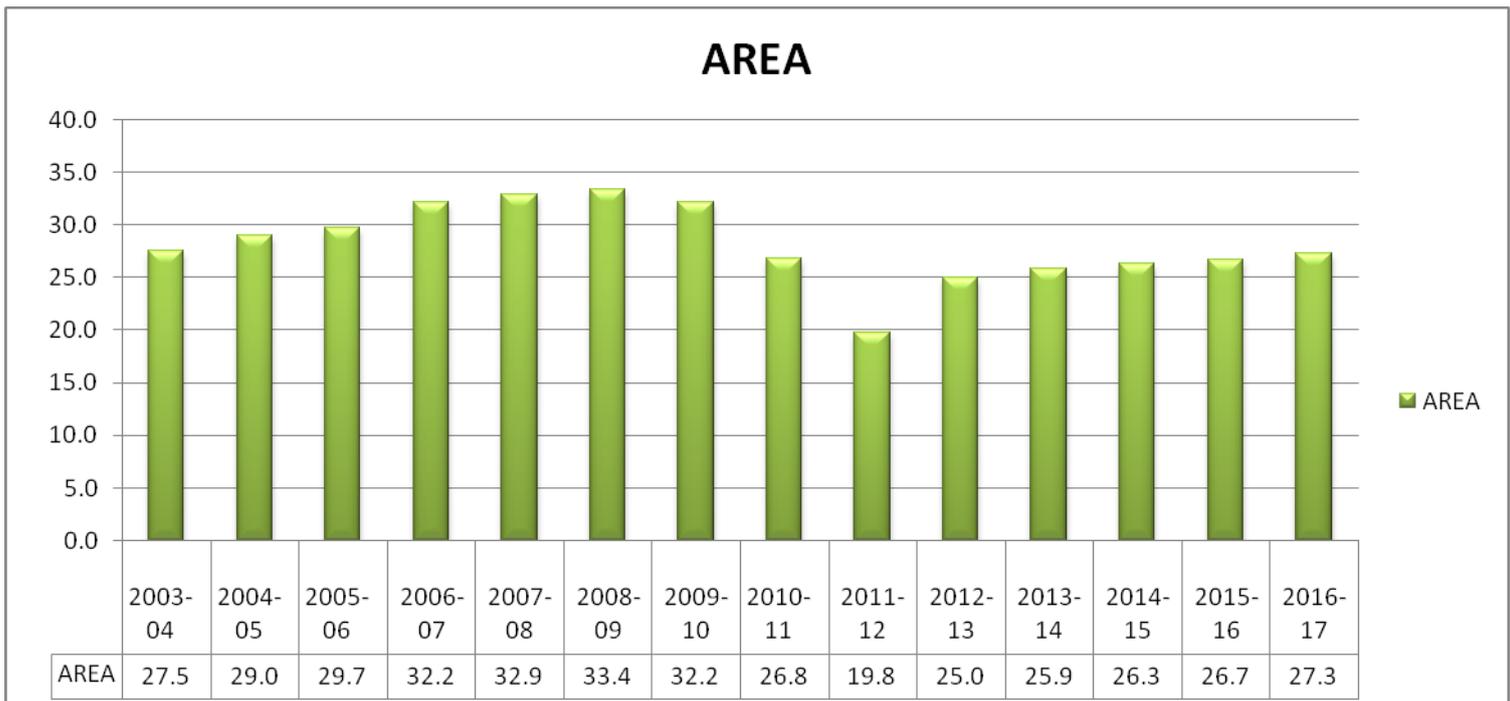
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## APPENDIX

### BANANA (PRODUCTION '000' TONNES) SINDH FROM 2003-04 TO 2016-17



### BANANA (Area '000' ) HECTARE SINDH FROM 2003-04 TO 2016-17



Collected Data from Direct Growers

## LIST OF GROWERS SURVEY

S:#	Name of Growers	Addresses		Cell no	Average disease incidence %
		District	Taluka		
1	Mr. Aijaz Bhatti	Thatta	Thatta	0321-7045983	3%
2	Mr. Ghulam Rasool	--do--	--do--	0321-3075405	2%
7	Mr. Muhammad Ashraf Arain	--do--	--do--	--	3%
8	Mr. Haji Muhammad Hassan Jakhro	--do--	--do--	0321-2158144	30%
15	Haji Muhammad Umer	Thatta	Ghora Bari	0321-6637113	0%
16	Mr. Abdul Ghani Lashari	Shaheed Banazirabad	Sakrand	--	3%
17	Mr. Shahnawaz Zanwer	--do--	--do--	--	3%
18	Mr. Ghulam Mustafa Zanwer	--do--	--do--	0304-3050500	2%
19	Mr. Muhammad Ishaque Kerio	--do--	--do--	0304-3801205	5%
20	Mr. Anwar Khaskheli Manager of Ghulam Qadir Chandio	Shaheed Banazirabad	Qazi Ahmed	0300-3224004	3%
21	Mr. Khadim Unnar	--do--	--do--	0300-3001181	3%
22	M.Alam	Mirpurkhas	Dagri	03062040727	0%
25	Shabir Rana	--do--	--do--	-----	0%
38	Yameen s/o Niaz Mohd	Mirpurkhas	Shuja Abad	03453700855	0%
39	Nawab Noor Ahmad Talpur	Mirpurkhas	Kot Ghullam Mohd	-----	0%

### Supervisor

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Contact no: 03063504786

## Primary data

### Publication

G. B. Sunil. Kumar. "Articulation of hepatitis B surface antigen in transgenic banana plants", *Planta*, 10/2005

Kondeti Subramanyam. "Very effective Agrobacterium-intervened change of banana cv. Rasthali (AAB) by means of sonication and vacuum invasion", *Plant Cell Reports*, 01/07/2011 *Plant Virus and Viroid Diseases in the Tropics*, 2014.

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