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Development of equipment suitable for low-cost micropropagation

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Abstract of project proposal

1.	**Title of the Project**	**:**	**Development of equipment suitable for low-cost micropropagation**
2.	**Principal Investigators and Co-Investigators**	**:**	**Dr. E.M.Muralidharan**
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I. INTRODUCTION

Micropropagation or the use of tissue culture techniques for clonal propagation of plants has been in use for a large number of plant species for more than half a century. It is the major means of commercial propagation in an increasing number of horticultural and ornamental crops since it has become more efficient or cheaper than the conventional methods. There are however several other plants in which tissue culture techniques have been reported in publications but in which a technology has never been standardised to enable commercial viability. It is clear that along with standardisation to scale up the lab scale technology there is a need to improve efficiency of the procedures to make it cost effective.

There is a tremendous potential for reduction in the capital costs of micropropagation by use of simpler and cheaper alternatives to equipment and infrastructure (Deberg, 1983; IAEA, 2002; Kodym and Zapata – Arias, 2001, Jain *et al*. 2005). Not only does it make the technology cost effective, but also puts it within the reach of small-scale entrepreneurs, hobbyists and educational institutions. Often equipment used is not designed specifically for tissue culture applications and therefore the scope for cost reduction and simplification exists. Such innovations have been attempted at the Department of Biotechnology in KFRI in the past and some of these adaptations have been used to carry out micropropagation (Muralidharan, 1995, 1998), this study is an effort to carry out further work in this direction.

In this context it is important to keep in mind that distributed all around the world is a community of tissue culture hobbyists - enthusiasts who carry out their culture in home laboratories with a minimum of facilities for micropropagation of a range of plants from insectivorous species to ornamental shrubs and trees. This they do by using the simplest of techniques and equipment but at a small scale. There is a lot to be learnt by examining their methods and evaluating the feasibility of the techniques for large scale micropropagation because it involves a top down approach of meeting the essential

requirements of sterile environment and control over the morphogenetic response but not necessarily conforming to high standards of precision and purity. Indeed in aspects such as use of plastic containers and innovative LED based lighting systems the lead comes from such hobbyists who are often specialists in other disciplines and without training in biology.

The present study looks at the modification of commonly used equipment such as laminar flow hoods, culture containers, distillation units, tissue culture racks etc. so as to make them more suitable for tissue culture applications.

Modified or redesigned equipment which can be used for micropropagation results in savings of capital and recurring costs. Some of the equipment can be considered for commercialisation. The cost saving result in reduced cost of production and therefore effectively brings a larger number of plants in the purview of commercial propagation especially in the small scale propagators.

Glass bottles and branded plastic tissue culture containers are used instead of borosilicate vessels in commercial tissue culture. Polypropylene and polycarbonate tissue culture vessels are commercially available in round, square and rectangular shapes. These are normally clear to partly translucent and are stackable to save space on culture shelves. Polycarbonate (PC) containers are clear and comparable to glass containers but are relatively more expensive and have shorter life when autoclaved repeatedly. Polypropylene vessels for plant tissue culture are cheaper but not available from Indian manufacturers. PP food grade containers are however commonly available for household use and for packing food products. These can be a cheaper alternative to commercial tissue culture containers although the rigidity of the material and design of closures leave much to be desired. A common drawback of most of the culture containers is the lack of good venting. Modifications in the design to permit better gas exchange would be desirable. Clear PP bags are yet another product commonly available in the market for packaging which can be autoclaved, is clear to permit light penetration and is amenable

to modification in size and shape with an electric heat sealer. These features make it a good choice for a simple and cheap alternative to culture containers.

To ensure consistent results and to avoid the risk of dissolved minerals interfering with the mineral composition of plant tissue culture media, double glass distilled water or ultra pure water is typically preferred. Distilling water is a energy intensive procedure entailing use of electricity and cooling water. Water quality being dependant on the locality and hardness of water being a common problem in many parts of the world it is of course necessary to purify water before it can be used for many laboratory procedures. Rain water is a good source of water that is adequate for media preparation but collection and proper storage of rain water in sufficient quantity requires suitable equipment which is not commonly available.

In localities where sunshine is available throughout the day and year round, the use of solar stills would be a good option for obtaining distilled water. Several simple solar still have been fabricated around the world for the purpose of producing potable water form sea water and ground water of uncertain quality. These could be adapted to produce pure water in sufficient quantities for a small tissue culture laboratory.

Light Emitting Diodes (LEDs) have the advantage over other light sources in being low in energy consumption, having long life and precise in the wavelength of light emitted. LED lamps are expensive but the prices are expected to fall as they being widely used. The latest generation of LEDs fall under the category of Superbright Surface Mounted LEDs which are ideal being small, lightweight, with a flat and low profile, resistant to shocks and highly durable. These are the type that is in common use in computers, mobile phones and in automobile lighting. They are also available as strips that can be installed in custom made configurations without much effort or skills other than soldering together the connections.

In the culture room LED have an additional advantage of generating little heat. The load on the air-conditioning is therefore reduced. It is also possible to precise control the

wavelength and the colour of the light provided to the cultures and thereby control the effect on morphogenesis and quality of the plants since the effect of different parts of the spectrum on the physiology of plant is well known. Commercial lighting options are yet to become widely available at reasonable cost but assembling with the components is an option that can save costs and permit configurations that suit the application.

It was with this background where a number of different options for cost reduction was possible that this study was undertaken to test modification of some of the equipment and devices used in plant tissue culture and combinations of different cost reduction measures for micropropagation.

II.MATERIALS AND METHODS

Modification /fabrication of equipment/devices were attempted in the following aspects:

i. Culture containers
ii. Water Distillation unit
iii. Lighting
iv. Tissue culture incubation rack

Cheaper, cost and energy efficient alternatives were used for effecting the modification but in such a manner that the bare minimum requirements of micropropagation are met and total costs kept low.

A. Culture containers:

As an alternative to borosilicate flasks, glass bottles and commercial plastic tissue culture containers, cheaper options were tested in this study.

1. Polypropylene (PP) food containers: PP food containers of various sizes were procured from the supermarket. Shoot cultures of teak and bamboo in solid and liquid media respectively were inoculated into these containers and growth compared to cultures maintained in standard glass bottles.
2. Clear polypropylene (PP) bags used commercially for packing grocery, snacks etc. were modified with a heat sealer to convenient sizes. For the smaller volumes the bags were modified to create a stand up pouch (Figure 1) of the type available in commerce for pickles and other preserved food. Commonly Low density Polyethylene (LDPE) is the material used for the purpose and PP pouches are not available.
3. Use of clear PP sheets as closures for bottles instead of the PP caps.

B. Water Distillation still:

1. A solar still of a simple design was tested for its suitability for use in the tissue culture laboratory as the source of water for media preparation.

   The still consists of a large plastic bucket water filled to half its capacity and with a measuring cylinder placed inside and weighted down to keep it in position when empty. An inverted conical chute of clear polypropylene film is placed with its tip positioned over the mouth of the measuring cylinder and the edges folded down over the mouth of the bucket and held in place with string to form an airtight cover. Some water is poured into the inverted cone to weigh it down and the setup is placed in bright sunlight. Water in the bucket is changed after a few days to prevent algal growth and to avoid salt buildup. The water collected in the measuring cylinder through evaporation and condensation on the surface of the plastic film cone is removed regularly.

2. A cooling and re-circulating system for conventional distillation units that saves on cooling water was fabricated as follows

   The cooling water line from the condenser of a quartz double distillation system was collected in a sump from which it was pumped and sprayed on the top of the clear polycarbonate roof of a tissue culture hardening chamber to achieve two purposes

   i. To lower the temperature of the chamber through evaporative cooling and
   ii. Bring down the temperature of the cooling water for recirculation to the distillation units.

   The overall efficiency of the distiller is expected to improve with the use of cooled water and a significant saving in the water used is achieved since otherwise it is allowed to go waste.

C. Lighting

Two approaches were taken to reduce the cost in lighting for maintenance of tissue cultures.

1. Different LED light sources were provided on culture room racks as alternative to the standard fluorescent tube light. Lamps consisting of a single row of superbright LED with reflector and fittings to replace T5 tube lights and LED strips with Surface mounted LED with no reflectors were used as light source for incubation of teak and bamboo shoot cultures. Evaluation of the efficacy was done by comparing growth under LED light with those under fluorescent tube lights acting as control.

2. Incubation of cultures in ambient conditions in an outdoor growth room made of double walled transparent and white polycarbonate sheets. Teak shoot cultures were placed in the chamber for three successive subcultures of 3-4 weeks each.

D. Tissue culture incubation rack:

To replace the conventional tissue culture growth room shelves which is typically a steel rack with 4-6 shelves of wire mesh, glass or metal sheet with a battery of fluorescent tube lights fixed on the underside of the shelves to illuminate the vessels kept below, two cost saving alternatives were tested.

1. Modified energy saving tissue culture rack.

   Culture room racks were fabricated with a modification from the conventional design. Instead of the usual horizontal alignment of four or more fluorescent lamps with or without fixtures at the bottom of each shelf to provide light to the shelf below, in the modified design the lamps were fixed vertically,(Figure 11). A pair of racks with 5 shelves each of 4 ft X 1.5 ft had a common railings in the middle on

which sockets were fixed permitting 6 lamps without any fixtures to be provided as common lighting source. Each shelf also had a single horizontally aligned lamp to provide illumination for the outer side. This arrangement therefore had 16 lamps instead of the 40 that the two conventional racks would require.

2. Turntable carousal tissue culture rack

A rotary culture rack was fabricated with four revolving carousels of 5 ft height consisting of four stacked circular (2 feet diameter) SS shelves (trays)fixed on a shaft at 1.5 ft intervals. The four carousels fixed on the frame with ball bearings and are connected to each other through pulley and belt and are driven by a 1 hp motor. A digital timer is used to operate the motor at variable intervals. Illumination is provided by vertically aligned fluorescent lamps placed in between the carousels. When in operation the culture bottles placed on the shelves on the rotating carousels are brought in proximity to the fluorescent lamps intermittently and for a duration that is determined by the intervals set on the timer. Thus a programme consisting of ON for 5 seconds and OFF for 30 minutes enables the carousel to turns by 45° every 30 minutes and complete one revolution in 2 hrs. All culture containers placed on the shelves are therefore exposed to constant light in this duration.

III.RESULTS AND DISCUSSION

A. Culture containers

Shoot cultures of teak and bamboo in solid and liquid media respectively were inoculated into disposable polypropylene (PP) food containers of various sizes were procured from the supermarket (Figure 1) and growth compared to cultures maintained in standard glass bottles. The main disadvantage noted for such containers was that because of the lower wall thickness, they were prone to deformation during autoclaving. If care was taken to ensure that vessels were not subject to much weight from above or pressure from the sides during the sterilization cycle, this problem could be avoided. Tightly closed lids were also to be avoided for the same reason. Although it carried a slight risk of increased contamination, the option of autoclaving the vessels and lids separately nested inside each other and wrapped with paper. Sterilized media is then poured into the vessels under the LAF bench. This option also saved a lot of space in the autoclave. If the vessels could be modified to incorporate a venting device the problem of deformation could be avoided besides improving the quality of the culture environment through enhanced gaseous exchange.

Figure 1. PP food containers with bamboo shoot cultures

Figure 2. Clear transparent closures made of PP sheets

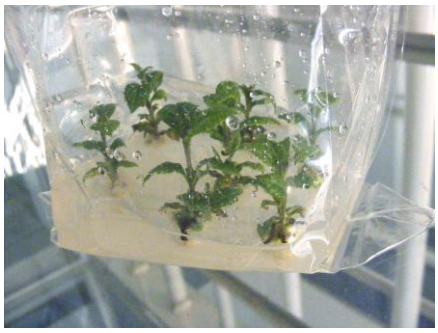

Figure 3. Teak shoot cultures in PP bags

Figure 4. Stand up pouches made from PP bags with heat sealer and with Bamboo shoot cultures

The PP sheet closure (Figure 2) for glass bottles were found to be a very inexpensive and convenient alternative to the rigid PP caps obtained commercially. Besides being clear and transparent they were easy to fabricate. Handling these closures was not as easy as the rigid caps since they were held in place with rubber bands. Cleaning of these closures were also rendered difficult especially if a double layer is used since cleaning and drying took longer. Disposing the closures after a single use was thus an option that would still be cheaper although undesirable because it contributes to generating plastic waste.

The use of clear polypropylene bags as culture containers is attractive for several reasons. Since PP bags are available in different thickness and sizes modification with a heat sealer give a measure of control over the size and shape of the containers that can be fabricated by the user according to the nature of the cultures. Cultures of teak and bamboo were successfully maintained on solid (Figure 3) and liquid media respectively in PP bags.

Stand up pouches (Figure 4.) have the advantage of being suitable for cultures that are typically carried out in bottles. These pouches the shape of which a familiar one in the form of the containers in which pickles and preserves are commonly marketed. Shoot cultures of teak and bamboo were successfully maintained in such vessels.

B. Distillation still

1. The simple water still was found to yield distilled water but efficiency of the process and the ease of operation was not satisfactory for routine use. Scaling up of the process with specially fabricated parts that could be assembled and disassembled easily is necessary for this still to find regular use in a laboratory. A design that incorporated a draining port at the bottom of the pure water still without having to dismantle the setup as well as a rigid transparent cone are some of the suggested modifications. There are however a number of designs for solar distillation stills which can be fabricated using different materials.

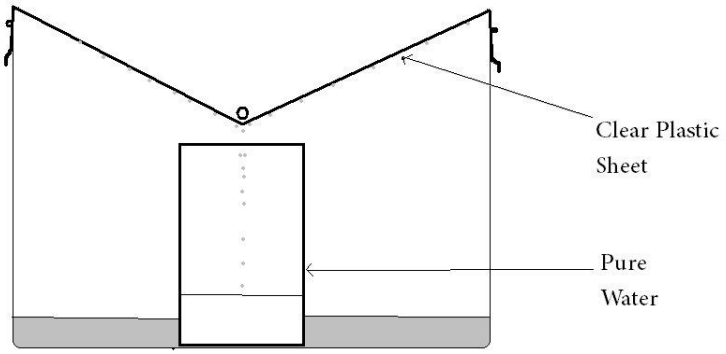

Figure 5. Schematic design of simple solar water still

2. The cooling water recirculation system for the conventional electrically operated laboratory distillation stills (quartz or borosilicate) was devised so that several such units could be assembled in a centralized unit and water from the condensers could be re-circulated for instead of being discarded into the drainage. This innovative system was devised to take advantage of the large surface area available in the form of the polycarbonate roof of the tissue culture hardening chamber. Not only does the system cool the chamber below ambient due to evaporative cooling of the discharge from the cooling condensers of the distillers but also re-circulates the cooled water back to the condensers.

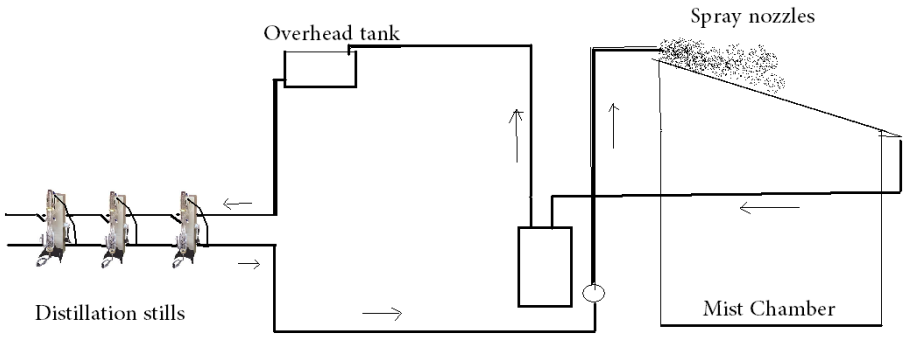

Figure 6. Schematic diagram of the cooling water recirculation system for distillation units

B. Lighting

Lighting contributes to a large share of the energy costs of the tissue culture laboratory. Besides the power consumed by the large number of lamps there is also the heating of the air that contributes to the load on the air-conditioning system. The use of LED lamp for displays and as indicators is common but their use for energy efficient lighting has only become popular in recent times. LED lamp assemblies for such purposes are still expensive and only mass production and use will bring down the costs. It was therefore interesting to examine the feasibility of using LED for lighting in the growth room to replace the conventional fluorescent tubelights.

Both the types of LED lamps were found to be suitable for use as the light source for tissue culture shelves and the cultures were observed to have no differences from cultures maintained under the fluorescent tubelights.

Although LED lamps are too expensive today to replace the tubelights the advantages of the new system in terms of long life and precise light output as well as the savings in energy are to be considered when planning for the future.

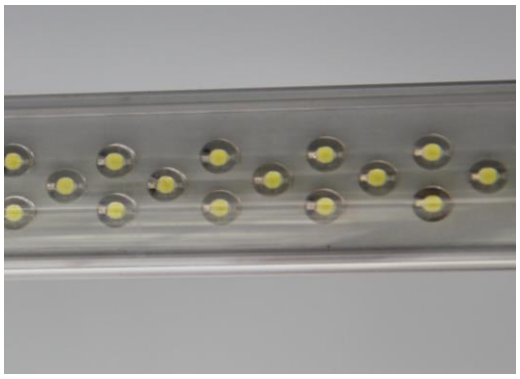

Figure 7. LED tubelight assembly that replaces fluorescent tubelights

Figure. 8 LED Striplight mounted on a PVC board.

Figure 9. : Outdoor culture room made of polycarbonate.

Figure: 10 Culture bottles maintained under outdoor conditions. Bottles are individually covered with a PP bag dust cover.

Growth room shelves:

As one of the options, culture room racks were fabricated with a modification from the conventional design. Instead of the usual horizontal alignment of four or more fluorescent lamps with or without fixtures at the bottom of each shelf to provide light to the shelf below, in the modified design the lamps were fixed vertically (Figure 11). The rack of 4 ft width had 6 lamps without any fixtures except for the sockets at both ends which were fixed on a common rail. The absence of fixtures especially the batten permits shadow free lighting and each lamp is utilized to the maximum without need for reflectors. Costs of the lamps as well as the energy costs are saved besides reducing the heating of the air since a smaller number of lamps are used in this design. There was not apparent effect of the orientation of the lamps on the cultures of teak and bamboo shoot cultures maintained for 3-4 weeks on either of the racks.

In a second option the turntable design reduced the lamps further and permitted the cultures to be intermittently exposed to light at regular intervals (Figures 12-14). The movement of the revolving turntable was however found to be jerky and the parking position of the trays kept shifting with successive revolutions. Use of a tacky mat prevented the culture bottles from shifting in position on the trays.

Rectifying this problem requires use of special stepper type motors which allows precise positioning, control of speed and obtain the high torque required for driving the heavy loads. Such motors are however expensive and only in the best quality motors can the jerky movement be avoided. In any case the total power consumption of the turntable mechanism including the lighting as well as that of the motor has to be lower than that of the stationary traditional rack where only the lamps are energy dependant. Since only the feasibility of the prototype design concept was tested in this study, no effort was made to source an efficient motor and gear system which is expected to be more expensive and was not available locally.

Future modification of the basic turntable design should focus on the efficiency of the turning system in terms of energy requirements and smooth and precise movements. Instead of the use of two sets of trays per lamp, the design could be made more efficient by having a quadruple configuration where a second row of turntables is aligned in in parallel so that lamps are in the centre of four sets of trays (Figure 15).

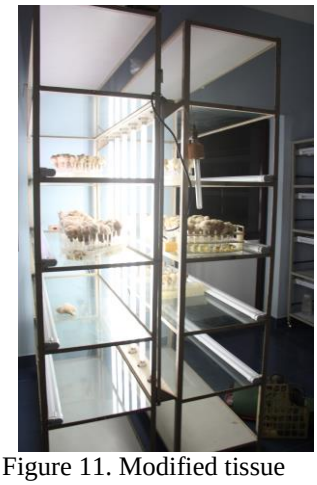

Figure 11. Modified tissue

Figure. 12: Turntable

Figure. 13 Another view of the

culture rack with vertically aligned lamps

tissue culture rack showing the 4 turntables with 4 trays and the vertically aligned lamps

rack with culture bottles

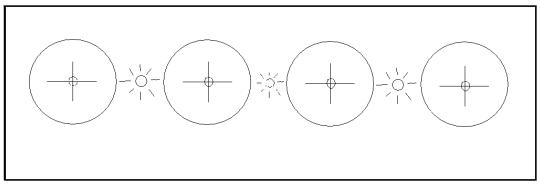

Figure 14 : Configuration of the turntable rack

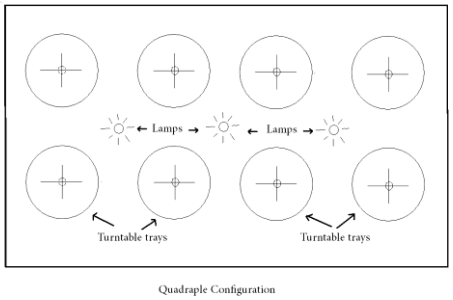

Figure 15 : Suggested improved configuration of turntable

IV. CONCLUSIONS

The low cost options tested and found successful in this study points to the possibilities of bringing down the cost of equipment used for routine tissue culture procedures. Not only are these options possible with simple and inexpensive fabrication but also found to be energy saving and thereby contributing to the reduction in the cost of production of plantlets. It would therefore contribute to making micropropagation more accessible to public who will be encouraged to take up tissue culture as a hobby or small scale commercial ventures. Small laboratories such as in educational institutions can also adopt some of these findings and save on costs.

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