Simple, efficient and problem solving techniques developed at SRC

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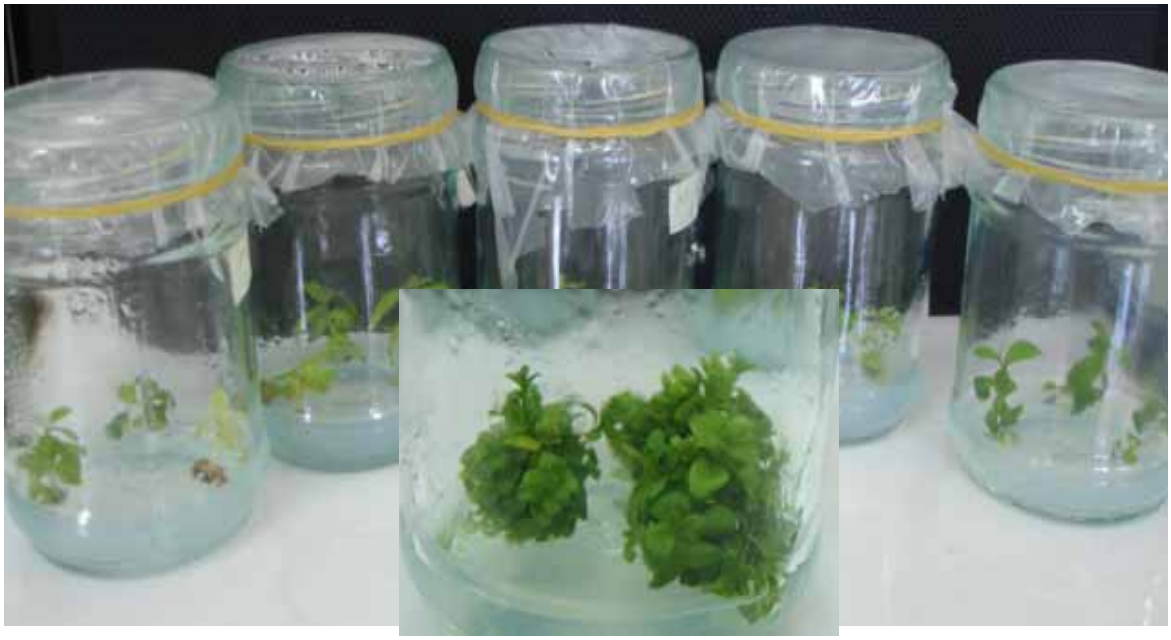
Plant tissue culture vessels and closures represent one of the important tools in plant tissue culture technology. Most of the work that has been conducted in this field utilized 150 × 25 mm culture tubes capped with polyprolidene closures with appropriate size. Such tubes and caps are not always available for researchers. Usually 25ml of nutrient medium is used to culture a single explants, and a large incubation space is required to house the tubes in commercial tissue culture laboratories, in addition to the need for stainless steel racks to hold the tubes during media preparation and culturing, as well as slanted plastic racks in the culture room to allow sufficient illumination for culture tubes.

To reduce the cost and solving the problem of non availability of some of these items which has to be imported, alternative and efficient techniques have been developed at SRC laboratories.

This report documents some of the techniques and substitute's items tested and developed with their results.

In the SRC plant tissue culture laboratory, we tried to use locally available 250 ml glass jars covered with 10×10 cm polyethylene plastic covers and fixed with rubber band. This system (the jar and plastic cover) was routinely used in our laboratory during the past three years in tissue and callus culture of various plant species belonging to different families and genera such as apple, pear, potato, carnation, grapes, tobacco, roses, nigella and ferns, utilizing liquid or agar solidified media.



The polyethylene nylon covers showed resistance to autoclaving conditions (i.e. 121^0C at 1.04 kg /Cm^2 for 15-20 minutes), and no contamination was observed over the 5% allowed range. Furthermore, no problems were encountered regarding the glass quality of the local jars, being non-pyrogen-free. This system, jar and nylon covers, allowed suitable gas exchange and illumination intensity and quality suitable for callus and tissue culture during the different stages of initiation, multiplication and rooting.

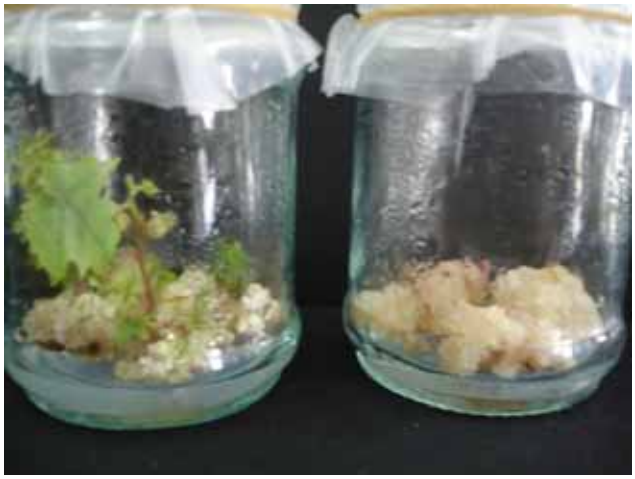
The **advantages** of this system are many folds, and some are listed bellow:

1. A total of 25 ml of the nutrient media is used to feed explants in the jar instead of one explants/tube

2. Less incubation area is needed for the incubation of the same number of plants. A total of 400 jars containing 5 plants (i.e. 2000 plants) can be seated in $1m^2$ directly on the salves in the incubation room, as compared to 400 tubes containing 1 plant in each, (i.e. 400 plants can be seated in the same of slanted racks.

3. Accordingly, a $40m^2$ of shelves(a common size 6×6 m incubation room designed in 4 stories shelves) can accommodate 16000 jars, or 16000 tubes. Therefore, one incubation room can be used for the production of 80,000 plants using jars as the culture vessels, while 5 incubation rooms are required for the production of 80,000 plants using culture tubes as culture vessels.

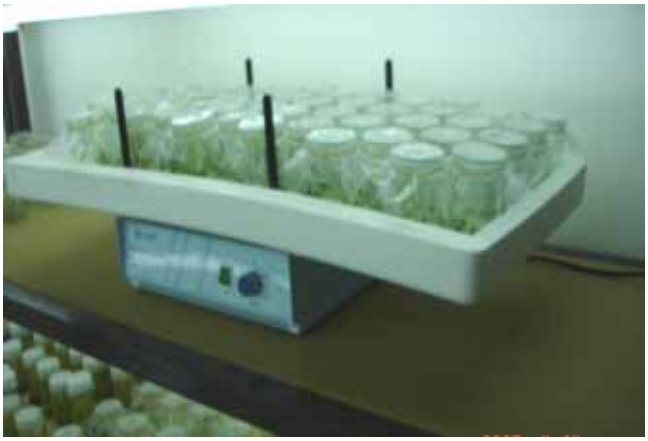
4. This implies that the preparation of 5 incubation rooms will add extra cost of production, since the incubation rooms requires air conditioners, fluorescent lamps, and shelves, in additions to construction and building costs, and electricity.



Cost comparison between various culture vessels and closures for the production of 10,000 plants is given below:

1- One x 3m of agricultural nylon covers (medium Gigue) made of polyethylene is sufficient to provide 300 cover measuring 10×10 cm each.
   The cost of the 1×3m nylon = 2000 ID (Iraqi Dinars)
   Thus the cost = 2000/300 = 6.66 (or 7 ID for short) /cover
2- Rubber band cost = 750 ID/250 rubber band/ bag
   = 3 ID
   Cost of cover + band = 10 ID
   For 10,000 plants = 10 ID× 2000 jars (since each jar will be used to culture 5 plants)
   =20,000 ID or 16 USD

Item	Price / Unit	Price / 10,000 plants	Price (USD)
1. Nylon covers + rubber band	7+3 = 10 ID	20,000 ID	16
2. Cover PVP, imported for 5 cm φ jars	1 USD	2,000	2,000
3. Cover PVP, local made for 5 cm φ jars	57 ID	154,000 ID	1,696
4. Jars, 250 ml mason jars, local market	1000 ID	2,000,000 ID	12,000
5. Jars, imported from Germany 250ml	6 USD	12,000	1,600
6. Jars , local manufacture 250ml	920 ID	1,840,000 ID	1,600



Test tubes as cultured vessels

1. Glass tube, local manufactory 150 x 24 mm	1000 ID	10,000,000 ID	8,400
2. Imported tubes (Pyrex) local market	3000 ID	30,000,000 ID	25,200
3. Nylon covers 5×5 cm	3.5 ID +3 ID rubber band= 6.5	65,000 ID	50
4. PVP caps for 150×4mm tube local manufactory	250 ID	2,500,000 ID	2,000
5. PVP kaput for 150× 24 mm tubes	10 cents	10,000 cents	1,000

The above comparison clearly indicates that the use of 250 ml mason jars (imported for the local market) and covered with a nylon covers costs a total of 8,480 USD/ 5 for the production of 10,000 plants (since 5 plants will be produced in each jar) which is equal to **1,696 USD** as compared to **8,450 USD** to produce the same number of plants if culture tubes are used. This means that the production of plants through tissue culture using jars covered with nylon covers is more than 4 times cheaper than using culture tubes. Furthermore, one may use the available glassware for tissue culture without having to depend on costly imported material.