

RITA[®]

Temporary Immersion System for Plant Tissue Culture



Main advantages of the Rita[®] technique

- ✓ Reduction in labor costs through simplified handling of plants and medium.
- ✓ Improved nutrition: the medium is in direct contact with the plants during immersion and a capillary film covers the plant throughout the remaining period.
- ✓ Very marked reduction in asphyxiation and tissue vitrification compared with permanent immersion.
- ✓ Complete renewal of the atmosphere at each immersion.
- ✓ Tissue division occurs during agitation due to bubbling.
- ✓ Control of the morphological process through modification of the frequency and duration of immersion.
- ✓ Protection of each apparatus guaranteed by air vents. Individual handling is possible. No risk of spread of contamination.

Achievements on some tropical crops

Improvements in comparison with culture on a semi-solid medium

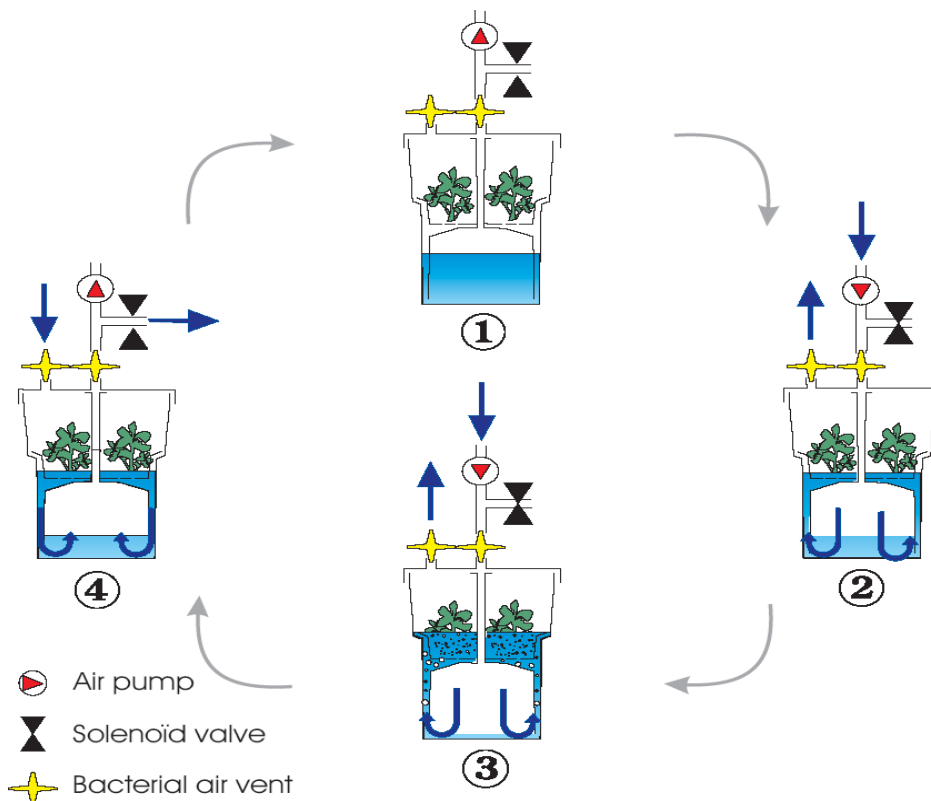
- ✓ Coffee *Microcuttings : the same multiplication rate in half the time.*

Embryogenesis : the cost of the procedure from embryogenic cell to germinated embryo is reduced by a factor of 10.
- ✓ Banana *Development and germination of somatic embryos : Each Rita® can produce 700 to 1000 plantlets ready to harden.*

Meristem proliferation : the 20-day proliferation rate can be multiplied by 2.5
- ✓ Rubber tree *Development of somatic embryos : 100 to 400 embryos per Rita® apparatus ready to germinate on a semi solid medium.*
- ✓ Potato *Microtuberisation : Production of 3 microtubers from a single node microcutting in 10 weeks. More than 50% of the microtubers weigh more than 0.5g.*
- Others *The RITA is also used for the multiplication of other plant species: Hemp, ornamental plants such as the Dragon tree, the Bamboo, succulent plants or market garden species such as Strawberries.*

How RITA[®] works?

RITA is an apparatus specially designed to carry out in-vitro plant culture using temporary immersion. It is made up of reusable and autoclavable components.



Temporary Immersion

Depending on their size, the plants are placed in the basket or on the netting and the nutrient medium is placed on the bottom of the container. Compressed air enters through the central tube creating over pressure on the liquid medium inside the bell. This pushes the liquid into the ring-shaped chamber formed by the vessel and the bell. The liquid passes through the holes in the basket and the meshes of the netting and immerses the plants. As soon as all the liquid medium reaches the upper compartment it is continuously agitated and oxygenated by the air flow. Over pressure escapes through the outlet at the edge of the cap. When the supply

of compressed air stops the pressure in the upper and lower compartments adjusts to atmospheric pressure via the outlets and the solenoid valve. The liquid medium returns to the bottom of the vessel.

Pressure application and air flow control

Compressed air must be oilless. It may be applied directly by an air pump or from an air tank. The over pressure must be lower than 0.2 bar. Air flow is sterilized through hydrophobic air vents on the inlet and the outlet ports. These air vents have an air flow rate that is as high as possible at atmospheric pressure to allow rapid readjustment of pressure in the two compartments. The air flow through each vessel must be controlled (0,8 to 1 l/mn) by a valve in order to reduce the rate of bubbling when plants are immersed. A jet should be used which allows control irrespective of whether the tubing is connected to a vessel or not.

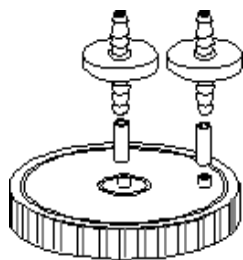
An air pump can operate several vessels by means of a manifold. The number of branches the manifold can have will depend on the power of the pump. Several manifolds can be connected to the same pump and may be individually programmed.

Programming

The frequency and duration of immersion periods vary greatly with the species and with required phenomena. Previous experimentations have shown these may range from 4 periods of 15 minutes per day to 1 period of 1 minute per week. Preliminary experimentation should be carried out to find optimum frequency and

duration in each particular case. Start and stop functions of the pump can be controlled by any programmable timer which allows the required number of on and off switchings per day or week and the required duration (resolution 1 or 15 mn).

Components and assembly plan of RITA®



R10 Air vent

R16 Vent tubings

R03 Cap



R13 Cap O Ring



R14 Central Tube O Ring



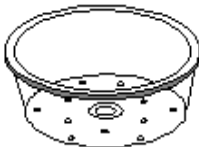
R04 Central Tube



R0702 Netting



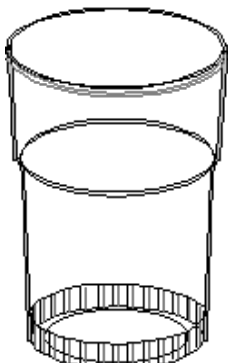
R15 Washer



R05 Basket



R06 Bell



R02 Vessel

Required Equipment



■ **Air supply:** air pump or compressor capable of supplying 1 liter/min (0.06m³/h) per Rita[®] at 200 mbar (hPa). Sterilisation by an air vent before each Rita[®].

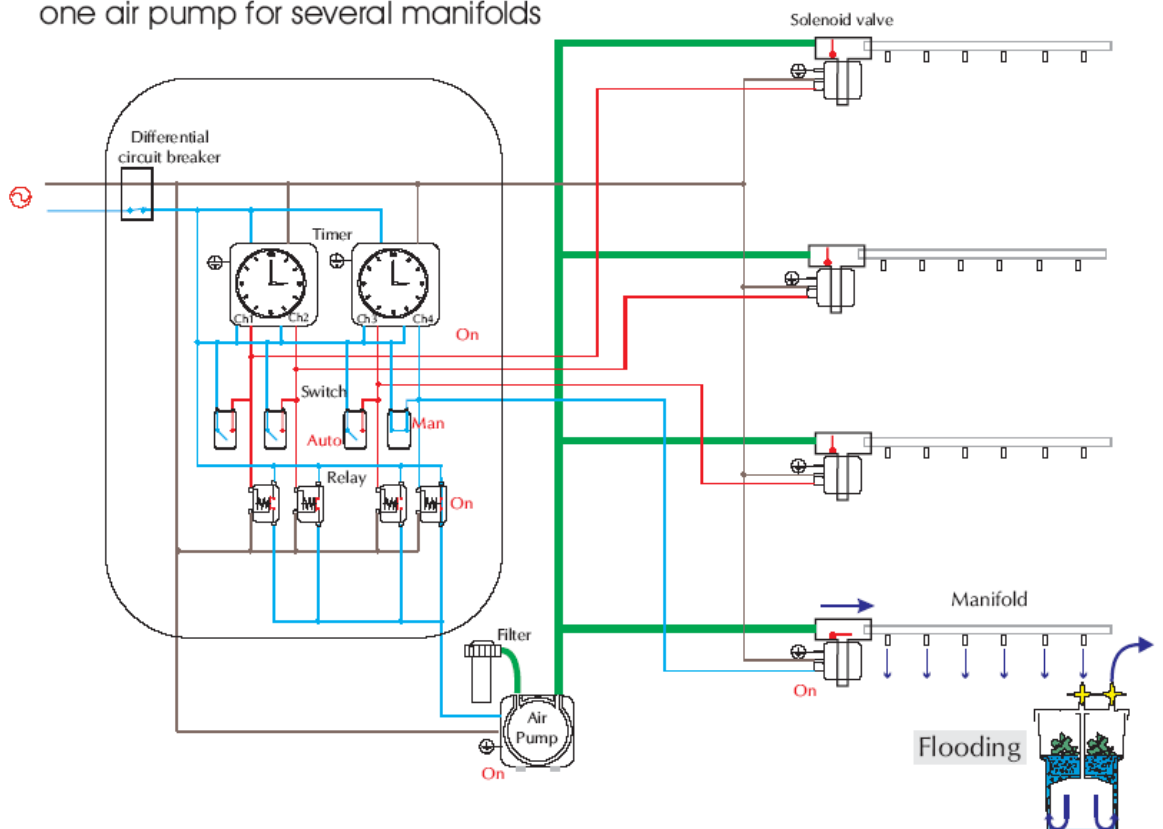
■ **Automation:** a timer with minimum of 1min/day, a manifold (includes a 3-way solenoid valve, nozzles and tubes).

We provide RITAs and manifold (for 6 or 20 RITAs) for 110V or 220 V.



Please contact us for industrial installation.

Electric and pneumatic installation :
 one air pump for several manifolds



RITA[®] handling

Cleaning

- For satisfactory cleaning the entire apparatus should be completely taken apart. Cleaning with detergent containing aromatic rings may induce cracking of the polysulfone during autoclaving. If you use polyurethane foam discs (no longer on sale), they are one use spare parts.

Sterilization

- Set up the RITA with its air vents (see assembly plan). The cap must not be closed too tightly before autoclaving to prevent any deformation.
- Add a few millilitre of water inside the RITA depending on the type of autoclave you use in order to ensure good pressure level.
- Protect the air vents against humidity by aluminium foils. Mark them at each autoclave cycle. We advice a maximum of 10 autoclave cycles.
- Autoclave following standard conditions (we cannot be more precise as conditions are dependant on the type of autoclave).
- Sterilize the medium separately (heat or filtration) and pour in the sterile RITA under laminar flow (200 ml per RITA at max.).

Medium change and plant transfer

Each apparatus together with its air vents can easily be disconnected from the compressed air network and carried to a laminar air flow cabinet for plant handling. Disinfecte the RITA thoroughly (insist on the margins of the cap).

The medium can be changed very simply in different ways without having to handle each plant separately:

- a new sterile vessel is prepared without its inside components (bell, basket, foam and central tube) and filled with new medium. The inside part of the previous vessel containing the plants is then placed inside the new vessel.