Aim of the experiment: To prepare sterilized MS media for the plant tissue culture and inoculation of explants to the media.

Principle:

Plant tissue culture is a technique used to maintain or grow plant cells, tissue or organs under sterile condition on a nutrient culture medium. Plant tissue culture is widely used to produce clones of an economically important plant in a method known as micro propagation. Different techniques in plants tissue culture may often certain advantages over traditional methods of propagation which are as follows-

- To produce large number of genetically uniform plants in a comparatively shorter period of time.
- For production of disease free plant material with the possibility of eliminating viral, bacterial and fungal contamination.
- To make clones of species which are very slow growing and very difficult to propagate by any other means.
- Requires small space for production and maintenance.
- To store germplasms for long-term basis
- To operate round the year i.e. it is independent of seasons.

Requirements:

1) Distilled water
2) MS major stock solution (10 mL)
3) Minor stock solution (1mL)
4) Iron (1mL)
5) Vitamin (1mL)
6) Myoenositol (0.02 g)
7) Sucrose (6 g)
8) Hormones(BAP=1.76 g and NAA=470 g)
9) Agar (1.6g)
10) 1N NaOH and 1N HCl

Procedure:

i) 100 mL distilled water is taken in a beaker.
ii) Major salts, minor salts, iron and vitamins are added to the distilled water and dissolved with the help of magnetic stirrer.
iii) After dissolving the compounds, plant growth regulator BAP (6-Benzylaminopurine)= 2µg/L and NAA (1-Naphthalene acetic acid)= 0.5µg/L are added.

iv) After properly mixed, the pH of the media is adjusted to 5.8 with the help of pH meter by adding 1N HCl or 1N NaOH.
v) Then the final volume of the media is maintained at 200 mL by adding distilled water.
vi) Finally, the MS media is now prepared and autoclaved at 121◦C at 15 lbs pressure.

Surface sterilization:

i) The explants leaves (rauvolfia tetraphylla) are taken for culture.
ii) They are then sterilized with 0.1% carbendizim fungicide for 5 minutes and rinsed with distilled water for 3 times.
iii) After then they are surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 4-5 minutes under laminar air flow (LAF) chamber and finally rinsed with autoclaved distilled water for 3 times.
iv) Finally, the explants are soaked with sterile tissue paper.

Inoculation:

i) 10 culture tubes are taken and marked the date of inoculation and the name of hormones which are added in the media as BAP/NAA.
ii) The prepared media are poured into each culture tubes in equal amount and left for solidify.
iii) The sterilized explants are cut into several pieces by sterilized blade before further treatment.
iv) After solidification, the pieces of explants are inoculated to each test tube and plugged with cotton plug.
v) All the above process is done in LAF chamber.
vi) Now, culture test tubes are kept in culture rack at 3000 lux light intensity, 60-70%RH and 25± 2◦C temperature.

Observation:

Callus is initiated in 5-7 days from the cut end portion of the explants. After 15 days, they are subculture in the same media with plant growth regulators. On repeated subculture in 15 days interval, the callus formation is takes place in entire explants forming a large mass of cells.