19.2 MATERIALS AND METHODS

Methanol, acetone, hexane, chloroform and ethyl acetate, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), TPTZ (2,4,6-tripyridyl-s-triazine), gallic acid, quercetin, ferrous sulphate and analytical grade chemicals supplied by Hi-Media, Merck and Sigma Chemicals were used.

19.2.1 Plant Collection and Surface Sterilization of Explants

The wild plant seeds of *S. grandiflora* were collected from Cuddalore, Tamil Nadu, India. Seeds were surface sterilized to produce contamination-free plants that can be maintained under aseptic conditions. Seeds were checked for viability and washed thoroughly under running tap water (15 min) without damage to the tissues. In order to avoid the interaction of microbes such as bacteria and fungi in plant tissue culture, seeds were then transferred to a beaker containing sodium hypochlorite (3%) with rapid shaking for 15–30 min and changing the solution at 10 min intervals. Then, the seeds were washed with distilled water and treated with 0.3% mercuric chloride for 2–5 min. The surface sterilized seeds were washed with sterile distilled water five times. The excess water on the seeds was removed by using sterile tissue paper before culture. The seeds were transferred to a semi-solid medium under aseptic conditions in a laminar flow chamber (Arjun 2011).

19.2.2 Preparation of Tissue Culture Media

MS (Murashige and Skoog 1962) Media used six individual stock solutions of macro, micro, minor, iron and vitamins, which were prepared and stored. The iron stock was stored in a black bottle to prevent photolysis of chemicals. All the stock solutions were stored in refrigerator and used within one year. Meso-inositol, cytokinin and auxin stock solutions were freshly prepared and used for a month. For preparation of medium, all the six stock solutions were mixed thoroughly with required amounts of sterile distilled water. Sucrose (3%, 30 g/L), 0.1% meso-inositol (100 mg/L) and the required amount of plant growth hormones were added to the medium and buffered by 1N HCl or 1N NaOH to adjust the medium pH to 5.6–5.8 before autoclaving. The medium was solidified by adding agar (0.8%, 8 g/L). Sterile distilled water was used to make the final volume. The medium was poured into culture vials and autoclaved at a pressure of 15 lbs for 15 min at 121°C. A photoperiod (16/8 h) light/dark condition (25 ± 2°C) under a cool white light (2000 lux) fluorescent tube was provided.

19.2.3 Callus Induction

Surface sterilized leaf explants were cut into small pieces (0.5–1.0 cm) barring the cut ends and transferred to MS basal medium. Three different auxins [(indole-3-acetic acid (IAA), α-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D)] cytokinins and 6-benzylaminopurine (BAP) at the same concentration (0.5 mg/L) were used in the callus induction. Leaf explants cultured on the MS