Abstract
Emblica officinalis commonly known as Indian gooseberry or amla is a moderate sized deciduous tree, belonging to Phyllanthaceae. Due to its exceptional ethnobotanical and ethnopharmaceutical use there is a great need to grow this plant in vitro for genetic development and continuous supply as these plants are seasonal. An efficient in vitro plant regeneration protocol was developed using nodal explant on M S media with different concentration of shooting and rooting hormones. Maximum multiplication of shoots was achieved on Murashige and Skoog’s medium supplemented with BAP (0.5 mg/l). The shoots were separated from cluster and subcultured for their elongation on the same medium. In vitro rooting was obtained on half strength MS medium supplemented with IBA (0.5 mg/l). Regenerated plants were successfully hardened and acclimatized.

Introduction
Micropropagation has been successfully employed for rapid production of uniform and superior quality planting material. Rapid and large scale clonal propagation of many fruit crops is now possible tissue culture. The regeneration can take place as extension or proliferation of shoot tip or axillary buds, direct production of organs from explant i.e. organogenesis or through the production of somatic embryos i.e. embryogenesis.

Phyllanthus Emblica (syn. Emblica officinalis), the Indian gooseberry, or amla from Sanskrit is a deciduous tree of the family Phyllanthaceae. The species is widely distributed in India and also grows in tropical and subtropical regions including Pakistan, Uzbekistan, Sri Lanka, South East Asia, China and Malaysia. The fruits of amla are widely used in the Aryuveda and are believed to increase defense against diseases. It has its beneficial role in treatment of cancer, diabetes, liver treatment, heart trouble, ulcer, anemia and various other diseases. Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective. The fruit is a very rich source of vitamin C. Its mineral and vitamin contents include calcium, phosphorous, iron, carotene, thiamine, riboflavin, and niacin [7]. The seeds of the Indian gooseberry contains a fixed oil, phosphatides, and an essential oil. The fruits, bark, and the leaves of this tree are rich in tannin. Amla is propagated sexually through seeds and vegetatively through grafting or budding [3]. Due to its high pharmacological value, crop improvement through genetic engineering and mass production of improved genotypes of this species require in vitro plant regeneration with the help of tissue culture techniques. In recent experiments aseptic cultures were established using various sterilizing agent and in vitro shooting and rooting were developed using nodal explant in M S media.

Objective of the Research
1) This practical is based on micro propagation studies of Emblica officinalis.
2) The study was focussed on in vitro shoot elongation and rooting in culture media.

Materials and Method
Plant Material
Nodal segment from a healthy mother plant were used as explant.

Sterilization
The picked nodal segments were kept under running water for about 15-20 minutes to remove soil particles and then rinsed with liquid detergent (Tween 20) for 5-10 minutes. Explants were then surface sterilized with fungicide
Bavistin (0.5-1.0%, w/v) for 20 minutes. Explants were then treated with antioxidants (ascorbic acid, PVP, and citric acid) for 15-20 min to minimise the release of phenolic compounds from explants.

Prior to inoculation, these explants were subsequently surface sterilized in the laminar air flow chamber with 70% ethanol, 0.1% mercuric chloride (HgCl2; w/v) and sodium hypochlorite solution (4-5%) followed by repeated rinsing with sterile autoclaved water [5,6].

**Culture media**
The surfaced sterilized explants were then aseptically inoculated on sterile MS medium (Murashige and Skoog, 1962) comprising 3% sucrose as carbon source and 0.8% agar as solidifying agent. The medium was also supplemented with various growth regulators [auxins- IAA (indole-3-acetic acid), IBA (indole-3 butyric acid), NAA, 2, 4-D (2, 4-dichlorophenoxyacetic acid) etc. and cytokinins- BAP(6-benzylaminopurine) [1], and pH of the medium was adjusted to 5.8 before addition of agar and sterilized by autoclaving at 1.08 kg/cm² pressure at 121°C for 16 minutes.

**Culture conditions**
The cultures were incubated at 25±2°C under cool, white and fluorescent light of 2000-2500 lux and relative humidity of about 55±5%. 16/8 hours photo and dark period were maintained in growth chamber, respectively [4]. The explants were regularly transferred into fresh media to check the browning of explants. Data on multiple shoot induction; elongation and rooting were taken and statistically analyzed. Observations were recorded periodically.

**Induction of Multiple shoots**
The aseptic nodal segments were cultured on MS medium supplemented with 0.75, 1.0, 1.25, 1.50, 1.75, 2.0 mg/l of BAP and Kn alone and/or in combination. Physiological conditions and number of shoots per explants were observed periodically. Maximum multiplication of shoots was noticed on MS medium congealed with BAP (0.5 mg/l) after 3-4 weeks of inoculation. In vitro elongation was also attained on the same medium.

**Establishment of Root cultures, Hardening and Acclimatization**
In vitro elongated shoots (6-7 cm.) with at least 3-4 nodes were taken out from the culture vessel and transferred to half strength MS medium with different concentration (0.5-8.0 mg/l) of auxins like IBA, IAA, 2,4-D, NAA for root induction [2]. Cleaned rooted plantlets were transferred to sterile soil mix prepared by mixing sand, peat moss and humus in a 1:1:1 ratio with 0.5 g/l Bavistin solution and subjected to photoautotrophic culture system for 20 days prior to the greenhouse transfer. After transferring to the temperature and humidity controlled greenhouse, plants were hardened for a month time. Subsequently, well-established plants were shifted to shaded nursery beds.

**Results and Discussion:**
Nodal segments proved to as good explants as it remains fresh even after the treatment antioxidants and HgCl2 as shown in following Fig 1.0

**Shoot Regeneration**
In Fig 2.0 it is shown that 3-4 multiple shoots from nodal explants in amla was obtained, when MS media was supplemented with BAP (5 mg/l) and IAA (0.5 mg/l) along with antioxidants. As shoot proliferation depends on concentration of auxin and cytokinin, axillary bud growth was also observed as shown in the Fig. 3.0. When MS media was supplemented with kinetin and IAA.
Shoot initiation

To enhance shoot regenerative potential, nodal explants were cultured on MS media supplemented with different cytokinin treatment. BAP has been reported as the most active and cheapest among cytokinins. When explants were reared on MS medium supplemented with BAP the rate of shoot development and size of shoot were increased. The shoot development was initiated within 1 week as shown in fig 4.0.

Establishment of Root Cultures

In vitro elongated shoots (6-7 cm.) with at least 3-4 nodes were taken out from the culture vials and transferred to half strength MS medium with different concentration (0.5-8.0 mg/l) of auxins like IBA, IAA, 2,4-D, NAA for root induction as shown in Fig 5.0. The plantlets were taken out from culture vessel without damaging the delicate root system and rinsed with distilled water to remove adhering agar and then transferred to polycups containing vermicompost and autoclaved soil.
During experiment there were two problems with nodal explant first the inherent contamination in the explant and second phenolic leaching. Most shoot cultures showed higher fungal infection with low bacterial contamination. Fungicide, Bavistin showing toxicity toward Ascomycetes and Basidiomycetes checked the fungal contamination upto 80%. Surface sterilization of explant with 1.0% HgCl₂ after treating with Bavistin was very effective in controlling contamination with good survival rate. However, when the concentration of HgCl₂ was increased explant turned brown and ultimately died. Use of antioxidants (PVP) and incubation of culture in dark was also beneficial for the removal of browning of explant medium. Shoot regeneration was enhanced by culturing the explant on M S media supplemented different cytokinins. BAP is reported to be the most active cytokinin. Maximum number of shoots (15.275±0.96) was obtained when nodal explants inoculated on MS medium supplemented with BAP (0.5 mg/l).

In vitro root induction was obtained when the elongated shoots were transferred on half strength of MS medium fortified with IBA (0.5 mg/l). IBA is more important plant growth hormone for the root induction in tissue culture. In vitro raised plantlets were taken out from the culture bottle and hardening and acclimatization was done.

Discussion:
Research in medicinal plants has gained a renewed focus recently. The prime reason is that other system of medicine although effective come with a number of side effects that often lead to serious complications. Plant based system of medicine being natural does not pose this serious problems. Though Emblica officinalis has various medicinal applications, but it is the need of hour to explore its medicinal values at molecular level with help of various biotechnological tools and techniques. Somatic embryogenesis plantlet regeneration is successful in many plant species. In amla, successful micropropagation method via organogenesis is reported (8). However, there is no repeatable protocol available for somatic embryogenesis method of plant regeneration on large-scale plant production (2).

There are several reports on in vitro shooting of E. Officinalis by using different explants such as hypocotyls, cotyledon, seed endosperm and adventitious bud but the best result was seen when nodal segment was used as explants on MS media supplemented with BAP (5 mg/l) and IAA (0.5 mg/l) along with antioxidants (PVP 700 mg/l).

In vitro root induction was obtained when the elongated shoots were transferred on half strength of MS medium fortified with IBA (0.5 mg/l). IBA is more important plant growth hormone for the root induction in tissue culture. In vitro raised plantlets were taken out from the culture bottle and hardening and acclimatization was done.

References


