Histological and biochemical changes in Aegle marmelos

Rajesh Pati

Available at: https://works.bepress.com/rajesh_pati/2/
Histological and biochemical changes in *Aegle marmelos* Corr. before and after acclimatization

Rajesh Pati, PhD

Central institute for subtropical Horticulture, Lucknow, India

e-mail: rajeshpati777@gmail.com
Introduction

- *Aegle marmelos* Corr., belongs to family Rutaceae, is more prized for its pharmacological virtues than its edible quality.
- Because of pharmacological importance, it’s become potential candidate for developing transgenics to enhance its medicinal properties.
- Maximum mortality of micropropagated plants occur during acclimatization phase because plantlets undergo rapid and extreme changes in physiological functioning, histological and biochemical changes.
- In order to investigate the actual reason of this limitation, test samples were collected at different stages of micropropagation of *Aegle marmelos* Corr. (*In vitro* stage, acclimation stage, and field established plants).

E-mail: rajeshpati777@gmail.com
Materials and Methods

• *Aegle mormelos* Corr. plants has been developed from mature tree using protocol standardized by Pati et al., 2008.

Anatomical studies

• Histological studies, 0.5 cm² leaf, stem and root samples of were collected from various cultural stages (8 weeks old *in vitro* and 16 weeks old acclimatized plants)

Fixation:

All the sample were dipped in Formaldehyde : Acetic Acid : Alcohol (5 ml : 5 ml : 90 ml).

Dehydration:

• Dehydrated through the series of t-butyl alcohol in ascending order (30% to 100%).

e-mail: rajeshpati777@gmail.com
Infiltration:

- The samples were passed through the graduated ethanol:xylene mixtures (100% t-butyl alcohol, 3:1, 1:1, 1:3, and 100% xylene) using automatic tissue processor electra (Yarco-YSI 104).
- Samples were embedded in molten paraffin wax (melting point 56°C) for 8 hrs in order to completely replace the xylene with paraffin wax.

Sectioning and staining:

- 15 µm thin sections were cut using microtome (Microm, HM 350S) and stained in 0.1% aqueous toluidine blue O.

Observation:

- The slides were observed under stereoscopic microscope (Leica DFC 320, Japan).
Biochemical Studies

• The chlorophyll (a, b and total) was estimated as per the method described by Arnon (1949).

• Nitrate reductase activity was estimated as per the method described Srivastava (1975).

• Total soluble protein was estimated as per the method described by Lowery et al. (1951).

• The reducing sugar was estimated as per the method described by Ranganna (1986).
Steps in micropropagation of *A. marmelos*

e-mail: rajeshpati777@gmail.com

Pati et al, 2008
(A) Whole mount of *in vitro* leaf showed open type stomata with fully turgid guard cells, (B) whole mount of acclimatized leaf showed partially closed stomata.
(A) T.S. of *in vitro* leaf, showed single layered epidermis with almost no cuticle and single layered and poorly developed palisade parenchyma with poorly developed vascular tissue.

(B) T.S. of *acclimatized* leaf, showed single layered epidermis with thick cuticle and well developed double layered palisade mesophyll while the lower side had spongy parenchyma with air spaces and sunken type stomata.

e-mail: rajeshpati777@gmail.com
Anatomy of stem

(A) T.S. of *in vitro* stem, had a polystelic structures, vascular bundles were arranged in a ring and they were conjoint collateral and open no corck cambium, showed primary medullary rays, uniseriate epidermis, no cuticle, distinct endoderm was absent while cortex was parenchymatous.

(B) T.S. of acclimatized stem, well developed cork cambium, parenchymatous pith but the pith was mucilaginous, distinct secondary growth with well developed wood and large woody vessels, epidermis was uniseriate and covered with cuticle, and cortex was collenchymatous.

e-mail: rajeshpati777@gmail.com
Anatomy of root

(A) T.S. of *in vitro* root, showed poorly developed pith, undifferentiated cortex with very less amount of storage tissue and no secondary growth at all.

(B) T.S. of *acclimatized* Root, had a well developed parenchymatous cortex with tannin cells, pericycle is made of stone cells vascular cylinder consisted of secondary xylem towards inner side and secondary phloem towards outer side, secondary xylem showed wide vessels scattered among trachieds, medullary rays were present while pith was negligible.

e-mail: rajeshpati777@gmail.com
Biochemical Studies

- The biochemical result showed that micropropagated plantlets produced significantly:
  - Low total chlorophyll (0.042 mg/g fresh weight)
  - Low reducing sugar (3.227%)
  - Low NR activity (1.353 NO2/h/g fresh weight)
  - Higher protein (0.048 μg/g) during *in vitro* phase
Conclusions

• The *in vitro* raised plants showed abnormal histological features like altered leaf mesophyll, absence of thick cuticle, sunken stomata, poorly developed stem and root histology.

• Photoautotrophic mode of nutrition during *in vitro* phase increased the survival rate during acclimatization compared to photoheterotrophic mode of nutrition.

• Photoautotrophism phenemoneon has substantial influence on the physiology and development of *in vitro* regenerated plantlets.
Related publication


  e-mail: rajeshpati777@gmail.com
Thank you

e-mail: rajeshpati777@gmail.com