PHYSIOLOGICAL STUDIES ON MICROPROPAGATION OF JACKFRUIT (Artocarpus heterophyllus L.)

By

NAGLAA ABDALLAH ABDELSALAM
B.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Cairo Univ., Egypt, 1999

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APPROVAL SHEET

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M.Sc. Thesis
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APPROVAL COMMITTEE

DR. HOSNY MOHAMED ABD EL-DAIM ..................
Professor of Plant Physiology, Fac. Agric., Benha University.

DR. MOHAMED RAMADAN ABDU EL LAH ...
Professor of Plant Physiology, Fac. Agric., Cairo University.

DR. NEVEEN BAHAA EL-DIN TALAAT SHAWKY.....
Associate Professor of Plant Physiology, Fac. Agric., Cairo University.

DR. EGLAL MOHAMED ZAKI HARAB ..........
Professor of Plant Physiology, Fac. Agric., Cairo University.

/ / Date:
SUPERVISION SHEET

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Professor of Plant Physiology, Fac., Agric., Cairo University

Dr. NEVEEN BAHAA EL-DIN TALAAT SHAWKY
Associate Professor of Plant Physiology, Fac. Agric., Cairo University

Dr. ABD-ELRAHMAN MOURSY GHALLAB
Prof. of Plant Physiology, Fac. Agric. Cairo University

Dr. MOHMAD REDA ABDELMAGED
Assistant Researcher Professor of Genetic Resources, Desert Research Center
To my loving family my husband,

Mohamed,

my sons karim and shady and my daughter jana.

Deep appreciation and love to my father,
mother in law, my sisters and brother and special
thanks to my sister Noha for fruitful help.

To the memory of late mother

prof. Dr. Nagwa Ali Mossa, my god blesses her soul.
First of all, I do indeed thank ALLAH ALRAHMAN ALRAHIEM who guided and aided me to bring forth to light this thesis. I would like to express my most deepest gratitude to Dr. Eglal Zaki, Professor of Plant Physiology, Department of Agricultural Botany, Plant Physiology Division, Faculty of Agriculture, Cairo University for her kind supervision this work, with the benefit of her guidance, the continuous help offered by her during the progress of this work and the preparation of the thesis. Also extend my thanks and appreciation to late Dr. Abd-Elrhman Ghallab, Prof. of Plant Physiology, Department of Agricultural Botany, Faculty of Agriculture, Cairo University and I hope to God that inhabited rest in peace, I do not forget standing my side, he passed away before the search is complete. My deep thanks and appreciation to Dr. Neveen Bahaa El-Din Talaat Shawky, Assistant Professor of Plant Physiology, Department of Agricultural Botany, Plant Physiology Division, Faculty of Agriculture, Cairo University for her supervision, grateful help and valuable scientific discussion encouragement during the progress of this work and preparation of the thesis. Particular thanks are due to Dr. Mohamed Reda Abdelmaged, Assistant Professor, of Tissue Culture, Department of Plant Genetic Resources, Ecology, Dry Land Agriculture Desert Research Center for his efforts and close supervision during the progress of the experimental part of this work. Great appreciation is also extended to all staff members of Agricultural Botany Department, Specially Plant Physiology Division, Faculty of Agriculture, Cairo University. Also my deep thanks to the all staff members of Desert Research Center. Finally I thank everyone helped me to perform this work.

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Title of Thesis: Physiological Studies on Micropropagation of Jackfruit (Artocarpus heterophyllus L.)
Supervisors:
Dr. Eglal Mohamed Zaki Harb
Dr. Neveen Bahaa El-Din Talaat Shawky
Dr. Abd elrahman Mousy Ghallab
Dr. Mohamed Reda Abdelmaged

ABSTRACT

This study was carried out in the Tissue Culture Laboratory at Genetic Resources Department, Desert Research Center, Ministry of Agriculture, Egypt, to establish an efficient protocol for rapid direct plant regeneration of Artocarpus heterophyllus L.

In order to optimize the establishment of both in vitro and ex vitro culture of Artocarpus heterophyllus L., shoot tips and nodal segments from mother plant were used. Best sterilization conditions were observed when shoot tip and nodal segment explants were surface sterilized by immersion in 30% clorox solution or in mercuric chloride at 0.2% solution containing 3-5 drops of Tween 20 for 15 minutes, followed by three times rinses in sterile distilled deionized water. For establishment stage, MS medium fortified with 3.0 mg/l BA gave the best result for explants survival % and shootlets number, while by using nodel segment explants, MS medium fortified with 2.0 mg/l BA gave the best results. For
multiplication stage, MS medium supplemented with 5.0 mg/l BA plus 2.0 mg/l Kin gave the best result for shootlets number. For elongation stage, MS medium fortified with 3.0 mg/l GA, gave the best result for the increase in shootlets length. Obtained shootlets were induced to roots and MS medium fortified with 1.0 mg/l IBA and 1.0 mg/l NAA gave the best result for roots number/plant, root length and rooted shoots %. Rooted plantlets were acclimatized to greenhouse condition with 100% transplant survival; moreover, successful ex vitro growth (about 70%) was achieved on peatmoss and sand at the ratio of 1:1(v/v).

**Key words:** *Artocarpus heterophyllus* L., micropropagation, shoot tip and nodel segment

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**INTRODUCTION**

The Jackfruit (*Artocarpus heterophyllus* L.), known as Jack tree, Jackfruit, or sometimes simply Jack or Jak, is a species of tree in the *Artocarpus* genus of the mulberry family (*Moraceae*). The jackfruit tree is well suited to tropical lowlands, and its fruit is the largest tree-born fruit, reaching as much as 80 pounds (36 kg) in weight, 36 inches (90 cm) in length, and 20 inches (50 cm) in diameter. The seed is 2-4 cm long and 1.25-2 cm thick and is white and crisp within. There may be 100 or up to 500 seeds in a single fruit. The Jackfruit tree is a widely cultivated and popular food item in tropical regions of India, Bangladesh, Nepal, Sri Lanka, Cambodia, Vietnam, Thailand, Malaysia, Indonesia, and the Philippines. Jackfruit is also found across Africa (e.g., in Cameroon, Uganda, Tanzania, Madagascar, São Tomé, Principe and Mauritius), as well as throughout Brazil, western central Mexico and in Caribbean nations such as Jamaica. Jackfruit is the national fruit of Bangladesh. The edible Jackfruit is made of, easily-digestible flesh (bulbs); A portion of 100 g of edible raw jackfruit provides about 95 calories and is a good source
of the antioxidant vitamin C, providing about 13.7 mg. Jackfruit seeds are rich in protein. The fruit is also rich in vitamin B6, potassium, calcium, and iron. The juicy pulp of the ripe fruit could be eaten either fresh or preserved in syrup. The fruit's isoflavones, antioxidants, and phytonutrients mean that jackfruit has cancer-fighting properties. It is also known to help cure ulcers and indigestion. The bulbs may then be enjoyed raw or cooked (with coconut milk or otherwise); or made into ice cream, jam, jelly, paste, or canned in sirup made with sugar or honey with citric acid added. The bulb of the unripe fruit is used as a vegetable and the seeds are roasted or fried and is sometimes called "vegetable meat".

Tissue culture techniques; micropropagation, provide a fast and dependable method for production of a large number of uniform plantlets in a short time and offer potential means not only for rapid mass multiplication of existing stocks but also for the conservation of important, elite and rare plants. It also provides novel approaches for the induction of genetic variability and production of plants which are resistant to biotic and abiotic stresses. Plant cell and tissue culture has contributed significantly to crop improvement and has great potential for the future. Micropropagation is being increased single used for large-scale production of many fruit, nut and ornamental trees. The Jackfruit is generally grown from seeds; however, the seeds are difficult to germinate even after a short period of storage. It is mainly propagation by seed which has resulted in immense variation in the population. Budding and grafting attempts have often been unsuccessful. Improvement of jackfruit tree through conventional breeding is limited due to high heterozygosity and long generation period. Attempts have recently been made to complement conventional breeding with modern biotechnological tools such as plant tissue culture, on the other hand, allows multiplication of the plant in a short period under the controlled conditions. Further, in conventional method of propagation through stem cuttings, each stem cutting produces only one
whereas in micropropagation thousands of plants can be produced from a single plant piece explants. Moreover, micropropagation can provide plantlets throughout the year irrespective of seasonal variations. Thus, micropropagation is an efficient and cost-effective method for rapid multiplication of jackfruit tree in a relatively short time and limited space. Therefore, the present investigation was undertaken to establish a protocol for regenerating a large number of plantlets from the nodal segment and shoot tip cultures of the Jackfruit tree. The experiment was conducted at Tissue Culture Laboratory at Genetic Resources Department, Desert Research Center, Ministry of Agriculture, Egypt, to standardize _In vitro_ protocol for multiplication of Jackfruit. In fact, we aimed at optimizing the organogenesis in Jackfruit (*Artocarpus heterophyllus* L.), in order to reduce the time required for the different production stages, focusing not only on _in vitro_ culture, but also on acclimatization and _ex vitro_ culture.

**Abstract**

This study was carried out, to establish an efficient protocol for rapid direct plant regeneration of *Artocarpus heterophyllus* L. Best sterilization conditions were observed when shoot tip and nodal segment explants were surface sterilized by immersion in 30% clorox solution or in mercuric chloride at 0.2%. For multiplication stage, MS medium supplemented with 5.0 mg/l BA plus 2.0 mg/l Kin gave the best result for shootlets number., gave the best result for the increase in shootlets length. Obtained shootlets were induced to roots and MS medium fortified with 1.0 mg/l IBA and 1.0 mg/l NAA gave the best result for roots number/plant, root length and rooted shoots %. Rooted plantlets were acclimatized to greenhouse condition with 100% transplant survival; moreover, successful _ex vitro_ growth (about 70%) was achieved on peatmoss and sand at the ratio of 1:1

*Key words:* *Artocarpus heterophyllus* L., micropropagation, shoot tip and nodel segment
REVIEW OF LITERATURE

The Jackfruit, Artocarpus heterophyllus L. of the family Moraceae. Jackfruit is a tropical fruit originating in India and the rain forests and mountains of Malaysia. It is cultivated widely at low elevations throughout India, in many parts of Southeast Asia, in the evergreen forest zone of West Africa, and in northern Australia as well (Azad et al., 2007). Moraceae family having five to six members can easily consume one Jackfruit at a time and satisfy their hunger. The Jackfruit tree is adapted to humid, tropical and subtropical climates. Jackfruit is the largest tree-borne fruit in the world, reaching 80 pounds in weight and up to 36 inches long and 20 inches in diameter. The exterior of the compound fruit is green or yellow when ripe. The interior consists of large edible bulbs of yellow, banana-flavored flesh that encloses a smooth, oval, light-brown seed. The seed is 3/4 to 1-1/2 inches long and 1/2 to 3/4 inches thick and is white and crisp within. There may be 100 or up to 500 seeds in a single fruit, which are viable for no more than three or four days. When fully ripe, the unopened Jackfruit emits a strong disagreeable odor, resembling that of decayed onions, while the pulp of the opened fruit smells of pineapple and banana (Morton, 1987). Jackfruit is the national fruit of Bangladesh and is one of the most common, important and delicious fruits in the country. It ranks third in area next to mango and banana and second in production (Anon, 2000). The edible portions are the pulp and seeds, which are considered as a good source of carbohydrates, proteins, Vitamins B1 and B2 and minerals (Burkill, 1997). The pulp of ripe Jackfruit is eaten fresh and used in fruit salads. It possesses high nutritional value; every 100 g of ripe fruit pulp contains 18.9 g
carbohydrate, 1.9 g protein, 0.1 g fat, 77% moisture, 1.1 g fiber, 0.8 g total mineral matter, 20 mg calcium, 30 mg phosphorus, 500 mg iron, vitamin A, 30 mg thiamin, and 84 calories (Samaddar, 1985). The Jackfruit also contains (Table 1) useful antioxidant compounds (Ko et al., 1998).

Propagation of Jackfruit plant from seeds is not widely accepted because of high heterozygosis. Clonal propagation through grafting of selected genotype is highly desirable but the number of plants produced by these conventional methods is relatively low (Samaddar, 1990).

Plant micro-propagation technology has been expanded and reclassified constantly all through the last 30 years, and now it has become a major tool for conservation of plant genetic resource. Tissue culture methods have been productively used for both propagation and conservation of many rare and critically endangered undomesticated plants. These protocols are especially useful when a species is hard to propagate using classical methods (Fay, 1992; Wala and Jasrai, 2003; Panayotova et al., 2008).

Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years,

### Table 1: Composition of Jackfruit (100 g edible portion).

<table>
<thead>
<tr>
<th>Composition</th>
<th>Young fruit</th>
<th>Ripe fruit</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (g)</td>
<td>76.2 to 85.2</td>
<td>72.0 to 94.0</td>
<td>51.0 to 64.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>2.0 to 2.6</td>
<td>1.2 to 1.9</td>
<td>6.6 to 7.04</td>
</tr>
</tbody>
</table>

become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites.

Small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in a continuous process. The parts of plants which are used for multiplying purpose are very small as compared with traditional vegetative multiplying therefore it is known as micro-propagation. It has a lot of benefits. Such as: high multiplying rate, small stresses on quantity of initial plants, minute demands on room and multiplying of plants despite seasons of the year (Kováè, 1995).

<table>
<thead>
<tr>
<th>Fat (g)</th>
<th>0.1 to 0.6</th>
<th>0.1 to 0.4</th>
<th>0.40 to 0.43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (g)</td>
<td>9.4 to 11.5</td>
<td>16.0 to 25.4</td>
<td>25.8 to 38.4</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>2.6 to 3.6</td>
<td>1.0 to 1.5</td>
<td>1.0 to 1.5</td>
</tr>
<tr>
<td>Total sugars (g)</td>
<td>–</td>
<td>20.6</td>
<td>–</td>
</tr>
</tbody>
</table>

**Minerals and vitamins**

<table>
<thead>
<tr>
<th>Total minerals (g)</th>
<th>0.9</th>
<th>0.87 to 0.9</th>
<th>0.9 to 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg)</td>
<td>30.0 to 73.2</td>
<td>20.0 to 37.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>27.0</td>
<td>54.0</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>20.0 to 57.2</td>
<td>38.0 to 41.0</td>
<td>38.0 to 97.0</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>287 to 323</td>
<td>191 to 407</td>
<td>246</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>3.0 to 35.0</td>
<td>2.0 to 41.0</td>
<td>63.2</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>0.4 to 1.9</td>
<td>0.5 to 1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>30</td>
<td>175 to 540</td>
<td>10 to 17</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>0.05 to 0.15</td>
<td>0.03 to 0.09</td>
<td>0.25</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.05 to 0.2</td>
<td>0.05 to 0.4</td>
<td>0.11 to 0.3</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>12.0 to 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The use of modern in vitro techniques on woody trees has opened new possibilities for rapid mass multiplication of the existing stocks of germplasm as well as for its ex situ conservation (Hussain et al. 2008; Kumari et al. 2009). Besides affording multiplication in limited time and space, the technique of tissue culture circumvents the limitations posed by the long regeneration cycle of woody trees and obviates the dependence on flowering and seed set (Bonga, 1985; Giri et al., 2004).

The application of tissue culture methods for improvement and large-scale propagation of fruit trees has been well demonstrated (Litz et al., 1985; James, 1988). Successful in vitro propagation of Jackfruit seedlings has been demonstrated (Rahman and Blake, 1988). Regeneration of plantlets from bud and nodal explants of mature Jackfruit trees has been reported (Jaiswal and Amin, 1990; Roy et al., 1990). The application of micropropagation techniques to the fruit crops is likely to continue in future in the production of new cultivars, difficult to propagate elite genotypes, and large quantities of root stocks (Hammerschlag, 1986).

Micropropagation of tree species offers a rapid means of producing clonal planting stock, woody biomass production, and conservation of elite and rare germplasm (Yeoman, 1986; Bonga, 1985). Plant tissue culture, is generally dependent for its success on the inclusion of plant hormones and plant growth regulators and/or other growth active substances, as one of the five classes of required substances, in the medium (Gamborg et al., 1976). Auxins, cytokinins, and Auxins–cytokinins interactions are usually considered to be the most important for regulating growth and organized development in plant tissue and organ cultures, as these two
classes of hormones are generally required (Evans et al., 1981; Vasil and Thorpe, 1994).

Micro-propagation is independent of season. As micro-propagation could be carried out throughout the year; production cycle can be scheduled to meet peak demands. For species that have long generation time, low levels of seed production, or seeds that do not readily germinate, rapid propagation is possible through tissue culture. The time required is much shortened, no need to wait for the whole life cycle of seed development (Azam et al., 2009).

According to reports concerning tissue culture, success of in vitro propagation of Jackfruit depends on the season, when explants are collected and on the source of explants, physiological state of plant and nutrient environment. In this circumstance an attempt has been made in the present study to investigate the effect of time of the year and growth regulators on shoot proliferation and subsequent development of Jackfruit shoot from matured tree.

**In vitro culture stages**

Plant tissue culture refers to growing and multiplication of cells, tissues and organs of plants on defined solid or liquid media under aseptic and controlled environment. The commercial technology is primarily based on micropropagation, in which rapid proliferation is achieved from tiny stem cuttings, axillary buds and to a limited extent from somatic embryos, cell clumps in suspension cultures and bioreactors. The cultured cells and tissue can take several pathways. The pathways that lead to the production of true-to-type plants in large numbers are the preferred ones for commercial multiplication. The process of micropropagation is usually divided into several
stages i.e., prepropagation, initiation of explants, subculture of explants for proliferation, shooting and rooting, and hardening. These stages are universally applicable in large-scale multiplication of plants. The delivery of hardened small micropropagated plants to growers and market also requires extra care (Harshal, 2014).

1. Establishment

a. Sterilization treatments

In this stage an explant is surface sterilized and transferred into nutrient medium. Generally, the combined application of bactericide and fungicide products is suggested. The selection of products depends on the type of explant to be introduced. The surface sterilization of explant in chemical solutions is an important step to remove contaminants with minimal damage to plant cells (Husain and Anis, 2009). Indeed, the major problems generally faced in culturing tissues from mature trees are contamination, recalcitrance of adult trees, hyperhydration, and browning or blackening of the culture medium and/or the explants due to leaching of phenolics which may be phytotoxic and cause necrosis of the explants (Bhojwani and Razdan, 1996). The most commonly used disinfectants are sodium hypochlorite (Tilkat et al., 2009; Marana et al., 2009) calcium hypochlorite (Garcia et al. 1999), ethanol (Singh et al., 2009) and mercuric chloride (HgCl₂) (Husain and Anis, 2009). Ahmad (2002) indicated that in Jackfruit explants from mature trees always have high numbers of exogenous as well as endogenous microbes. The action of HgCl₂ may be through lysis of cells of microorganisms or it may act on thiol groups in microbial enzymes. Like NaOCl, HgCl₂ is known to be a powerful antimicrobial agent. Treatment with 0.10% HgCl₂ for 5–7 min is used

Shoot tips of *Citrus junos* were sterilized in 0.1-2% sodium hypochlorite with 0.01 % Tween for 1 to 30 minutes. The most effective sterilizing procedure was 1% sodium hypochlorite for 10-15 minutes followed by 4 washes in distilled water (Auoh et al., 1991).

Amin (1992) by using Jackfruit shoot tip observed that lower concentration of 0.1% HgCl₂ was suitable for sterilization, but prolonged treatment (more than 10 minutes) manifested *in vitro* tissue killing.

Good aseptic establishment of cultures and proliferation of cultures were obtained from Jackfruit explants treated with HgCl₂ at 0.2% for 4 min or at 0.1% for 10 min (Chavan et al., 1996).

Mannar et al. (2006) said that in Jackfruit, the apical buds remain covered with a cap like structure called stipule. Unopened buds with one or two leaves along with shoot tip were collected from the selected plant and placed in water and then brought into the laboratory and used as plant materials. After removing the expanded leaves, the materials were washed thoroughly under running tap water. The materials were then suspended in 0.7% PVP polyvinylpyrrolidone solution, an antiseptic plus detergent containing 2% sucrose and were agitated by a magnetic stirrer for 15 minutes. They were then washed thoroughly to remove PVP. After that the plant materials were transferred to the laminar air-flow cabinet. Then the plant materials were dipped in 70% ethanol for 30 seconds and immediately washed with autoclaved sterilized distilled water. This was followed by surface sterilization with 0.2% mercuric chloride (HgCl₂) for 10 minutes followed by washing with sterile double distilled water giving 3-5 changes. The surface water of treated material were then dried with the autoclaved tissue paper and kept for few minutes in laminar air-flow on an open Petridish for final drying.
Hamed et al. (2007) reported that Jackfruit explants were surface sterilized at first using 3 g/L mercuric chloride for 1 min., and then rinsed with sterilized distilled water for three times. After that shoot tips were continued surface sterilization using 40% Clorox (5.25% sodium hybochloride) for 20 min. While both the intermediate and basal nodes were surface sterilized using 50% and 70% Clorox for 20 min. respectively. All surface sterilized solutions contained one drop of Tween 20 per 100 ml solution used as surfactant.

El-Zaher (2008) obtained good results with 0.30% HgCl₂ for 5 min in Jackfruit who stated that HgCl₂ at 0.20% for 4 min or at 0.10% for 10 min gave good sterilization results in shoot tip cultures of Jackfruit. Comparison of the two sterilizing agents showed that NaOCl gave a maximum survival rate of 70% and a minimum mortality rate of 10% after treatment of 10% NaOCl, while HgCl₂ gave a maximum survival rate of 63.33% after treatment of 0.10% HgCl₂ and a minimum mortality rate of 6.66% after treatment of 0.01% HgCl₂.

Azam, et al. (2009) after removal of expanded leaves from the Jackfruit shoot apices and nodes, They washed thoroughly under running tap water for 30 minutes and then treated with Tween 80 plus Savlon for 10 minutes and again rinsed with distilled water. The explants were surface disinfected with 0.1% HgCl₂ (for 5 to 15 minutes) followed by seven times rinsing with sterile distilled water in a laminar air flow cabinet. The explants were finally trimmed, prepared and cultured on to the medium. A survival rate of 82.22% was observed when the excised explants were surface sterilized with 0.1% HgCl₂ solution for 10 minutes.

Shoot tips 3-5 cm long and nodal segments collected from Jack fruit seedling were surface sterilized in order to control contamination by Khan et al. (2010). Chlorox was used with the following concentration (0.5, 0.75, 1.0, and 1.25%) for 15 minutes with two drops of Tween 80. From the results it is evident that (0.75 % NaOCl) gave the maximum survival (46.66 %), followed by (1.0 % NaOCl) with 26.66 % survival while the minimum survival (6.6 %) was observed in (1.25 % NaOCl). It is clear from results that infections were
reduced (0.0 %) with an increase in concentration of NaOCl (1.25 %). Results showed that both higher and lower concentrations greatly affected the cultured shoot tip explants. Lower concentrations enhance contamination whereas higher concentrations were toxic to the plant as it damaged the tissues. Ashrafuzzaman et al. (2012) said that Jackfruit explants were surface sterilized at first using 3 g/L mercuric chloride for 1 minute, and thoroughly rinsed with sterilized distilled water for three times then continued surface sterilization using 5.25% sodium hypochlorite for 20 minutes. Shoot tips and individual nodes from Cordyline sp. were excised with a scalpel and used as explants. Isolated explants were washed with detergent followed by three rinses in distilled water and these were treated with bactericide (streptomycin) and then washed with distilled water. They were surface sterilized with 0.1% HgCl2 for 5 minutes and then washed 3-4 times with sterile distilled water in laminar air flow cabinet (Chinnu et al., 2012). *Eriobotrya japonica* shoot tips (3–4 cm in size) of loquat cultivar Mardan were collected from the orchard of Tret (Murree, Pakistan) to be used as explants for *in vitro* culture establishment. After removing leaves, hairs, and dirt, they were placed under running tap water with one drop of Tween 80 and detergent for 30 min to remove any foreign contaminants. After washing, shoot apices were dissected and surface sterilized with different concentrations of NaOCl (5%, 7%, 10%, 12%, 14% (v/v)) for 12 min and HgCl2 (0.01%, 0.05%, 0.10%, 0.20%, 0.25% (w/v)) for 2 min in a laminar air flow hood. Data regarding the effect of disinfectants on surface sterilization of loquat shoot tips after 28 d treatment with 10% NaOCl showed significant results with an increased survival percentage (70%). Treatment with 14% NaOCl resulted in minimum survival (2%) and maximum necrosis (90%). Generally, necrosis increased as the concentration of NaOCl increased which shows that higher concentrations of NaOCl damage young tissues. Fungal and
bacterial contamination was high (86.66%) after treatment with 5% NaOCl, but absent after treatment with 14% NaOCl. The highest NaOCl concentration badly damaged the explants and decreased the survival rate. HgCl₂ significantly reduced contamination (to 3.33%) after treatment of 0.25% HgCl₂ but increased mortality (to 53.3%). Percentage survival was highest (63.33%) after treatment of 0.10% HgCl₂ followed by 0.20% HgCl₂ (43.33%). The percentage survival after treatments of 0 and 0.05% HgCl₂ was not significantly different (40%). Increases in the concentration of HgCl₂ showed increasing necrosis and mortality, but a decline in contamination. HgCl₂ is one of the strongest disinfectants used in culture establishment. From the above results, authors inferred that treatment with NaOCl at 10% performed better than treatment with HgCl₂. NaOCl is also a readily available and economical sterilant (Abbasi et al., 2013).

Arya et al. (2013) found that Dalbergia sissoo nodal segments measuring 2-3 cm containing axillary buds were first surface sterilized using 0.1% HgCl₂ for 15 min, after which they were washed with sterile distilled water 3-4 times for further inoculation in medium.

Lalitha et al. (2013) used the nodal region measuring about 2-3 cm of mulberry as an explant. Then, they washed the explants in running water for 2-3 times and again washed with mild detergent and rinsed thoroughly with distilled water. The explants were surface sterilized by keeping them in 70% ethanol for 5 minutes. After washing 2-3 times with water, they were treated with 0.1% (w/v) of systemic fungicide, Bavistin for 10 minutes. Subsequently, the explants were washed and kept in 0.1% (w/v) of HgCl₂ for 10 minutes in an orbital shaker at 100 rpm. Finally, the explants were serially washed 2-3 times with sterile double distilled water to remove traces of sterilizing chemicals before inoculation.

Mahdi et al. (2014) showed that excised Musa sapientum explants were surface sterilized with 0.1% HgCl₂ to avoid contamination where survival rate increased according to the increase of treatment period from 10 minutes to 15
minutes. Explants treated for 15 minutes showed the best survival rate (90%) and prolonged treatment showed tissue blackening.

*Artocarpus chaplasha* Roxb shoots were defoliated and apices of two three nodes were taken. They were then washed thoroughly under running tap water for 30-40 minutes, washed with liquid detergent for another 15-20 minutes, and then with a solution of 5% w/v Aseptic Savlon for 10 minutes. After repeated washing with distilled water the explants were finally treated with 0.2% HgCl₂ for 10 minutes in the laminar air flow cabinet and washed for three times with autoclaved double distilled water (Rahman *et al.*, 2014).