Tissue Culture of *Artocarpus heterophyllus* L., an Underutilized Fruit of Bangladesh

Presented by

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Contents

Introduction

- Jackfruit, a dicotyledonous compound fruit (4.5-20 kg) of jackfruit tree (*Artocarpus heterophyllus*); an evergreen tree of subtropical and tropical countries.
- The national fruit of Bangladesh, locally known as ‘Kathal’.
- Usually bears between June to August though a few year round varieties exist.
- Yearly yields only around 279.5 thousand metric tons.
- This fruit is commonly referred as ‘poor man’s food’.
- A family having five to six members can easily consume one jackfruit at a time and satisfy their hunger.

- Considered as a good source of carbohydrate, proteins, Vitamins B_1 and B_2 and minerals.
- This is an underutilized fruit due to lack of proper and customized consumption and shortage of techniques for higher yield.
- Durable timber known as Jackwood.

Objectives of the Research

- The Jackfruit plant usually propagates from seeds but is unacceptable for heterozygosis.
- Clonal propagation through air laying, grafting, budding is not a desirable approach for large scale production.
- Tissue culture techniques are the most effective procedure for large-scale propagation of fruit trees.

- Per capita of fruit consumption in this country is seriously low (**26.8** gm/per day only) compared to other developed countries.
- Bangladesh persists very high – among the highest in the world for child malnutrition rates-
  - 50.5% children aged <5 years stunted for age
  - 42.7 % children aged <5 years underweight for age.
- The jackfruit variety that can yield fruits throughout the year may be a way to increase the total yield of jackfruit by several folds.
- From large scale cultivation, Jackfruit can be available year round to the poor people as a cheap fruit, which can ultimately reduce malnutrition rate of Bangladesh.
- ICUC (International Center for Underutilized Crops) have examined the contribution of underutilized plants, which can lead to meeting the Millennium Development Goals (MDGs), pointing out the vital role of biodiversity in the efforts to rid the world of poverty and hunger. FAO has also concluded that combating hunger and malnutrition is a necessary precondition for meeting any of the other MDGs.

Material & Methods

This experiment was carried out at the laboratory of Plant Tissue Culture Laboratory of PROSHIKA, Bangladesh during June, 2006 to February, 2007.

Explant Preparation & Sterilization

Newly sprouted healthy shoots of 10-15 days-old were collected

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Shoot apices and nodes were excised

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Washed under running tap water for 30 minutes

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Treated with Tween 80 and Savlon for 10 minutes
and rinsed with distilled water

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Surface disinfected with 0.1% $HgCl_2$ for 10 minutes
followed by seven times rinsing with sterile distilled water
in a laminar air flow cabinet.

Shoot Proliferation Stage

- Sterilized explants were implanted vertically on MS medium supplemented with different concentrations (0.2 to 4.5 mg/L) of 6-benzylaminopurine (BAP).
- All media were supplemented with 3% sucrose and 0.8% Difco Bacto-agar.
- The pH was adjusted to 5.8.

Multiple Shooting Stage

- Explants were cultured on MS medium fortified with different concentrations and combinations of BAP (3.0, 3.5, 4.0 mg/l) and Kinetin (Kn) from 0.2 to 3.0 mg/l.
- We observed the synergistic effects of auxin and cytokinin for multiple shoot formation.

Root Induction Phase

- Individual shootlets were cultured on ½ MS medium enriched with Indole-3-butyric acid (IBA) ranging from 0.2 to 2.0 mg/l for the induction of root.

Hardening and Adaptation Stage

- Plantlets were gradually hardened through periodic exposure to the lower relative humidity and sunlight for seven days
- Then transferred to greenhouse.
- Plantlets were taken out from the culture tubes and washed carefully under running tap water for complete removal of media.
- Every single plantlet was transplanted to small plastic bags containing different soil formula. They were-

  I. Only garden soil

  II. Garden soil + Sand (1:1)

  III. Garden soil + Sand + Cow dung (1:1:1)

  IV. Garden soil + Sand + poultry liter (1:1:1)

  V. Garden soil + Organic fertilizer (1:1)

  VI. Garden soil with hot water (100^{o}C) treatment

  VII. Garden soil with formalin treatment (.01% formalin into soil)

Open Field Observation

- Acclimatized plants were observed for proper establishment in soil for fifteen days.
- Then field observation was done for two months from January`07 to February`07 at one week intervals.

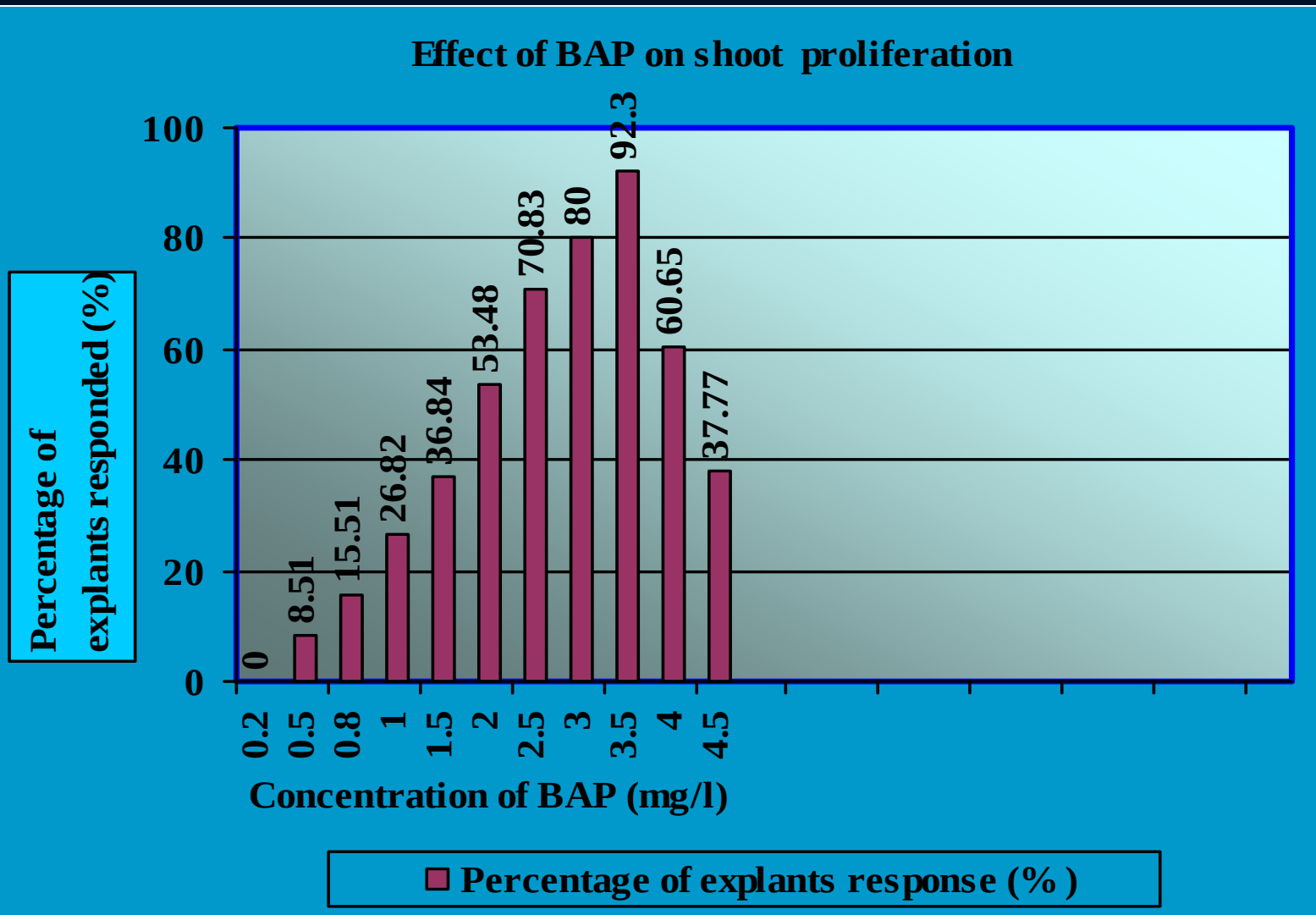
Results & Discussion

Explant Sterilization

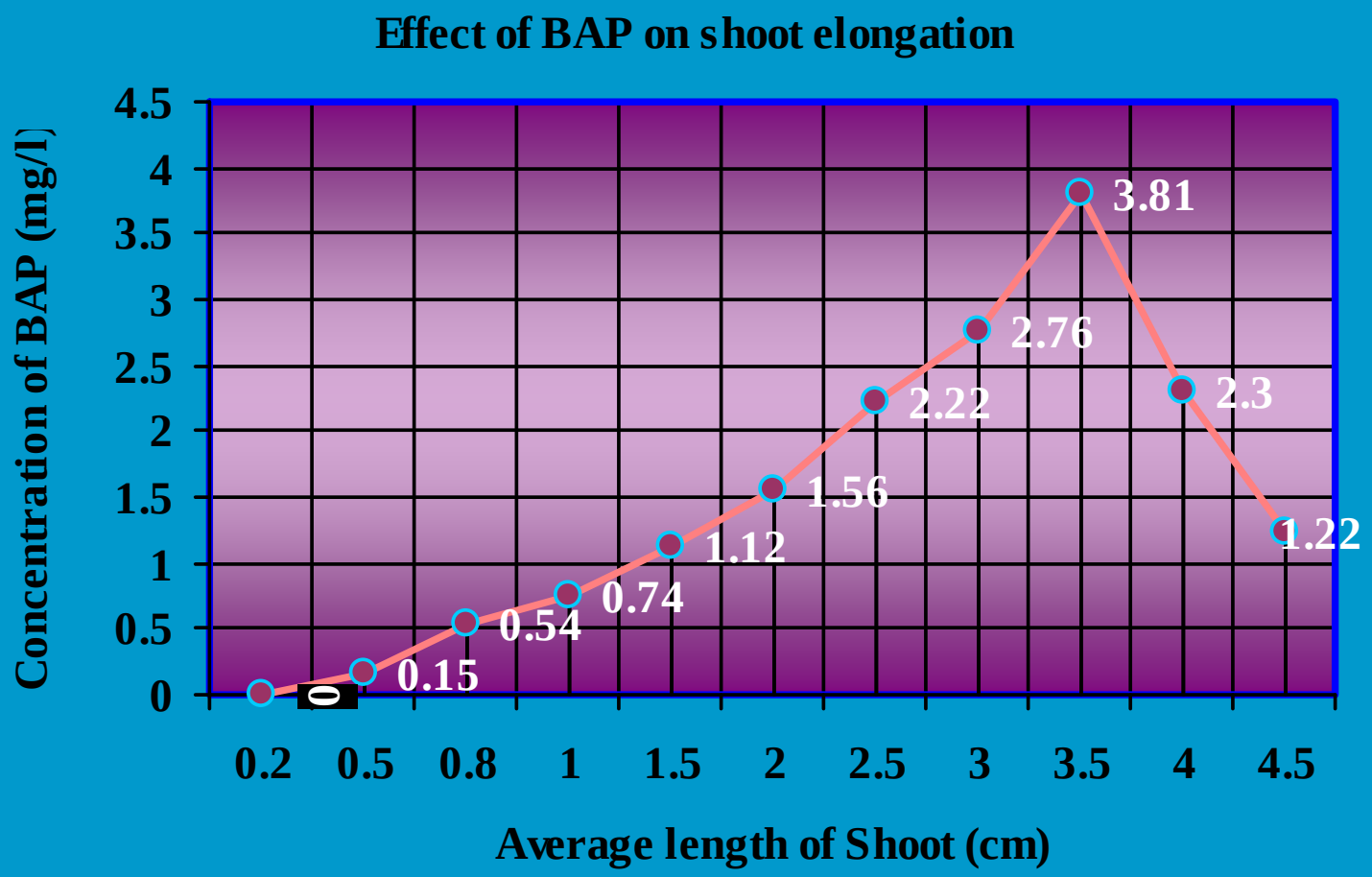
- Highest percentage (82.22%) of survival rate was observed when the excised explants were treated with 0.1% $HgCl_2$ solution for surface sterilization for 10 minutes.

Shoot Proliferation Stage

- Highest frequency of sprouting and adventitious shoot proliferation (92.30%) observed when (**Figure-A1)** implanted on MS medium supplemented with BAP at 3.5 mg l^{-1} and the highest length of shoots were measured at 3.81 cm (Graph-2).



Graph-1: Effect of different concentrations of BAP on shoot proliferation from shoot tip & nodal explants



Graph-2: Effect of BAP on shoot elongation

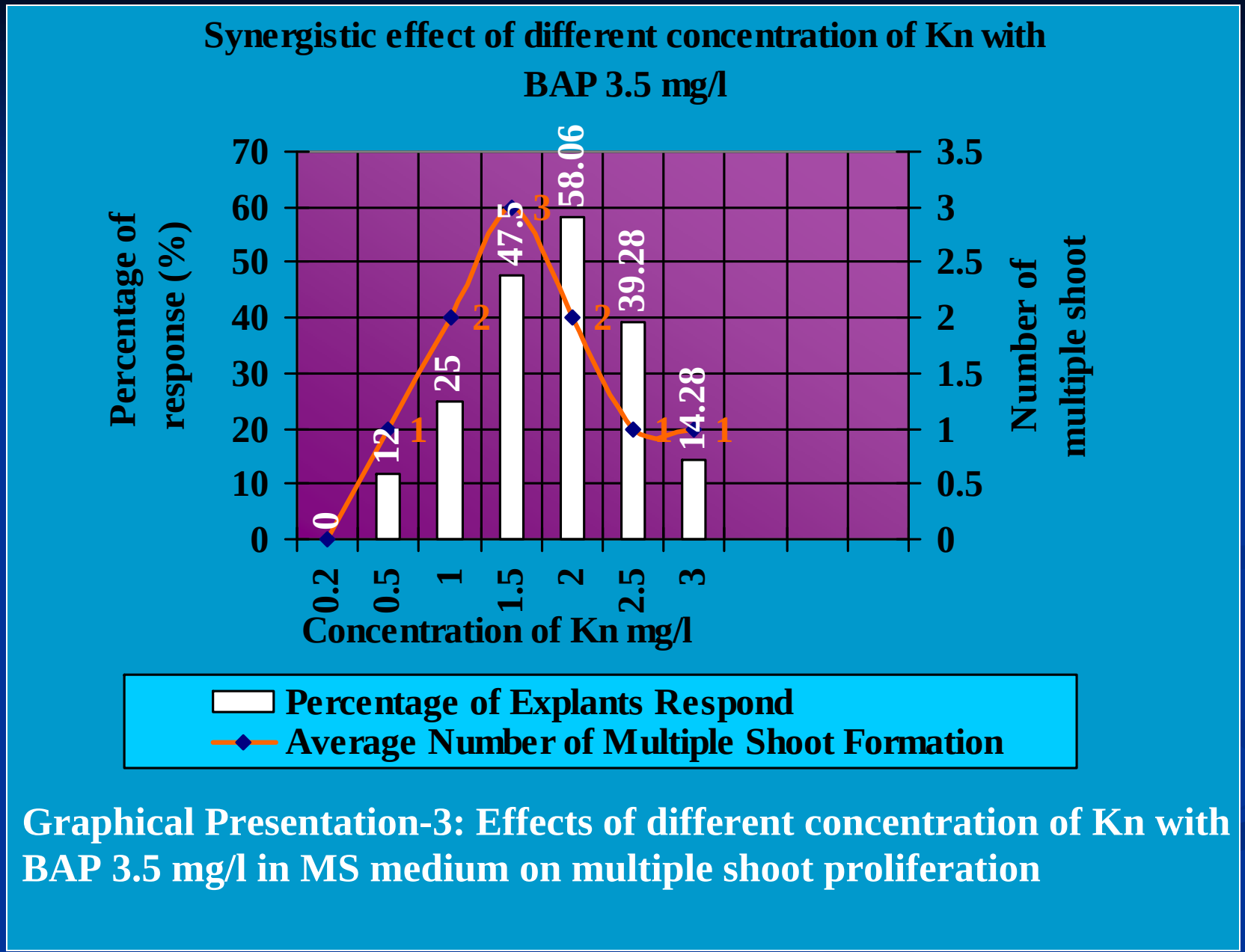
- The percentage of shoot proliferation decreased (from 92.30 % to 37.77%) with increase of BAP concentration. Shoot elongation was examined through the gradual increase of hormonal concentration along with effects it had on rapid proliferation of shoots.
- Higher and increased concentration of BAP produced shooting but excessive amount caused reducing effect on proliferation and caused leaves to become curled.

Figures 1 &2:

1. Regenerated micro-shoot on MS medium fortified with BAP 3.5 mg/l after 2 weeks of culture.
2. Shoot proliferation after 8th subculture in the same medium

Multiple Shooting Stage

- Synergistic effects of cytokinin and auxins were observed. Auxiliary and adventitious shoots grew out from each shoots and lateral branches developed and the result indicated that the highest percentage (58.06 %) of response was observed at 3.5 mg/l BAP and 2.0 mg/l of Kn, which showed two multiple shoots having average length of 1.54 cm(Graph-3).



Graphical Presentation-3: Effects of different concentration of Kn with BAP 3.5 mg/l in MS medium on multiple shoot proliferation

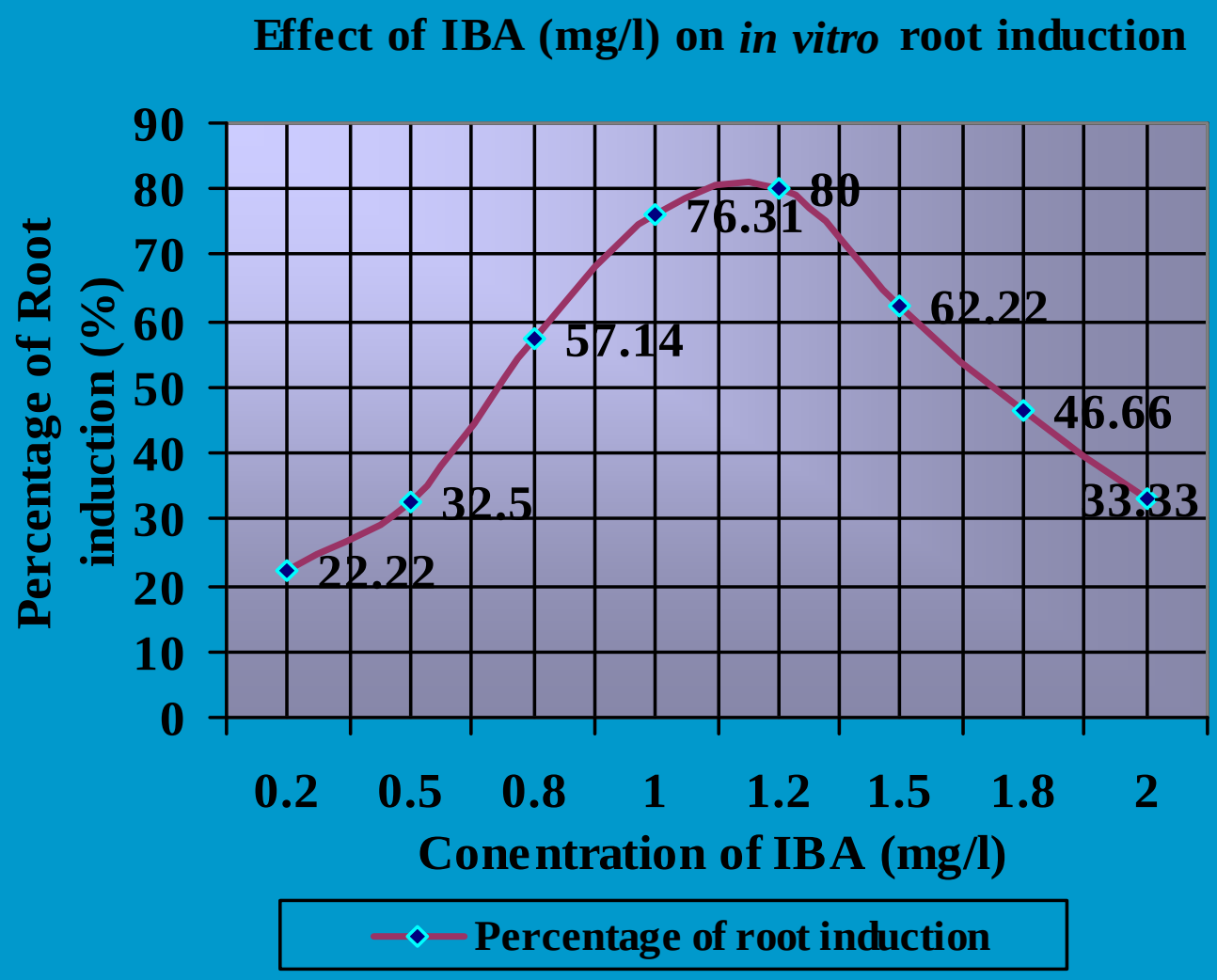
Figures 3 & 4: 3. Clusters of profuse branching of proliferated shoots on MS + 3.5 mg/l BAP + 1.5 mg/l Kn

4. Clump of proliferated auxiliary buds on MS + 3.5 mg/l BAP + 1.5 mg/l Kn

Root Induction Phase

- Rooting occurred at all the concentrations of IBA, but maximum response (80%) was obtained when shootlets were incubated on MS enriched with IBA 1.2 mg l-1 (**Table 7**). This concentration induced highest average number of roots (6.10) and root length (3.2 cm).

Figures 5 & 6: Adventitious rooting of shootlets on MS medium enriched with 1.2 mg/l IBA



Graphical Presentation-4: Root induction in shootlets on MS enriched with BAP 1.2 mg/l

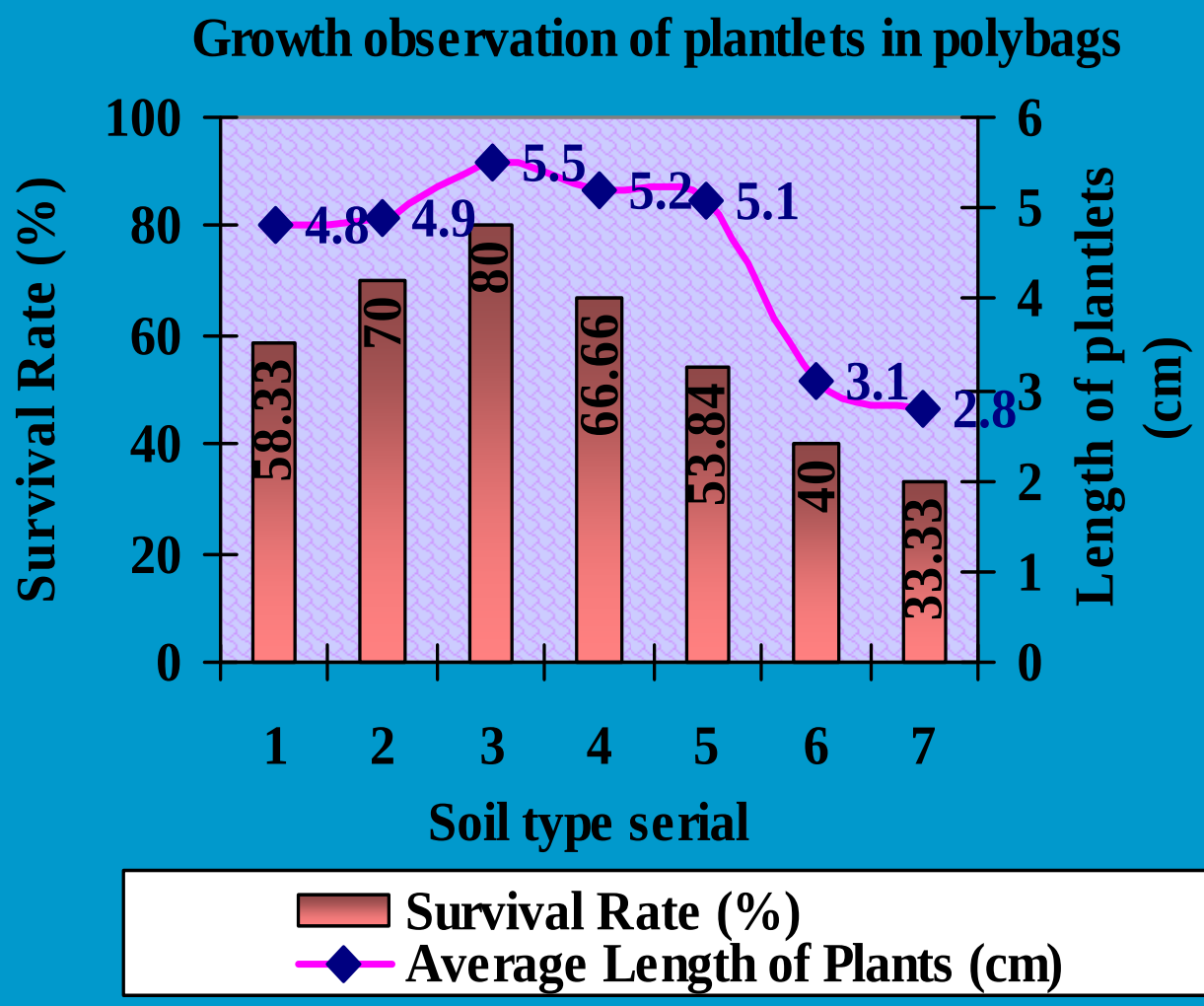
Hardening & Acclimatization

- In vitro raised plantlets 4-5 cm length were transferred to the hardening room for a week. Then they were transplanted in to different earthen pot media and were kept in net house.

- After fifteen days observation from transplantation in soil, highest survival rate of 80% was found from the soil formula containing garden soil, sand and cow dung (1:1:1).

Field Observation

- After sixty days observation period, the transplanted plantlets resembled the general morphological characteristics of the donor plants but showed detectable variation in growth on the basis of formulated soil type (Graph 4).
- Highest length (5.50 cm) and highest number of leaves (4.60) were observed from soil type containing garden soil, sand and cowdung (1:1:1).



Experimental Soil formula Serial
1. Garden soil
2. Garden soil + Sand (1:1)
3. Garden soil + Sand + Cow dung (1:1:1)
4. Garden soil + Sand + poultry liter (1:1:1)
5. Garden soil + Organic fertilizer (1:1)
6. Garden Soil(100^{o} C hot water treated)
7. Garden soil (0.01% formalin treated)

Graphical Presentation-4: Growth observation of plantlets in poly bags

Figure 7: Eight weeks old micropropagated plants established after acclimatization in a mixture (1:1:1) of garden soil, sand and cow dung

Conclusion

With this experiment, a protocol for the micropropagation of *A. heterophyllus* was established from shoot tip and nodal explants, which could be used to raise fruit garden, reforestation program and to trim down malnutrition on one hand and on the other hand, this finding would be a milestone for genetic transformation studies and conservation of endangered varieties.

THANK YOU
LADIES AND GENTLEMEN