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National Academy of Agricultural Science (NAAS) Rating : 3.03

# Studies on Hardening of Tissue Culture Propagated Plants of Jamun *cv.* AJG-85

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***ABSTRACT**: Jamun (Syzygium cuminii L.) is an evergreen multipurpose tree. Micropropagation has definite and indispensable advantages over conventional method of vegetative propagation. One of the major obstacles in the application of tissue culture methods for plant propagation has been difficulty in successful transfer of plantlets from the laboratory to the field. Hardening is an integral and vital activity of the whole process of tissue culture technology. Improper hardening leads to the failure of whole technology and the industry itself. Success in hardening is must for an industry for its survival. Studies were, therefore, carried out to develop protocol for successful acclimatization of in vitro propagated plantlets of Jamun cv. AJG-85. Different media viz., coco pith, vermicompost, perlite, vermiculite, soilrite, sand alone or in combination were tried for primary hardening under polytunnel for four weeks. Secondary hardening was carried out using different potting media combinations under shadehouse for eight weeks. The media mix of perlite + vermiculite + cocopeat (1:1:1 v/v) and cocopeat alone proved superior for primary hardening. For secondary hardening with perlite + red soil (1:1 v/v) and vermiculite + red soil (1:1 v/v)was found superior fallowed by cocopeat + Red soil (1:1 v/v) and perlite + vermiculite + cocopeat (1:1:1 v/v).*

## INTRODUCTION

Jamun (*Syzygium cuminii* L.) is an evergreen multipurpose tree. It is an important minor fruit belonging to the family Myrtaceae consisting of over 75 species and native of India. It is also known as Black Plum, Java Plum, Indian blackberry, Jambolan, *etc.* It is tall and evergreen tree (Fig.1) distributed throughout India for its edible fruits. Jamun is highly valued for its fruits, seeds and leaves and are recommended to control diabetes, dysentery, diarrhoea, edema, ringworm, fever, *etc.* Apart from antidiabetic preparations, the seeds are also known to be very good concentrate feed to the cattle as they are rich in protein, carbohydrates and calcium. The timber is used for making plywood and agricultural implements as it is durable.

Micropropagation has definite and indispensable advantages over conventional method of vegetative propagation in ensuring extremely rapid rate of multiplication, not season bounded, require only limited quantities of plant tissues as a source of explants and also requiring limited space. It not only provides economy of time and space but also gives greater output and allows further augmentation of elite, disease free propagules. One of the major obstacles in the application of tissue culture methods for plant propagation has been difficulty in successful transfer of plantlets from the laboratory to the field. Hardening is an integral and vital activity of the whole process of tissue culture technology. Improper hardening leads to the failure of whole technology and the industry itself. Success in hardening is must for an industry for its survival. Studies were, therefore, carried out to develop protocol for successful acclimatization of Jamun *cv.* AJG-85.

## MATERIAL AND METHODS

The *in vitro* rooted plantlets were removed from culture bottles after 4 weeks incubation. The agar gel adhered to the roots of plantlets was washed off and treated with Bavistin 1 % + Streptomycin sulphate 0.05 % for 10 minutes. Plantlets with 2-3 pairs of leaves were used for hardening studies. The hardening of plantlets was carried out in two stages namely primary hardening of 4 weeks period under poly-tunnel environment and secondary hardening of 8 weeks under shade house.

Different media viz., cocopeat, perlite, vermiculite, soilrite, sand, perlite + vermiculite +

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**Figure 1: A superior Jamun *cv*. AJG-85**

cocopeat (1:1:1 v/v) were evaluated for primary hardening. *In vitro* rooted plantlets were transferred to protrays filled with different types of potting media and placed under polytunnel having hot and humid condition in nethouse for 4 weeks.

For secondary hardening, black coloured polybags of 4 cm width and 15 cm length were filled with different media along with red soil in equal proportion and then sterilized with carbendazim 1%. Later primary hardened plantlets were transferred to polybags and kept under shade house having 50% shade for 8 weeks.

The observations were recorded on *per cent* survival, shoot length (cm), number of leaves/ plantlets, length of leaves (cm), breadth of leaves (cm), number of primary roots, number of secondary roots and length of primary roots (cm) at $4^{th}$ and $8^{th}$ weeks after primary and secondary hardening, respectively. Completely randomized design (CRD) was employed for the experiments and data in percentages were transformed to arcsine values for statistical analysis. The data were subjected to ANOVA as suggested by Panse and Sukhatme (1967). Critical difference values were tabulated at one *per cent* probability where "F" test was significant.

## RESULTS AND DISCUSSION

During primary hardening better plantlets establishment was observed with the media mixure of perlite + vermiculite + cocopeat (1:1:1 v/v) and cocopeat alone (Table 1a & b: Fig. 2 & 3). This may be due to better aeration, water holding and nutrient supplying capacity of the media. These finding are in close agreement with the report of Manjusha and Sathyanarayana (2008) in stevia. Sujatha *et al.* (2008) of the opinion that the tissue culture hardening techniques using low cost inputs like coir pith, soilrite, cow based organic preparation have yielded favourable results and has also reduced the cost of hardening in crops like banana, anthurium, orchids, calla lily and so on.

For secondary hardening, perlite + red soil (1:1 v/v) and vermiculite + red soil (1:1 v/v) was found superior followed by cocopeat + Red soil (1:1 v/v) and perlite + vermiculite + cocopeat (1:1:1 v/v) (Table 2a & b: Fig.4). Probably, perlite and vermiculite might have helped in improving physical and chemical properties of the media, consequently resulted in better growth of jamun plantlets. The successful establishment in *in vitro* plantlets of neem was accomplished when perlite was used as a potting medium (Upadhyaya, 1995). Jackfruit was successfully hardened with high percentage of survivability using vermiculite as potting media (Rajmohan and Mohankumaran, 1988). The effectiveness of vermiculite as a potting medium has been reported in pomegranate (Muralikrishna, 1998). Successful acclimatization of satin wood (*Chloroxylan swietenia*) ramets was accomplished in vermiculite and sand (Umer *et al*., 1993).

The final stage in the micropropagation which decides the success of the micropropagation is hardening. This is the most crucial stage where humidity needs to be maintained and it should be insect and disease-free environment. A period of acclimatization maintaining high humid conditions is essential for the plantlets to adapt to the outside environment during which the plantlets undergo morphological and physiological adaptation enabling them to develop typical plant-water control mechanisms (Grout and Aston 1977 and Sutter *et al*., 1985).

After primary hardening, there was no further decline in the survival *per cent* and it remains constant thereafter. This suggest that the plant require about 4 weeks to adapt themselves to typical plant water control mechanisms, especially the stomatal regulation and development of proper vascular connections between the shoot and root for better establishment of the plants (Fig. 2 &3).

It is evident that the media mixure of perlite + vermiculite + cocopeat (1:1:1 v/v) and cocopeat alone proved superior for primary hardening, whereas, for secondary hardening perlite + red soil (1:1 v/v) and vermiculite + red soil (1:1 v/v) was found better fallowed by cocopeat + Red soil (1:1 v/v) and perlite + vermiculite + cocopeat (1:1:1 v/v).

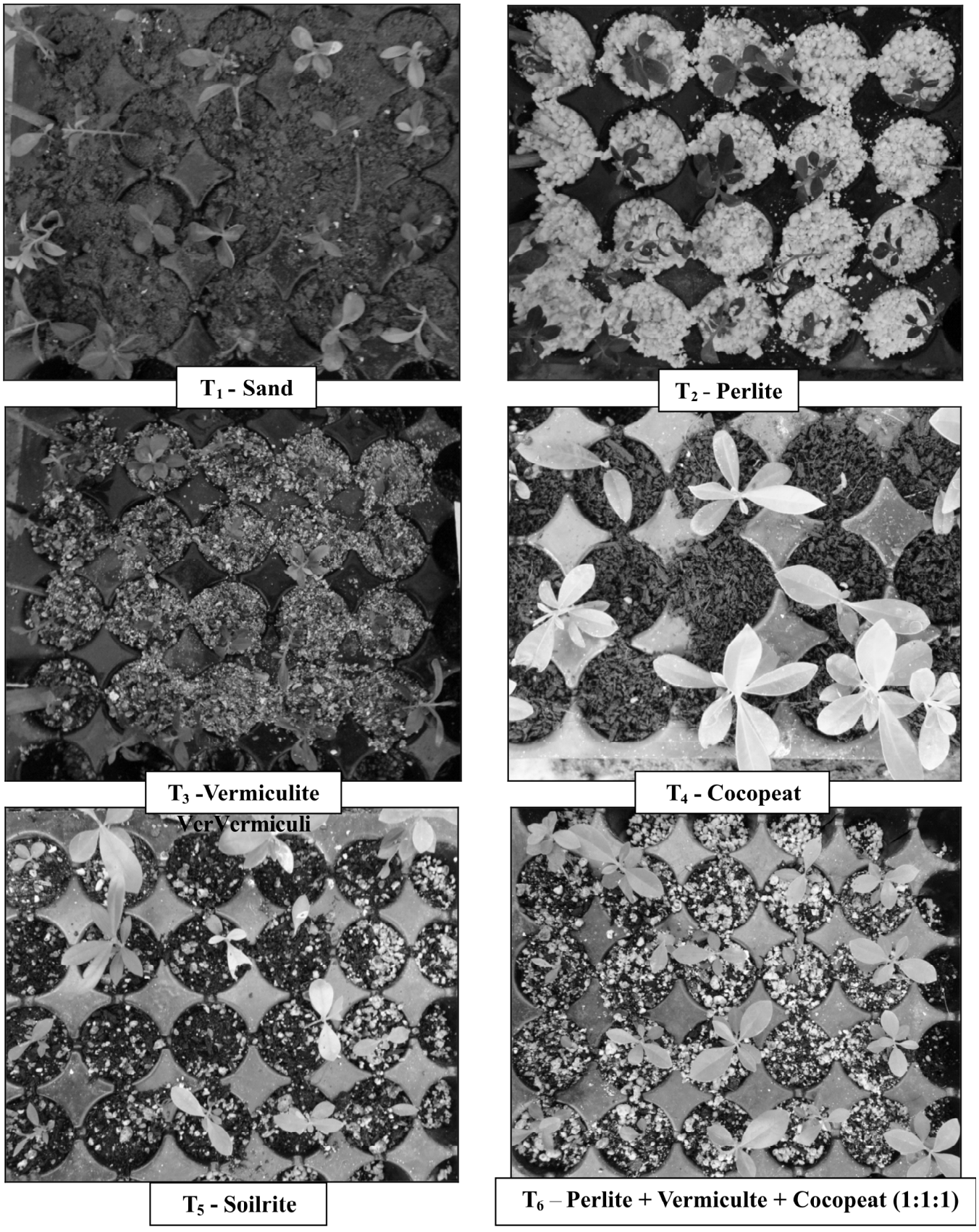


**Figure 2: Different media used for primary hardening**

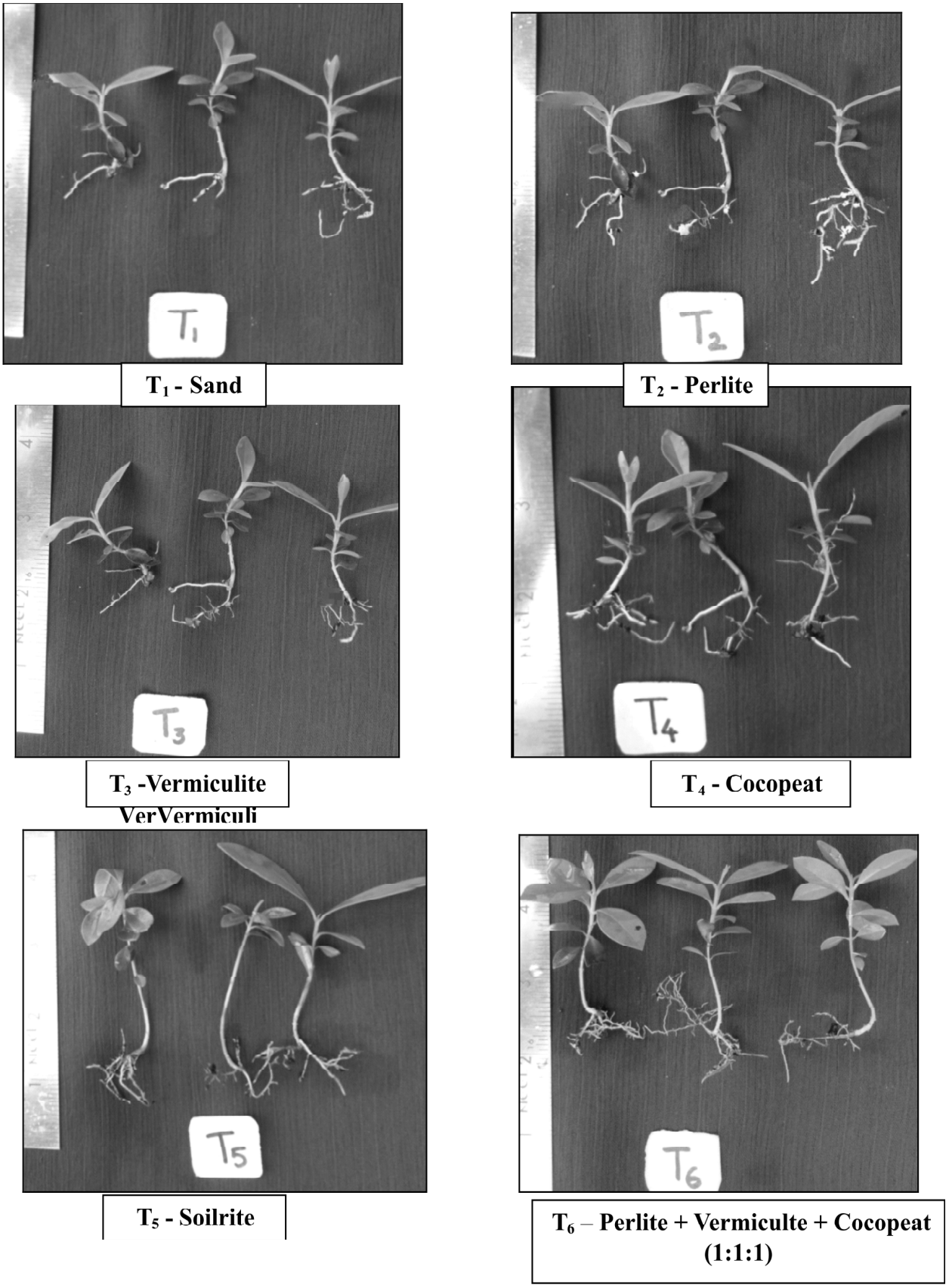


**Figure 3: Primary hardened plantlets of Jamun *cv.* 'AJG-85'**

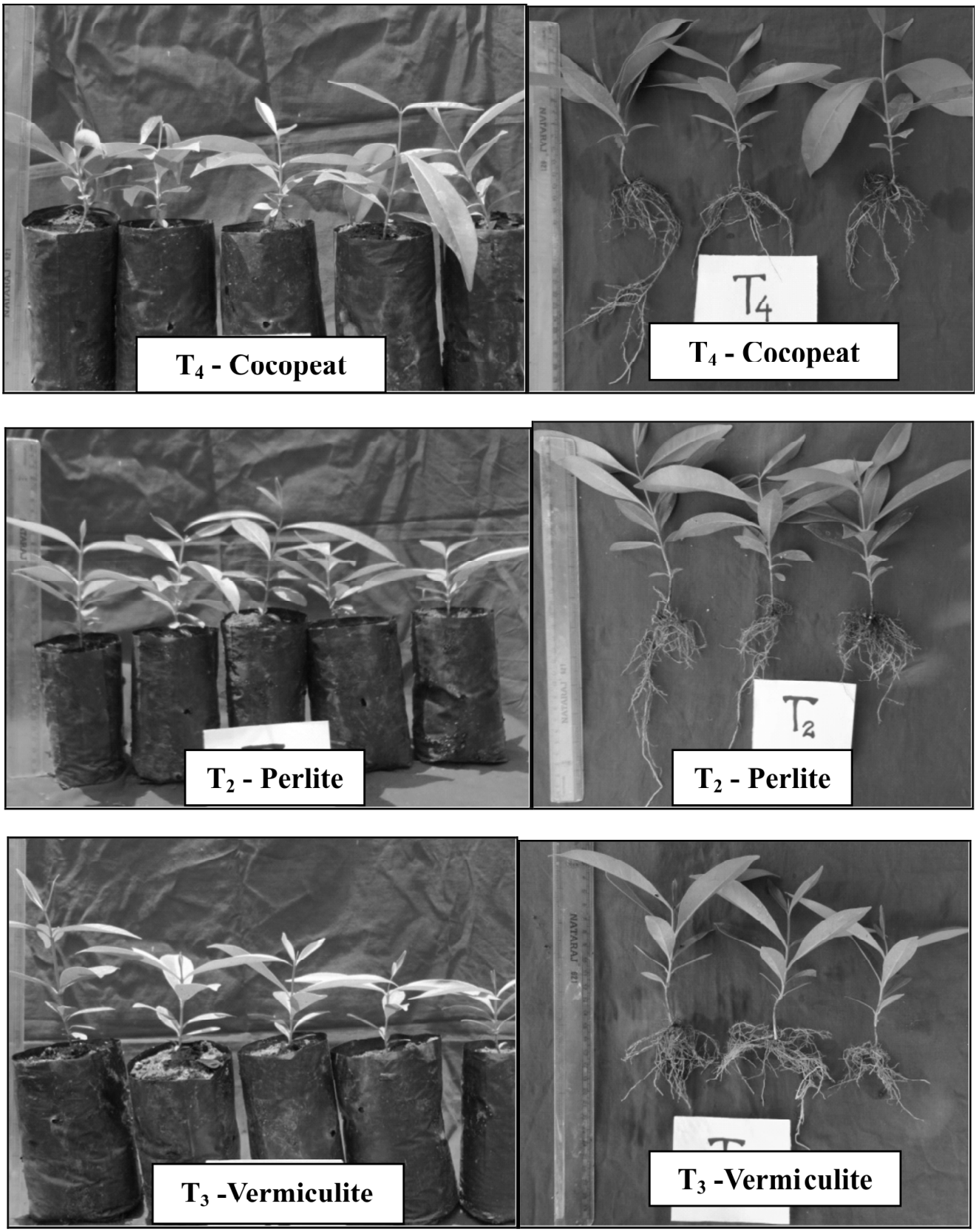


**Figure 4: Secondary hardened plantlets of Jamun *cv.* 'AJG-85'**

**Table 1a**
**Effect of potting media on primary hardening of Jamun plantlets of *cv.* 'AJG-85'**

| | | *Plant establishment after 4 weeks of hardening* | | | | |
|---|---|---|---|---|---|---|
| *Sl. No.* | *Treatments* | *Survival (%)* | *Shoot length (cm)* | *Number of leaves* | *Length of leaves (cm)* | *Breadth of leaves (cm)* |
| $T_1$ | Sand | 70.00 (57.10)* | 5.73 | 10.60 | 3.04 | 1.09 |
| $T_2$ | Perlite (P) | 75.00 (60.27) | 7.10 | 11.50 | 2.99 | 1.42 |
| $T_3$ | Vermiculite (V) | 75.00 (60.27) | 4.76 | 9.80 | 2.72 | 0.96 |
| $T_4$ | Cocopeat (C) | 80.00 (63.44) | 4.53 | 10.55 | 2.43 | 1.20 |
| $T_5$ | Soilrite | 70.00 (57.10) | 5.70 | 11.85 | 2.65 | 1.06 |
| $T_6$ | P+V+C (1:1:1) | 80.00 (63.44) | 6.43 | 12.50 | 2.97 | 1.29 |
| | S.Em± | 4.36 | 0.35 | 0.48 | 0.11 | 0.05 |
| | CD at 1% | NS | 1.03 | 1.44 | 0.33 | 0.14 |

*The values given in parenthesis are arcsine transformed values ($Sin^{-1}$"X/100). NS- Non significant

**Table 1b**
**Effect of potting media on primary hardening of Jamun plantlets of *cv.* 'AJG-85'**
*Root establishment after 4 weeks of hardening*

| *Sl. No.* | *Treatments* | *Number of primary roots/plantlets* | *Number of secondary roots/plantlets* | *Length of roots (cm)* |
|---|---|---|---|---|
| $T_1$ | Sand | 4.25 | 15.85 | 3.85 |
| $T_2$ | Perlite (P) | 4.05 | 15.55 | 4.12 |
| $T_3$ | Vermiculite (V) | 3.95 | 16.85 | 3.56 |
| $T_4$ | Cocopeat (C) | 4.05 | 13.35 | 3.62 |
| $T_5$ | Soilrite | 3.98 | 12.00 | 3.75 |
| $T_6$ | P+V+C (1:1:1) | 4.44 | 15.00 | 3.97 |
| | S.Em± | 0.15 | 0.79 | 0.09 |
| | CD at 1% | NS | 2.36 | 0.27 |

NS- Non significant

**Table 2a**
**Effect of different potting media on secondary hardening of Jamun plantlets *cv.* 'AJG-85'**

Plant establishment after 4 weeks of primary hardening

| *Sl. No.* | *Treatment* | *Survival (%)* | *Shoot length (cm)* | *Number of leaves* | *Length of leaves (cm)* | *Breadth of leaves (cm)* |
|---|---|---|---|---|---|---|
| $T_1$ | Sand # | 65.00 (53.93)* | 7.13 | 10.15 | 8.61 | 3.28 |
| $T_2$ | Perlite (P) | 100.00 (89.73) | 9.09 | 12.70 | 8.76 | 2.93 |
| $T_3$ | Vermiculite (V) | 100.00 (89.73) | 7.86 | 11.10 | 7.78 | 2.42 |
| $T_4$ | Cocopeat (C) | 95.00 (83.17) | 8.58 | 13.20 | 10.15 | 3.45 |
| $T_5$ | Soilrite | 75.00 (63.68) | 8.53 | 10.00 | 8.79 | 3.21 |
| $T_6$ | P+V+C (1:1:1) | 80.00 (66.84) | 8.72 | 12.70 | 10.25 | 3.64 |
| | S.Em± | 5.89 | 0.17 | 0.32 | 0.14 | 0.07 |
| | CD at 1% | 17.51 | 0.51 | 0.95 | 0.40 | 0.22 |

*The values given in parenthesis are arcsine transformed values ($Sin^{-1}$"X/100).
# Red soil was included in all treatments in equal proportions except $T_6$.

**Table 2b**
**Effect of different potting media on secondary hardening of Jamun plantlets *cv.* 'AJG-85'**

*Root establishment after 4 weeks of primary hardening*

| *Sl. No.* | *Treatment* | *Number of primary roots* | *Number of sec. roots* | *Length of roots (cm)* |
|---|---|---|---|---|
| $T_1$ | Sand | 8.00 | 104.55 | 16.73 |
| $T_2$ | Perlite (P) | 5.60 | 67.25 | 18.22 |
| $T_3$ | Vermiculite (V) | 4.40 | 63.80 | 10.70 |
| $T_4$ | Cocopeat (C) | 8.60 | 126.30 | 14.59 |
| $T_5$ | Soilrite | 7.00 | 84.60 | 10.26 |
| $T_6$ | P+V+C (1:1:1) | 8.70 | 160.00 | 14.51 |
| | S.Em± | 0.19 | 1.76 | 0.37 |
| | CD at 1% | 0.56 | 5.24 | 1.10 |

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