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Development of *ex-situ* conservation protocol of Ceylon gooseberry [*Dovyalis hebecarpa* (Gardner) Warb.]

E. M. U. I. Ekanayake and K. M. C. Fernando*

Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya 81100, Sri Lanka.

**Email: menaka@crop.ruh.ac.lk*

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ABSTRACT

Ex-situ conservation techniques are used effectively to preserve many threatened plant species including Ceylon gooseberry from imminent extinction. The present study was conducted to evaluate an integrated conservation approach which includes seed treatments, seedling establishment and rooting of stem cuttings of Ceylon gooseberry (Dovyalis hebecarpa). Three experiments were set up at the Faculty of Agriculture University of Ruhuna from September 2020 to January 2021. Six seed treatments (seed clipping, cold water soak, hot water treatment, rubbing with fine sand, wood ash, and sandpaper) were used to induce germination of seeds. Germination percentage and germination time were significantly different among treatments. The highest final germination percentage (53%) was recorded when seeds were clipped. The best potting mixture for the seedling growth was observed as topsoil: sand: coir-dust: compost, 1:1:1:2 ratio having 60% seedling survival rate. The commercially available PGR and Aloe vera gel were used to induce rooting in semi-hardwood cuttings. Total root length, number of roots, germination percentage, root and shoot vigour were significantly different among treatments. Ceylon gooseberry seedlings and rooted cuttings could be produced in large scale and establish in the field by adopting the propagation protocol developed in the present study.

Keywords: Ceylon gooseberry, *Ex-situ* conservation, potting mixture, seed treatments, semi-hardwood cuttings

INTRODUCTION

The rapid loss of biodiversity is one of the most critical environmental challenges faced by the present generation. Ceylon gooseberry (*Dovyalis hebecarpa*) is also a threatened plant species that belongs to the family Salicaceae. *Dovyalis hebecarpa* is a perennial shrub growing up to 4-6 meters high with spherical shape berries known as Ceylon gooseberry, or *Ketembilla*. It is well grown in lower mountain rain forest areas in Sri Lanka. Ceylon gooseberry has far-reaching, unique medicinal qualities and it is a source of anthocyanin and other phytochemicals with great potential to be used for drug preparation (Bochi *et al*., 2014). The potential health benefits of phenolic compounds present in Ceylon gooseberry are very important for human health and now investigating the antioxidant functions and disease prevention ability of the fruit (Bochi *et al*., 2015). Ceylon gooseberry fruits contain high antioxidant volume and are acidic fruits. Flavonoids and anthocyanins are the main bioactive compounds responsible for the antioxidant activity of the fruit. In folk medicine, Ceylon gooseberry is often used to medicate infections, eye problems, and diarrhoea. According to Bochi *et al*. (2015), this is a good source of phytochemicals that could be used for the human to provide defence against free radicals. Moreover, the exocarp of the fruit contains higher levels of secondary plant metabolites than the pulp. Hence, exocarp is a promising source of natural pigments and antioxidants for commercial applications. About 40% of plants are now in the threat of extinction. Current literature lacks information about the *ex-situ* conservation methods of this fruit. Therefore the objective of the present study is to develop an integrated approach to *ex-situ* conservation of Ceylon gooseberry.

MATERIALS AND METHODS

The present study comprises three experiments. All experiments were carried out at the Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka from September 2020 to January 2021. Plants were grown under controlled conditions in a protected plant house with average temperature of 40^0C and light intensity of 25,000 lux.

Experiment 1

Effect of different seed treatments on germination

The objective of this experiment was to find out the best seed treatment for rapid and quality germination of Ceylon gooseberry seeds. There were six different mechanical and physical seed treatments, *viz.,* T_1-rubbing with fine sand, T_2-rubbing with wood ash, T_3-rubbing in sandpaper, T_4-cold water treatment, T_5-hot water soak and T_6-seed clipping. A completely randomized design was used to set up the experiment with five replicates. Fully mature fresh fruits were collected from well-grown gooseberry plants in Matara district belongs to the low country wet zone of Sri Lanka. Seeds collection was done in September 2020. The length and width of fruits and seeds were measured using a vernier caliper and fresh weights were measured using an electronic balance. Seeds were extracted from ripening fruits and altogether 150 seeds were randomly selected for applying treatments. Then 25 seeds were subjected to each treatment with five seeds per replicate. For the first treatment river sand with 0.125-0.25 mm particle size was used after sieving by a mesh and rub gently for 10 minutes to remove the slimy coat of the seeds and soften the seed coat. As the second treatment timber wood ash was used and it was also gently rubbed for 10 minutes. For the third treatment, sandpaper was used and rubbed the seed sample carefully for 5 minutes. For cold water soak, seeds were soaked 12 hours in cold water while as hot water treatment, seeds were immersed in a 30^0 C water bath for 30 minutes was practiced. Seed clipping was done by damaging the seed coat slightly using a cutter from the pointed end. Then all seeds were wrapped in wet cotton for 2 weeks and during this period germination status was recorded.

Daily germination was recorded for 14 days. Seeds with about 2mm radical were considered as germinated seeds. The final germination percentage (FGP; Equation 1), germination rate index (GRI; Equation 2), mean germination time (MGT; Equation 3), and mean daily germination (MDG; Equation 4) were calculated using standard equations (Aravind *et al.*, 2020; Dharmasena & Arunakumara, 2020) at the end of the experiment.

$$FGP = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ sown} \times 100 \quad \text{... (1)}$$

$$GRI = N1/T1 + N2/T2 + N3/T3 + ... + Nn/Tn \quad \text{..... (2)}$$

N1, N2, N3,……, Nn = number of germinated seeds at a time (days)

T1, T2, T3, …, Tn = number of germinated seeds at a specific time (not the cumulative number).

$$\text{MGT} = \frac{\sum_{i=1}^{k} NiTi}{\sum_{i=1}^{k} Ni} \quad \text{.... (3)}$$

Where; Ti = time taken from the beginning to the ith observation

Ni = number of germinated seeds at the ith time (not the cumulative number, only take the corresponding number relevant to the ith observation)

k = last time of seeds germination

$$\text{MDG} = \frac{Final\ germination\ percentage}{Total\ number\ of\ days} \quad \text{....... (4)}$$

Experiment 2

Effect of different potting mixture for growth and development of seedlings

The objective of this experiment was to select the best potting mixture for seedling growth and survival of Ceylon gooseberry. Germinated seeds of the 1st experiment were sown in nursery trays with compost: sand 1:1 ratio. During the nursery period of three weeks, all the environmental conditions and agronomic practices were equally made to the seedlings to remove any residual effects gained due to various seed treatments. Seedlings which were maintained in nursery trays for three weeks transferred to pots with different potting mixtures (T_1- sand: topsoil: coir-dust: compost 1:1:1:1, T_2- sand: topsoil: coir-dust: compost 1:1:1:2, T_3- sand, topsoil, compost 1:1:1, T_4- sand: coir-dust: compost 1:1:1). Same sized healthy seedlings were selected and planted according to randomized complete block design with five replicates.Survival percentage and a "vigour" scale for the seedlings were recorded after 4 weeks. As the "vigour" scale, (fully burning -on leaves and wilted nature = 0, partially burning sign-on leaves and partially wilted =1, less than 5 healthy leaves without new buds = 2, less than 5 leaves with new buds = 3, more than 5 leaves with new buds and side branches = 4, more than 5 leaves with new buds, side branches and thorns = 5). Similar visual score was used by Engelbrecht *et al*. (2007) to measure the wilting nature of seedlings due to drought stress.

Experiment 3

Effect of commercially available hormone and *Aloe vera* gel on rooting of semi-hardwood cuttings

The objective of this experiment was to find out the effect of commercially available plant growth regulator (PGR) and the *Aloe vera* gel on rooting of stem cuttings of Ceylon gooseberry. Semi-hardwood cuttings of 4-6 inches in length were established in single propagators of size 16''×5''. All the cuttings were in same length and same number leaves with half removed leaf blade and having few active buds (Ambagaspitiya *et al*., 2020). Five different hormone treatments were used (T_1 - commercial PGR- Rapid root® containing 0.3% Indole 3-butyric acid), T_2 - dipped in *Aloe* gel for 2 minutes, T_3 - dipped in *Aloe* gel for 5 minutes, T_4 - dipped in *Aloe* gel for 10 minutes, and T_5 - without any treatment (control). In the case of *Aloe vera* gel, *Aloe* leaves have been separated from the mother plant two days before extracting gel. The lower portion of the base, the tapering top and the sharp spines of the leaf margin were removed while only the mucilage layer was extracted avoiding vascular bundles, the top rind and the bottom rind (Chandegara and Varshney, 2012). Gel paste was prepared without adding water or any substitute.

General potting mixture of topsoil: sand: coir-dust: compost 1:1:1:1/4 was used for filling single propagators and completely randomized design was applied. Five experimental units with 3 replicates were used for each treatment and data were collected after 2 months of initiating single propagators. Survival percentage (Equation 5), the average length of roots (cm), the average number of roots, roots, and shoot "vigour" were evaluated at the end of the trial while lengths of roots were measured manually. "Vigour" scales were introduced to measure the roots and shoot development. As root "vigour" scale, (no callus formation = 0, callus formation = 1, callus and roots initiation = 2, callus and less than five adventitious roots = 3, callus and more than five adventitious roots = 5) and as shoot "vigour" scale, (brown color dried stem = 0, moist stem with a green color = 1, moist stem with green color and less than 3 new buds = 2, moist stem with green color and more than 3 new buds = 3, moist green color stem with more than 3 new buds and less than 2 new leaves = 4, moist green color stem with more than 3 new buds and more than 2 new leaves) were recorded destructively at the end of two months.

$$\text{Survival percentage} = \frac{\textit{Number of rooted} \frac{and}{or} \textit{callus formed stem cuttings}}{\textit{Total number of cuttings used}} \times 100 \quad ..... (5)$$

Statistical analysis

All the data were analyzed using ANOVA by SAS. Final germination percentage data of experiment 1 and survival percentage data of experiment 3 were subjected to arcsine transformation before analysis. To compare the means of treatments Duncan's Multiple Range Test at 5% probability was used.

RESULTS AND DISCUSSION

Table 1 shows the average fresh weight of fruits and seeds of Ceylon gooseberry. The fruit and seed weight varied in the range of 5-7 g and 55-75 mg, respectively. The average size of fruit was 17.17± 1.83 mm in transversal diameter and 16.72±1.97 mm in longitudinal diameter. Similar fruit characteristics evaluation of *Elaeagnus latifolia* L was done by Rymbai *et al*. (2020). *Dovyalis* fruit color varies during ripening from green to intense brown color and fruits reach their maximum size and biomass accumulation point when the exocarp color is brown while seed removal for propagation should be done when fruits are yellowish-green in hue (Villa *et al*., 2019).

Table 1: Average length, width and fresh weight of fruits and seeds

	Transversal diameter (mm)	Longitudinal diameter (mm)	Weight
Fruit	17.17± 1.83	16.72±1.97	5-7 (g)
Seed	3±1.25	4±1.55	55-75 (mg)

The highest final germination percentage (53%) was recorded in seed clipping treatment (T_6) which is in significant with fine sand rubbing treatment (T_1) (Fig. 1). The lowest germination percentage was observed from hot water treatment (T_5) and sandpaper rubbing (T_3). Duval & Nesmith (2000) reported enhanced seed germination of watermelon after done clipping and removing the seed coat. It was found that pre-soaking for more than 12 hours does not improve germination. Fathurrahman *et al*. (2015) investigated that hot water treatment can reduce germination due to embryo damaging when increase the immersing time for *Albizia saman* plant.

Germination rate index (GRI) defines as the number of seeds germinated within a day and here the highest GRI was recorded from seed clipping (T_6, 0.69) and rubbing in fine-sand treatments (T_1,0.63) (Fig. 2). These two treatments indicate the highest and fastest germination (Table 3). The benefits and weaknesses of GRI have been discussed by Mayer (2000).

The shortest time (5 days) was recorded in seed clipping treatment (T_6) and fine sand rubbing treatment (T_1) while the longest time is taken for germination by seeds treated with hot water (T_5) and sandpaper rubbing (T_3)(8 days) (Table 2). In general, the lowest mean of germination time gives the fastest germination. Hence, the highest germination was recorded in T_6 while the lowest was recorded from T_5. However, T_3, T_5 and T_1, T_6 pairs are not significantly different from others (Fig.1).

The highest mean daily germination (12.80 %) was recorded from seed clipping (T_6) while it was not significantly different with the treatment where seeds were rubbed with fine sand (T_1). The lowest MDG (2.5%) was observed from the hot water treatment (T_5) (Table 2). Similarly, the germination measurements are used to make the interpretations and proper decisions during comparisons. Time, rate and, homogeneity can be measured, depicting the dynamics of the germinations. These characteristics are important to predict the success of a species based on the germination within a time frame. Therefore germination is permitting the recruitment of a plant species in a particular ecosystem (Ranal and De Santana, 2006). According to Hartmann *et al.* (1997), mechanical seed treatments are the best for breaking seed coat dormancy. In the present experiment also gave better result in mechanical and physical seed treatments and the findings was supported from the result of Dahanayake (2015) for 'cardamom seeds'.

In experiment 2, the highest survival percentage and seedling "vigour" were showed by T_2 treatment having sand: topsoil: coir-dust: compost 1:1:1:2 and supporting our results by Khater (2015) who noted the importance of compost for the seedling growth, The lowest survival percentage and seedling vigour were observed from the potting mixture (T_3) prepared with sand, topsoil, compost 1:1:1 and reason may be due to the lack of coir-dust in the mixture and so compaction can be happened (Figure 3A and 3B). Nazari *et al*. (2011) has recommended coir-dust as an efficient substitution for potting mixtures. High total pore spaces and water holding capacity of coir-dust may help to grow seedlings in pots and it has been found that growth index shoot and root dry weight of majesty palm increases in coir-dust media (Meerow, 1995).

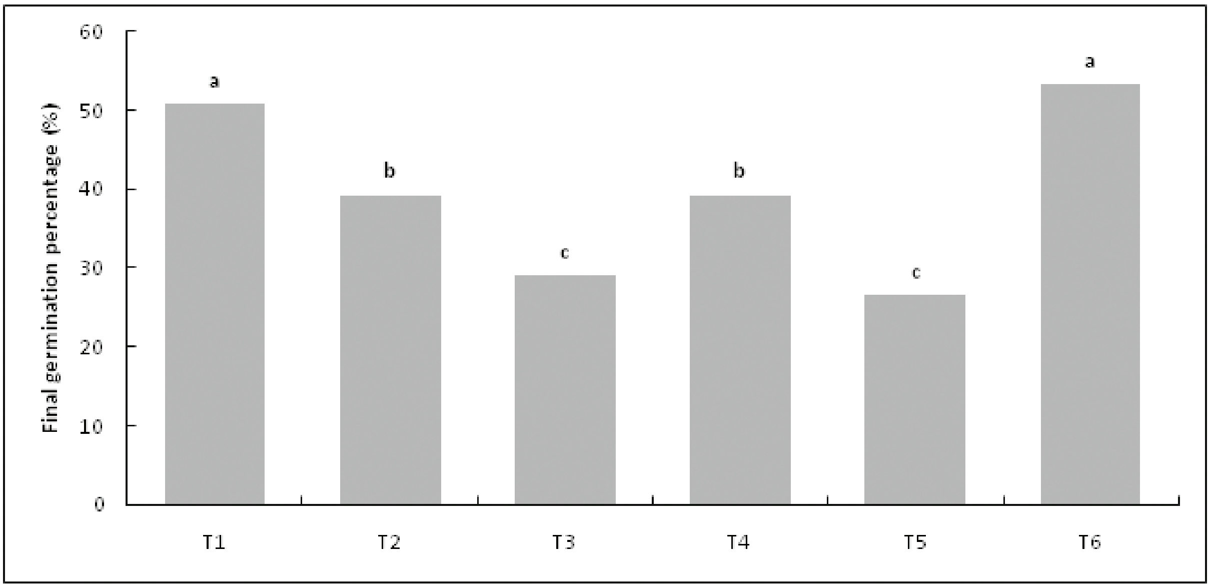

Fig. 1: Effect of different seed treatments on final germination percentage of Ceylon gooseberry seeds.
(T_1-rubbing with fine sand, T_2-rubbing with wood ash, T_3-rubbing in sandpaper, T_4-cold water treatment, T_5-hot water soak and T_6-seed clipping)
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

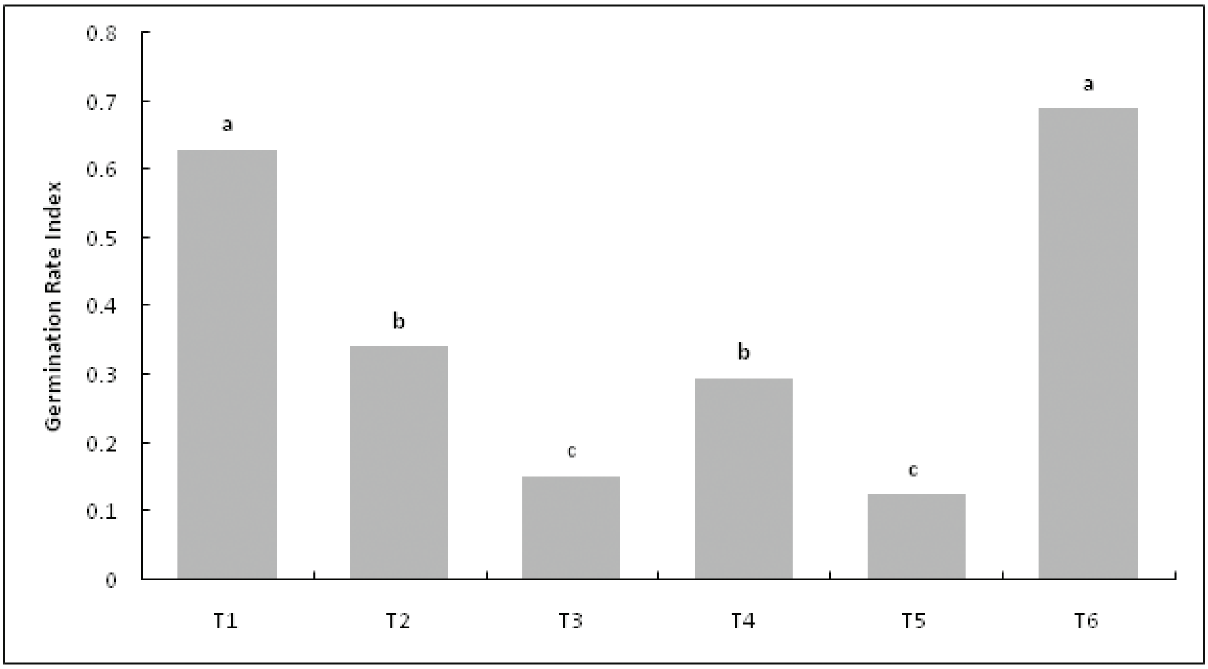

Fig. 2: Effect of different seed treatments on germination rate index of Ceylon gooseberry seeds
(T_1-rubbing with fine sand, T_2-rubbing with wood ash, T_3-rubbing in sandpaper, T_4-cold water treatment, T_5-hot water soak and T_6-seed clipping)
Means with similar letters are not significantly different from each other in $\alpha = 0.05$

Table 2: Average time for initiating germination, mean germination time and mean daily germination of Ceylon gooseberry seeds

Treatments	Average time for initiating germination (Days)	Mean germination time	Mean daily germination
T_1	5	4.796[d]	12.0[a]
T_2	6	5.8[c]	6.66[b]
T_3	8	7.9[a]	3.00[c]
T_4	7	6.6[b]	5.71[b]
T_5	8	8.0[a]	2.5[c]
T_6	5	4.6[d]	12.8[a]
CV	21.2	3.197	12.10

(T_1-rubbing with fine sand, T_2-rubbing with wood ash, T_3-rubbing in sandpaper, T_4-cold water treatment, T_5-hot water soak and T_6-seed clipping).
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

Table 3: Average number of roots, the average length of roots, root vigour, and shoot vigour of semi-hardwood cuttings under different treatments

Treatments	Average number of roots	Average length of roots	Root vigour	Shoot vigour
T1	3.75[a]	6.22[a]	4.50[a]	4.25[a]
T2	1.33[c]	1.20[c]	1.33[c]	1.50[c]
T3	2.44[b]	4.01[b]	2.66[b]	2.33[b]
T4	3.69[a]	5.97[a]	4.55[a]	4.50[a]
T5	1.17[c]	0.59[d]	1.16[c]	1.16[c]
CV	9.35	8.53	10.10	12.25

(T_1- commercial PGR- Rapid root® containing 0.3% Indole 3-butyric acid, T_2 - dipped in *Aloe* gel for 2 minutes, T_3 - dipped in *Aloe* gel for 5 minutes, T_4 - dipped in *Aloe* gel for 10 minutes, and T_5 - without any treatment -control)
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

In experiment 3, the commercial IBA hormone treatment (T_1) and dipping in *Aloe vera* gel for 10 minutes (T_4) were not significantly different from each other for the rooting percentage of semi-hardwood cuttings of Ceylon gooseberry which were giving the highest rooting percentage (Figure 4). Control treatment without any hormone application (T_5) was showed the lowest rooting and our findings are fine-tune with the (Almeida *et al.*, 2004) who used IBA for air layering of Ceylon gooseberry for getting better success.

The highest average number of roots, length of roots, root, and shoot "vigour" were observed from commercial PGR applied cuttings (T_1) and cuttings were dipped in *Aloe vera* gel for 10 minutes (T_4) (Table 3). Increasing in dipping times of stem cuttings in *Alo vera* gel may caused higher accumulation of plant metabolites in the cuttings that increased their rooting numbers and length. According to Mirihagalla and Fernando (2020), root length, the number of roots and root "vigour" of the semi-hardwood cuttings of *Citrus aurantifolia* treated commercial PRG (Rapid root®, 0.3% Indole 3-butyric acid; IBA) and dipped cuttings ends in fresh *Aloe vera* gel for two and five minutes were not significantly different. Shidiki *et al.* (2019) showed that IBA, *Aleo vera* gel, and coconut water showed comparable influence on root initiation while number of primary roots was highest in *Aloe vera* gel treatment. They were dipped in 2 minutes in each treatment. Similarly, Uddin *et al.* (2020) showed that the highest root development and survival percentages were recorded from IBA

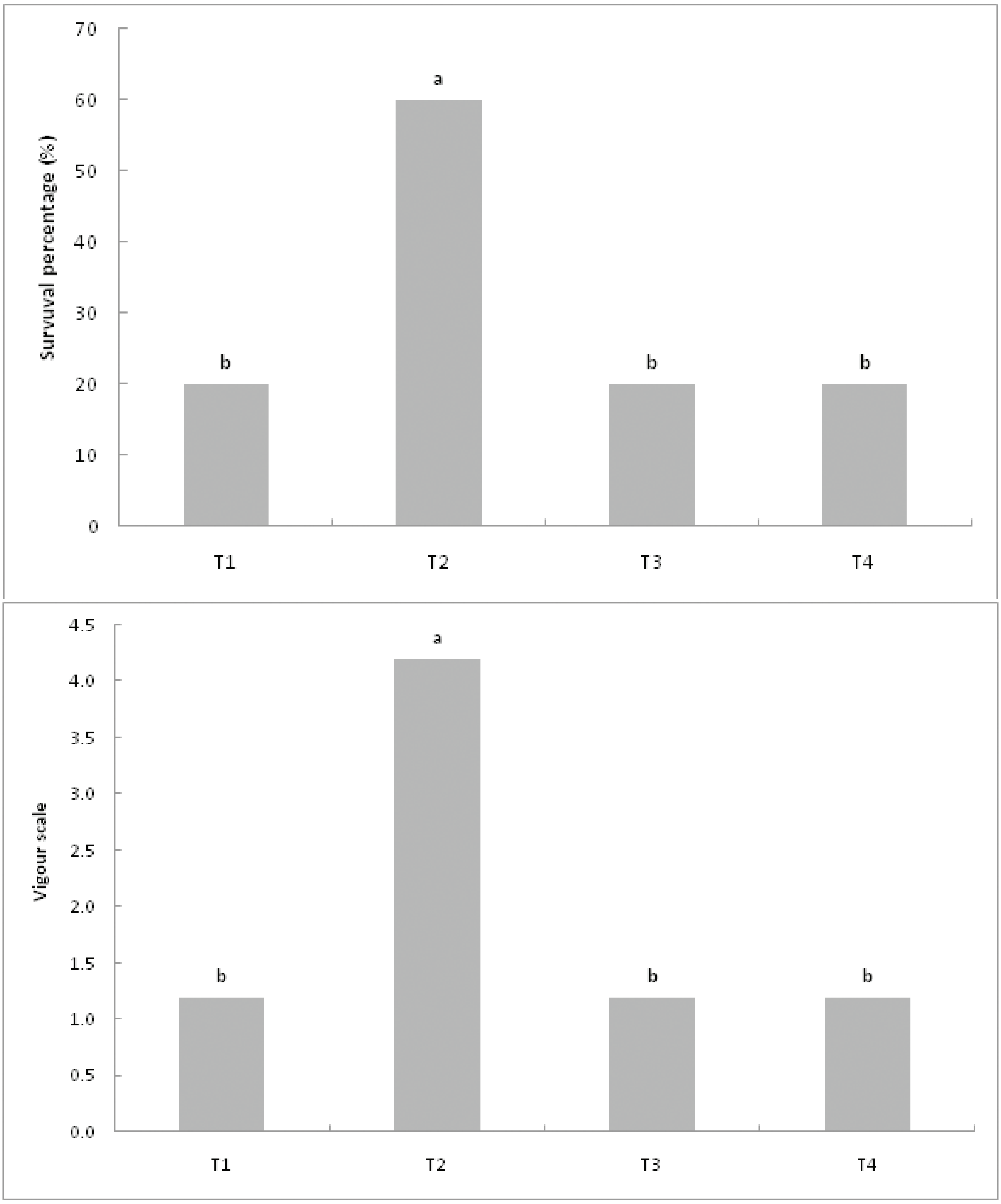

Fig. 3: Effect of different potting mixtures on (A) survival percentage of seedlings and (B) "vigour" of seedlings
(T_1- sand 1: topsoil 1: coir-dust 1: compost 1, T_2- sand 1: topsoil 1: coir-dust 1: compost 2, T_3- sand 1, topsoil 1, compost 1, T_4- sand 1: coir-dust 1: compost 1)
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

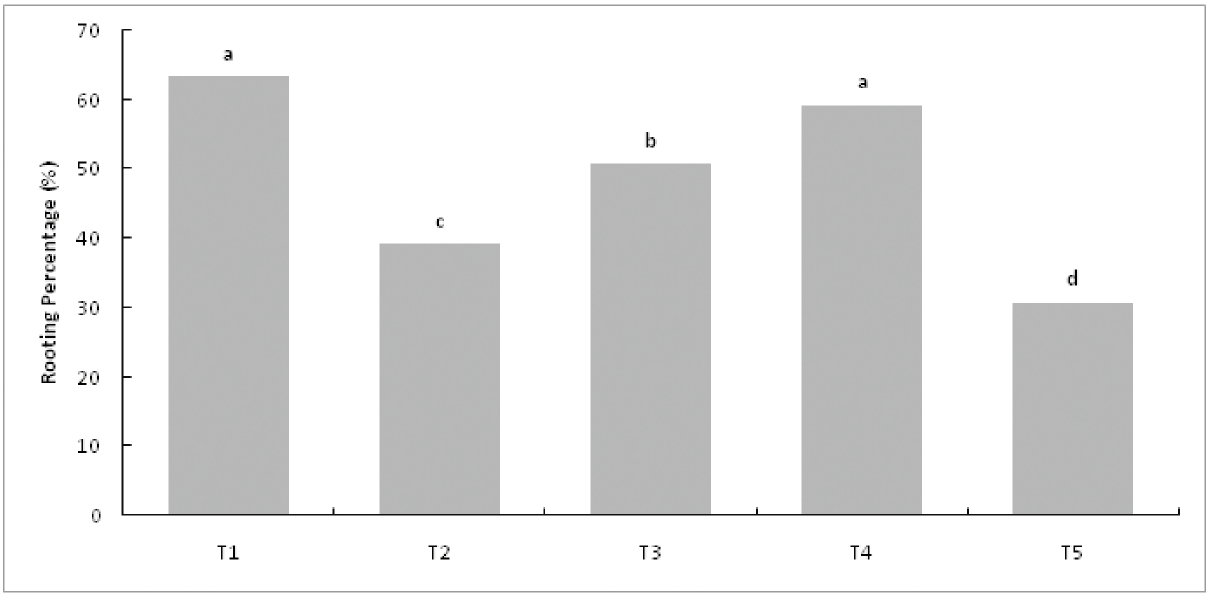

Fig. 4: Effect of different treatments on rooting percentage of semi-hardwood cuttings of Ceylon gooseberry two months after establishment
(T_1- commercial PGR- Rapid root® containing 0.3% Indole 3-butyric acid, T_2 - dipped in *Aloe* gel for 2 minutes, T_3 - dipped in *Aloe* gel for 5 minutes, T_4 - dipped in *Aloe* gel for 10 minutes, and T_5 - without any treatment -control).
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

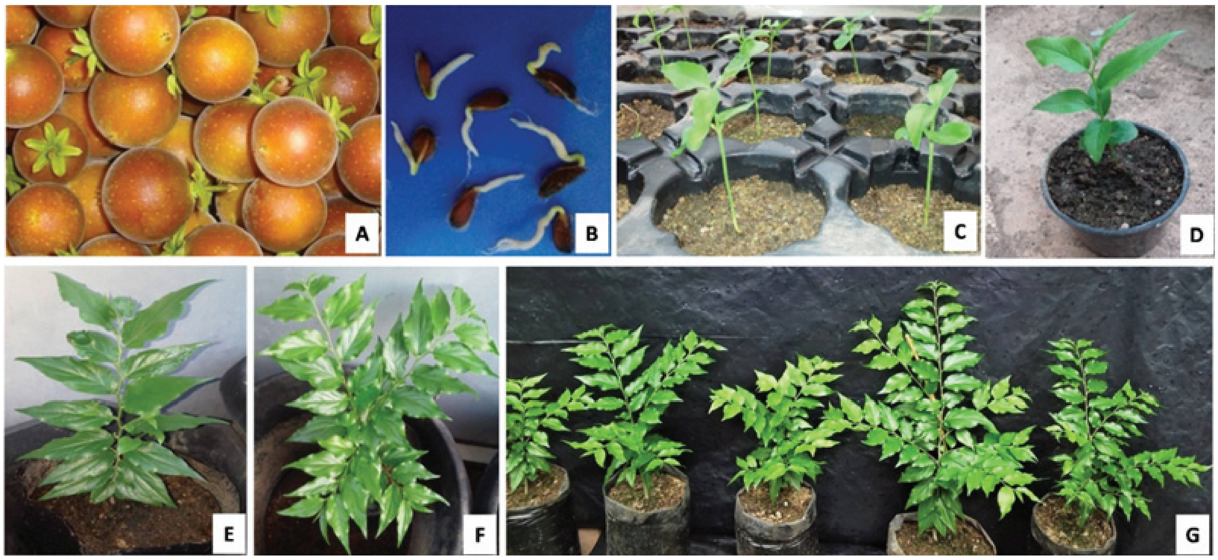

Plate 1: (A) Ripen fruits of Ceylon gooseberry (B) germinated seeds (C) seedling tray nursery (D) transferred seedling to a pot (E) two months old seedling (F) three months old seedling (G) seedlings are ready to be transplanted in the field

which results were closed to natural *Aloe vera* gel treatment. The lowest rooting and "vigour" were showed in the control treatment without any hormone or PGRs (Table 3). *Aloe vera* gel contains mainly polysaccharides and many other compounds like carboxypeptidase, minerals, glucose, vitamins, amino acids, auxins, and gibberellins (Shariff Moghaddasi and Verma, 2011). The use of cuttings for propagation is a fast method and it allows bringing favorable mother characteristics having an agronomic interest (Pourghorban *et al.*, 2019).

CONCLUSION

Conservation of Ceylon gooseberry (*Dovyalis hebecarpa)* species is essential by using propagating techniques since it is gradually disappearing from the natural habitats. According to the results of three experiments, it can be concluded that the physical and mechanical seed treatments can be used to enhance seed germination while potting mixture prepared by sand: topsoil: coir-dust: compost 1:1:1:2 could be used for seedling growth. Commercially available PGRs like Rapid root® (0.3% Indole 3-butyric acid) and cutting ends dipped in *Aloe vera* gel for 10 minutes could be recommended for inducing rooting of semi-hardwood cuttings. Furthermore, for rooting of Ceylon gooseberry, commercial PGRs can be effectively replaced by *Aloe vera* gel. The commercial scale production of Ceylon gooseberry seedlings and rooted cuttings can be achieved by following the propagation protocol developed in the present study.

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