International Journal of Minor Fruits, Medicinal and Aromatic Plants. Vol. 6 (1) : 20- 24, June 2020

Variation of shoot regeneration capacity of cuttings of selected *Cinnamomum verum* genotypes

M. R. Prathibhani[1], R. Azad[1<%] and S. Geekiyanage[2*]

[1] Faculty of Graduate Studies, University of Ruhuna, Matara, Sri Lanka
[2]Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka
[<%]Current Address: Plant Biotechnology Division, National Institute of Biotechnology, Dhaka, Bangladesh
**Email: sudarshanee@agbio.ruh.ac.lk*

Received : 17.11.2019 ; Revised : 05.02.2020 ; Accepted : 10.02.2020

ABSTRACT

Vegetative propagation is practiced for elite planting material of cinnamon, which needs extensive labour. This study is aimed to determine the shoot regeneration capacity of cuttings of three Cinnamomum verum genotypes. Cuttings were collected from one cinnamon accession from Faculty of Agriculture, University of Ruhuna and, Commercial varieties Sri Gemunu and Sri Wijaya from National Cinnamon Research and Training Center, Pallolpitiya, Sri Lanka. Perimeter of cuttings was measured to group the cuttings as 2-3 cm, 4-6 cm and 7-8 cm. Three replicates of each genotype were taken from each perimeter group for a completely randomized design (CRD). Cuttings were kept in tap water filled jam bottles. A cutting consisted with 1 to 5 nodes. The length of a cutting varied from 20-30 cm. Cuttings were placed in natural open environment at Faculty of Agriculture from May, 2019. Sri Gemunu did not produce buds for the experimental period of 8 weeks. Sri Wijaya produced one bud only from a cutting of 6-8 cm perimeter group, which did not develop in to a shoot. Cinnamon accession from Faculty of Agriculture produced buds in all three replicates of 7-8 cm perimeter group at 3-4 weeks. Bud regeneration frequency of 4-6 cm group was 2/3. All buds of above accession developed into shoots. None of the genotypes produced buds in 2-3 cm perimeter group. Above results provide an insight on genetic diversity of cinnamon, which would be useful in developing method of efficient plant material production in long run.

Keywords : Cinnamomum verum, shoot regeneration, Sri Gemunu, Sri Wijaya

INTRODUCTION

Sri Lankan cinnamon (*Cinnamomum verum*) is a unique spice in export market. Sri Lanka is the world's largest true cinnamon producer. The export volume of cinnamon from Sri Lanka was 14 692.8 Mt in year 2017 (Sri Lanka Customs, 2017). *Cinnamomum* genus in Family Lauraceae consists of about 250 species and sub-species (Mabberly, 2008). There are seven wild cinnamon species endemic to Sri Lanka, which could be used as genetic resources for crop improvement of cultivated cinnamon (Sitharan, 1984; Kumarathilake *et al*., 2010). Sri Lankan cinnamon germplasm exhibits a wide morphological and chemical diversity (Azad *et al*., 2016; Azad *et al*., 2019; Azad, 2017). Two varieties of cinnamon named *Sri Gemunu* and *Sri Wijaya* from Department of Export Agriculture, Sri Lanka (2019) are based on the oil yield and quality parameters. Plant material is selected from the mother plant having desirable characters of erect stems with smooth bark, vigorous growth, pest and disease resistance, good peeling qualities, good fragrance and taste, higher dry bark yield, higher leaf and bark oil yield and oil quality. Seed propagation is the most common propagation method for cinnamon in Sri Lanka, according to the Department of Export Agriculture, Sri Lanka (2019). Cinnamon fruits are 15 to 20 mm in length (Azad *et al*, 2019) and each fruit contains a seed. Farmers collect well-ripened fruits from desirable mother plants. Recalcitrant cinnamon seeds can be kept viable for about six months under dark conditions by mixing with sand and storing in air tight poly bags. Higher seed germination percentages could be obtained if sown immediately after harvesting (Kannan and Balakrishnan, 1967).

Protogynous dichogamy in cinnamon is a reason for the cross pollination. There are two types of cinnamon plants, named A and B in any natural population. In type A plants and type B plants, the first flowering occurs in the morning and in the afternoon respectively. Cinnamon flowers are bisexual (Joseph, 1981). The stigma is receptive

Table 1: Record of shoot initiation of three perimeter groups of the cinnamon accession collected from Faculty of Agriculture, University of Ruhuna, Sri Lanka

Perimeter above 5 cm from the base	Replicate 1	Replicate 2	Replicate 3
7-8 cm	3 red colour and 1 green colour shoots	Only one green bud	4 red colour shoots and 5 green colour buds
4-6 cm	One green shoot	5 green colour shoots and 5 green colour buds	No green colour buds or shoots
2-3 cm	No green colour buds or shoots	No green colour buds or shoots	No green colour buds or shoots

during the first opening and anthers dehiscent at the second opening. Azad *et al* (2015) reported the possibility of cross pollination by comparing the mother plants and their progenies for age independent leaf morphological characters of leaf shape, leaf base and leaf apex. None of the seedlings were 100% similar to their mother plants. New phenotypes for leaf shape and leaf base were produced. Elite plant material to mother plant cannot be obtained from seeds due to cross pollination. Such seeds result in a heterogeneous cinnamon population.

Vegetative propagation is carried out through cuttings or layering to overcome the effect of cross pollination for elite and true to type planting material. Semi hard wood stem cuttings (with 1 to 2 leaves) of 2.5- 4.0 cm in length, are planted in poly bags filled with a mixture of equal parts of top soil, cow dung, sand and coir dust. Pots are kept in propagators maintaining 100% RH to prevent water loss from cuttings. Prevention of pest damages and fungal infestations are important inside the propagator. After thirty days, cuttings with shoots are placed outside in a shady place and watered regularly until about 30 cm heights. A ring bark is removed from semi hard wood shoots for the air layering. Rooting hormones should be applied on wounded area. Moist coir dust is wrapped on it with a sheet of polythene. It takes 40-60 days for rooting. Well rooted layers can be separated and transplanted in poly bags. Azad *et al* (2019) conducted an eco-geographical survey in major cinnamon growing districts in Sri Lanka and collected stem cuttings for the establishment of a core-collection at Faculty of Agriculture, University of Ruhuna. The average shoot regeneration percentage of collected 269 accessions was 47.76% after one month of planting. Only 80% of accessions survived until field planting. A protocol for micro-propagation of cinnamon using explants of axillary buds was reported from *in-vitro* grown seedlings. An embryonic axis with half of the cotyledon portions were excised from sterilized seeds with 15% Clorox for 20 min and inoculated in half strength MS medium supplemented with (1.5 mg L^{-1}) BAP, (0.2 mg L^{-1}) IAA and activated charcoal (1.0 g L^{-1}). After 14 days of culture establishment micro stem cuttings were transferred to the full strength MS medium supplemented with (0.1 mg L^{-1}) NAA, (4.0 mg L^{-1}) BAP and activated charcoal (1.0 g L^{-1}) for root initiation. Rooted plantlets were acclimatized using coir dust as the potting medium for maximum survival of 90% (Subasinghe *et al.*, 2016). However, the available measures of vegetative propagation provide a limited number of planting material for farmers due to low shoot regeneration rate in addition to the requirement of intensive labour and long period of propagators.

Development of alternative methods of vegetative propagation is required to fulfill the heavy demand for planting material by cinnamon growers, which cannot be supplied completely due to the inability to supply for the demand. Most farmers depend on seeds, which result in a heterogeneous population leading to depletion of quality of yield. Plant tissue culture techniques for cinnamon propagation are practically not feasible due to high cost of production. Vegetative propagation, being the most common alternative

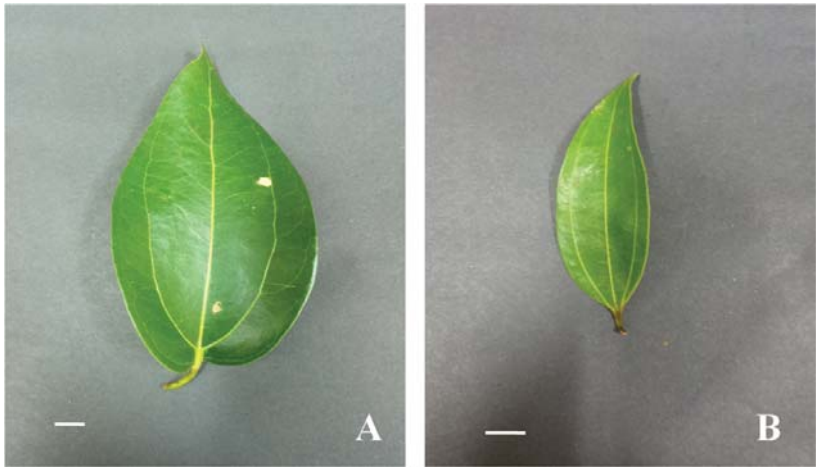

Figure 1: *Sri Gemunu* (A) and *Sri Wijaya* (B) leaves. Scale: 1 cm

Figure 2: Shoot regeneration from cinnamon cuttings in water for 4 weeks. A: 4-6 cm perimeter cuttings, C: cinnamon accession, G: *Sri Gemunu* and W: *Sri Wijaya* B: 7-8 cm perimeter cuttings, C: cinnamon accession, G: *Sri Gemunu* and W: *Sri Wijaya,* C:c_1; more than 8 cm, c_2; 7-8 cm, c_3; 4-6 cm cuttings of cinnamon accession from Faculty of Agriculture, University of Ruhuna

Figure 3: Two cuttings of the cinnamon accession collected from Faculty of Agriculture, University of Ruhuna on soil after one month of transplanting. (A) Cutting from above 8 cm perimeter group (B) Cutting from 7-8 cm perimeter group (C) Cutting of above 8 cm perimeter group at flowering

method, requires intensive labour and propagators, while only semi hard wood is effective. This study is an initial attempt on developing an alternative shoot regeneration method based on genetic potential of different cinnamon genotypes to avoid above drawbacks.

MATERIAL AND METHODS

Cuttings of one cinnamon accession were collected from Faculty of Agriculture, University of Ruhuna, Sri Lanka (GPS: 6.061445 N, 80.567595 S) and, cuttings of *Sri Gemunu* and *Sri Wijaya* were collected from National Cinnamon Research and Training Center, Pallolpitiya, Sri Lanka (GPS: 6.028302 N, 80.559563 S). Perimeter of cuttings was measured to group the cuttings as 2-3 cm, 4-6 cm and 7-8 cm. Cuttings consisted of three replicates of each genotype were taken from each perimeter group for a completely randomized design (CRD). Cuttings were kept in tap water filled jam bottles. A cutting consisted with 1 to 5 nodes. Cuttings varied from 20-30 cm in length. Cuttings were placed under shade of trees in natural open environment at Faculty of Agriculture from May, 2019. Shoot regeneration capacity of cuttings of different thicknesses from three cinnamon genotypes was determined in open environment. Two commercial varieties of *Sri Gemunu* and *Sri Wijaya* were included in order to check their potential for alternative method (Figure 1). Bud break and shoot regeneration was recorded from the cuttings. Cuttings with shoots were transplanted directly on soil for rooting.

RESULTS AND DISCUSSION

During this study, commercial variety *Sri Gemunu* did not produce buds for the experimental period of 8 weeks. *Sri Wijaya* produced one bud only from a cutting of 7-8 cm perimeter group, which did not develop in to a shoot. Above results suggests that two commercial varieties may not be suitable for the above method. Cinnamon accession from Faculty of Agriculture produced buds in all three replicates of 7-8 cm perimeter group at 3-4 weeks (Figure 2). Bud regeneration frequency of 4-6 cm group was 2/3 (Table 1). All buds of above accession developed into shoots. None of the genotypes produced buds in 2-3 cm perimeter group.

The transplanted cuttings with shoots further developed leaves at the end of the first month on soil (Figure 3; A and B). The fungal growth was observed around the basal area of the cutting edge after one month of transplanting suggesting further investigations on cinnamon and associated fungi on root development and nutrient uptake. The transplanted cutting of the 8 cm perimeter group flowered after four months. The cutting belonged to flower type A (Figure 3; C).

The age of the cinnamon accession from Faculty of Agriculture may be different from the two commercial varieties. Further, the commercial varieties were originated through vegetative propagation and the accession from the Faculty of Agriculture must be seed borne as it was grown in the wild. Therefore, the differential shoot regeneration potential among three genotypes may be due to physiological differences led by above factors. However, above results would not exclude the provision of an insight on potential genetic diversity of cinnamon, which would be useful in developing method of efficient plant material production in long run.

REFERENCES :

Azad, R. 2017. Genetic diversity of Cinnamomum verum germplasm in Sri Lanka. PhD thesis, University of Ruhuna, Sri Lanka.

Azad, R., Jayasekara, L., Ranawaka, R.A.A.K., Senanayaka, G., Kumara, K.LW., Pushpakumara, D.K.N.G. and Geekiyanage, S. 2019. Development of a core collection for Sri Lankan cinnamon germplasm based on morphological characterization using an eco-geographical survey. *Australian J. of Crop Sci.,* **13** (09): 1473-1485.

Azad, R., Ranawaka, R.A.A.K., Senanayake, G., Kumara, K.W., Pushpakumara, D.K.N.G., Wijesinghe, K.G.G. and Geekiyanage, S. 2016. Morphological variation of cinnamon (*Cinnamomum verum* Persl) germplasm in Matara District of Sri Lanka. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **2**(1):6-14.

Azad, R., Senanayake, G., Kumara, K.L.W., Ranawaka, R.A.A.K., Pushpa-Kumara, D.K.N.G., Wijesinghe, K.G.G. and Geekiyanage, S. 2015. Morphological variation within progeny and deviations from mother plant reveal the allele richness in *Cinnamomum verum* germ-plasm: a case study from Deiyandara, Matara collection at the early vegetative stage *Tropical Agricultural Research and Extension*, **18**(4):163-167.

Department of Export Agriculture, Sri Lanka., 2019. Cinnamon. [Online] Available at: <http://www.exportagridept.gov.lk/web/index.php?option=com_content&view=article&id=128&Itemid=159&lang=en> [Accessed 11 October 2019].

Joseph, J. 1981. Floral biology and variation in cinnamon. In S. Vishveshwara (ed.) *Proc. PLACROSYM c!*, ISPC, CPCRI, Kasaragod, India, pp. 431-434.

Kannan., K. & Balakrishnan., S. 1967. A note on the viability of cinnamon seeds (*Cinnamomum zeylanicum* Nees). *Madras Agric. J.* **54**: 78-79.

Kumarathilake, D.M.H.C., Senanayake, S.G.J.N., Wijesekera, G.A.W., Wijesundera, D.S.A. and Ranawaka, R.A.A.K., 2010. Extinction risk assessment at the species level: national red list status of endemic wild cinnamon species in Sri Lanka. *Tropical Agricultural Research*, **21**(3): 247-257.

Mabberley, D.J. 2008. *Mabberley's plant-book: a portable dictionary of plants, their classifications and uses*. 3rd edition. Cambridge University Press.

Sri Lanka Customs., 2017. *Sri Lanka Customs Annual Performance Report 2017*. Sri Lanka Customs.

Sritharan, R. 1984. The study of the genus Cinnamomum. M. phill thesis, Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

Subasinghe, S., Hettiarachchi, C.S. and Iddagoda, N. 2016. In-Vitro Propagation of Cinnamon (*Cinnamomum verum* Presl.) Using Embryos and in Vitro Axillary Buds. *Journal of Advanced Agricultural Technologies*, **3**(3):164-169.