Model for Tree Volume Estimation and Determination of Root-Shoot Relationship and Biomass Production of *Annona Glabra* in Thalangama Tank, Sri Lanka

PG Dikwaththa [1*], DT Jayawardana[1] and D Pindeniya[2]

[1]Department of Forestry and Environmental Science, University of Sri Jayewardenepura, Sri Lanka
[2]Wetland Management Division, Sri Lanka Land Reclamation and Development Corporation

Abstract

Tree volume is one of the most important and difficult variable in an ecosystem. It has to be predicted using a reliable method since all the management decisions are taken on the volume production of the tree. *Annona glabra* is a widely spread invasive species which can significantly impact native communities and ecosystems in Sri Lanka by widely reducing biodiversity. The objectives of the study were to (a) construct a volume predication model, (b) determination of the root and shoot ratio, (c) measure biomass of *A. glabra*, and root: (d) determine shoot variation with DBH, tree height and tree volume of *A. glabra* for making suitable management decisions. Random sampling was used in 0.01ha sample plots on Thalangama tank to collect the data of 60 individual trees stratified by age into two age classes of 7 years (2009-2016) and 12 years (2004–2016). All the reliable tree measurements of each tree were measured by using standard methods. Using these measurements, log volume was estimated by Newton's formula and the volume of final section was calculated by assuming as a cone. The tree volume was then calculated by adding all the section volumes together. Total biomass was estimated using fresh weight and dry weight of destructive tree samples. Estimated mean total tree biomass for 7 years and 12 years old *A. glabra* were 6.98 kg per tree (±0.42) and 7.59 kg per tree (±0.40) respectively. Estimated root: shoot ratio for 7 years and 12 years *A. glabra* in Thalangama tank area were 1: 2.6 and 1: 2.4 respectively. Model for tree age prediction was built as a function of tree height while tree volume prediction model was built as a function of tree height and basal area using SPSS. The best models were selected based on R^2 value and distribution of the standardized residual. Accordingly, selected models for tree age and tree volume with the R^2 values were 94% and 92% respectively. Finally, it is recommended that the volume prediction model of *A. glabra* able to use as management decision tool in Sri Lankan wetlands.

somatic embryos, to identify suitable medium and growth regulator combination for producing viable somatic embryos, to compare the effect between liquid and solid medium, to compare different tea cultivars responses and the role of Silver nitrate in *se*.

In this study first time, the somatic embryos were obtained from *ex vitro* nodal explants in Tea Research Institute of Sri Lanka. This is the first report that testing effect of Silver nitrate on promoting *se* of tea. Hence, this study has provided a novel approach to induce *se* as an alternative method of vegetative propagation of tea.

Materials and Methods

The research was conducted at the tissue culture laboratory of the plant breeding division of Tea Research Institute (TRI), Talawakelle. Different explants (cotyledons, stem cuttings, leaf callus, leaf segments) from different cultivars (TRI2024 and TRI2043) were used to induce somatic embryos directly from cotyledons (mature and immature) and *ex vitro* stem (nodal and internodal) cuttings of TRI2024 cultivar on solid media and indirectly from *in vitro* leaf callus of both TRI2024 and TRI2043 cultivar on both solid and in liquid media and *ex vitro* leaf

were inoculated inside the culture vessels containing different plant growth regulator combination in culture media. The cultures were kept under controlled conditions for incubation. After that they were sub cultured at regular intervals onto fresh media.

The cultures were observed carefully and morphological changes were recorded. Qualitative measurement (colour, callus induction, texture) and quantitative measurement (somatic embryo induction, weight of callus and surface area of callus) were taken for analysis. Number of culture vessels which had induction of somatic embryos among total number of replicates in every treatments were taken in a percentage as a quantitative measurement. The CRD (Completely Randomized Design) was used as the experimental design for all experiments (in experiment 1 and 2, two factorial CRD with treatment and explant as factors; in experiment 3, three factorial CRD with cultivar, treatment and media formulation as factors and in the experiment 4, two factorial CRD with treatment and media formulation as factors).

Ten replicates were taken for each treatment. The somatic embryo induction and callus induction percentage values were transformed

Table 1: The different explants, media and their formulation with different plant growth regulators combinations (mg/L)

Explants		Media form	Callus Induction	Callus Proliferation	Somatic embryo Induction
Cotyledon (TRI2024)	Mature and Immature	Solid	----N/A----		(M1)- 2 BAP + 0.2 NAA
					(M2)- 2 BAP + 0.2 NAA + 8.6 AgNO$_3$
Callus (TRI2024 and TRI2043)	*In vitro* leaf callus	Solid and Liquid	2 BAP + 3 NAA		(M3)- 2 BAP + 3.5 NAA
Leaf (TRI2024)	3$^{rd.}$ leaf		2 BAP + 3 NAA + 8.6 AgNO$_3$		(M4)- 2BAP + 3.5 NAA + 8.6 AgNO$_3$
Nodal (TRI2024)	Nodal and Inter Nodal	Solid	----N/A----		(M5)-0.5 BAP + 3 GA$_3$ + 0.1 IBA
					(M6)-0.5 BAP + 3GA$_3$ + 0.1 IBA + 8.6 AgNO$_3$
					(M7)- 2 BAP + 0.2 NAA

explants of TRI2024 cultivar on both solid and in liquid media with different plant growth regulators (Table 1).

The explants were sterilized by using disinfectants inside the laminar flow. Then they

using Arcsine transformation before statistical analysis. Data were analyzed by ANOVA. Both quantitative and qualitative data were subjected to ANOVA and mean separation procedure was done using SAS version 9.2 for windows and

results were interpreted at 5% probability level. The comparison of mean was done with LSD test at $P < 0.05$.

Results and Discussion

Se of tea varies with explants and their maturity stages, plant growth regulator combinations of the media and their formulations and also different cultivars. All the explants were responded well to *se* by tissue culture under *in vitro* conditions.

Experiment 1: Direct somatic embryogenesis in cotyledons

Somatic embryos were observed in immature cotyledons on MS medium contained 2 mg/L BAP + 0.2 mg/L NAA + 8.6 mg/L $AgNO_3$ after 8 weeks of culture. In this experiment, based on response of morphological changes and somatic embryo induction sample%, immature cotyledon responded better than mature cotyledon after 8 and 10 weeks of culture. Similar results reported by Bano *et al.*, (1992) the potential of embryogenesis in immature cotyledon segments were more responsive than mature ones. On the contrary, Abeywardana, (2016) reported that mature stage of cotyledons gave more response than immature cotyledons. This may be due to the lesser accumulation of reserve materials in the late immature cotyledons.

Experiment 2: Direct somatic embryogenesis from ex vitro stem cuttings

Somatic embryo induction was firstly observed in nodal explants 5 weeks after culturing while the internode explant started somatic embryo induction after 6 weeks. Somatic embryos were observed 10 weeks after culturing (Plate 1).
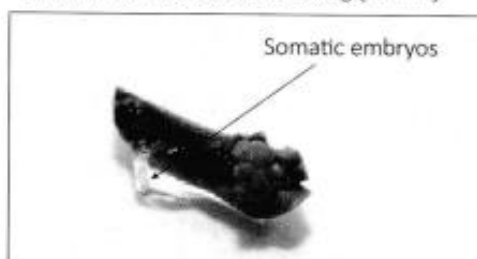

Somatic embryos

Plate 1: Development of somatic embryos from nodal explant after 10 weeks on M1 medium

Similar observations were reported by Akula and Dodd (1998). The highest somatic embryos were obtained from nodal explants on MS medium contained 0.5 mg/L BAP + 3 mg/L GA_3 +

0.1 mg/L IBA. On the contrary, MS medium contained 0.5 mg/L BAP + 3 mg/L GA_3 + 0.1 mg/L IBA + 8.6 mg/L $AgNO_3$ highly responded to internodal explants. Ishizaki *et al.* (2000) stated that Silver nitrate has been used extensively in various plant species to induce somatic embryogenesis. Moreover, MS medium contained 0.5 mg/L BAP + 3 mg/L GA_3 + 0.1 mg/L IBA and nodal explants are also suitable for direct *se*.

Experiment 3: Somatic embryo induction from in vitro leaf callus

Callus induction and callus proliferation were observed in leaf callus of TRI2024 cultivar on solid medium contained 2mg/L BAP + 3mg/L NAA meanwhile somatic embryo induction was observed firstly in leaf callus of TRI2024 cultivar on solid medium contained 2mg/L BAP + 3.5mg/L NAA + 8.6 mg/L $AgNO_3$. Among the cultivars, TRI2024 (Mean value 423.5) was reported as better response for callus growth weight for the leaf calli than TRI2043 (Mean value144.3) (Figure 1). These results indicate that, solid media suitable than liquid media and TRI2024 cultivar suitable than TRI2043 cultivar for somatic embryo induction via callus.

Experiment 4: Callusing from from ex vitro leaf segments

In the *ex vitro* leaf cultures, the cut ends of cultured leaf segments became slightly swollen and yellowish white in the liquid leaf cultures after 2 weeks of culturing. Subsequently, the swollen tissues burst and callus initiation was observed firstly at the cut ends during the 3rd week in liquid medium which was supplemented with 2mg/L BAP + 3mg/L NAA + 8.6 mg/L $AgNO_3$. In a similar experiment was conducted
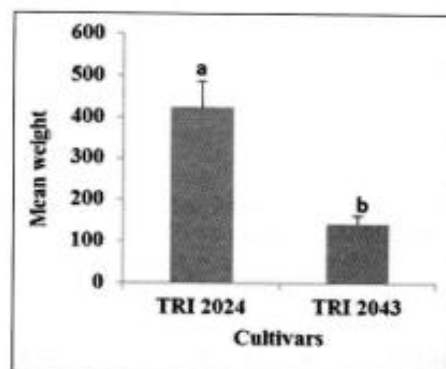

Figure 1: Mean weight of callus among TRI2024 and TRI2043 cultivars

by Seran (2006) supported the results of present

study. The highest callusing (mean callusing 55.56%) was derived in liquid MS medium contained 2mg/L BAP + 3mg/L NAA. Liquid MS medium with 2mg/L BAP + 3mg/L NAA was reported better for callus induction and proliferation.

Conclusion

The immature cotyledon explants on MS medium contained 2 mg/L BAP + 0.2 mg/L NAA + 8.6 mg/L AgNO$_3$ and nodal explants on MS medium contained 0.5 mg/L BAP + 3 mg/L GA$_3$ + 0.1 mg/L IBA were suitable for direct *se*. The *in vitro* leaf callus of TRI2024 cultivar on solid MS medium with 2mg/L BAP + 3mg/L NAA was reported for better callus induction and callus proliferation and solid MS medium with 2mg/L BAP + 3.5mg/L NAA + 8.6 mg/L AgNO$_3$ was reported for the better somatic embryo induction. The *ex vitro* leaf segments in liquid MS medium contained 2mg/L BAP + 3mg/L NAA was reported for better callusing. Silver nitrate was found to be beneficial in immature cotyledons, *ex vitro* internodal explants, *in vitro* leaf callus of TRI2024 cultivar to induce *se*. Results obtained so far clearly indicate that propagation of tea by *se* is a distinct possibility in the near future.

References

Abeywardana, D. A. S. R., Ranaweera, K. K., Ranatunga, M. A. B., & Warnasooriya, W. M. R. S. K. (2016). Somatic Embryogenesis Protocol for Tea (*Camellia Sinensis* (L.) 0 Kuntze).

Akula A and Dodd W.A (1998) Direct somatic embryogenesis in a selected tea clone, 'TRI-2025' (*Camellia sinensis* (L). O. Kuntze) from nodal segment. Plant Cell Rep. 17: pp. 804-809.

Bano Z, Rajaratnam S and Mohanty BD (1992) Somatic mbryogenesis in cotyledon culture of tea (*Thea sinensis* L.) *Journal of Horticultural Science* 66: pp. 465-470.

Gunasekare, M.T.K. (2008). Planting Material. Pp34-49: In A.K.N.Zoysa (ed.) *Hand book on Tea*. Tea research institute, Thalawakelle, Sri Lanka.

Ishizaki, T., F. Komai, and K. Msuda. 2000. Exogenous ethylene enhances formation of embryogenic callus and inhibits embryogenesis in cultures of explants of spinach roots. *Journal of the American Society for Horticultural Science*. 125:pp. 21–24.

Seran, T.H., Hirimburegama, K. and Gunasekare, M.T.K (2006). Somatic Embryogenesis from Embrogenic Leaf Callus of Tea. *Tropical Agriculture* 18: pp.28-35.