Callus Induction using Leaf Explants and Development of Thin Layer Chromatographic Fingerprint Profile for Secondary Metabolites of *Gyrinops walla* Gaertn.

S. Selvaskanthan[a], C. Kehelpannala[b], U. L. B Jayasinghe[b], D K.N.G Pushpakumara[ac] and J.P. Eeswara[ac*]

*[a]Postgraduate Institute of Agriculture, University of Peradeniya, [b]National Institute of Fundamental Studies, Kandy, Sri Lanka, [c]Department of Crop Science, Faculty of Agriculture, University of Peradeniya (*jpeeswara@yahoo.com/jpe@pdn.ac.lk)*

Gyrinops walla Gaertner., an endemic plant species, produces agarwood, is widely used as an ingredient in the cosmetic industry. The recent high demand for *G.walla* has created large scale harvesting and consequently had classified as an endangered species. Application of plant cell culture techniques is the best alternative for production of secondary metabolites while ensuring survival of the species. Induction of callus serves as a basis for *in vitro* secondary metabolite production. Development of a fingerprint using thin layer chromatography (TLC) is a potent tool to identify the phytochemical constituents in a sample due to its simplicity and reliability.

In the present study, the effect of 2,4-dichlorophenoxyaceticacid (2,4-D) on callus induction potentials and growth rates was investigated by inoculating *G. walla* leaf explants on Murushige and Skooge (MS) medium supplemented with 1.0 mg/L Benzyl Amino Purine (BAP) and different levels of 2,4D (0.1, 0.5, 1.0 and 2.0 mg/L). MS medium supplemented with 1.0 mg/L BAP and 0.5 mg/L 2, 4D showed highest callus induction (100%) with fastest growth rate (0.261 g/week) and cell doubling time (2.66 weeks).

Thin layer chromatographic profiles in different solvent systems, of freeze dried leaves, stem, bark and callus, extracted with hexane, EtOAc and MeOH were observed at UV 256 nm. A higher number of spots were observed in the MeOH extracts of all callus lines and plant parts, under 15% MeOH: chloroform solvent system and compounds appearing at R_f=0.26 and 0.45 could be possible markers for *G.walla*. Our study has devised a callus induction medium, identified the TLC fingerprint profile and possible markers for secondary metabolites of *G.walla*.

Acknowledgement: National Research Council of Sri Lanka–NRC/2017/17