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Effect Of Plant Growth Regulators On Production Of Callus In *Azadirachta Indica* And Characterization Of Resulting Calli

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Abstract—**Exploitation of *Azadirachta indica* has increased in the past few years due to its potential role as biopesticide, insecticide and pharmaceutical properties. As an alternative way to overcome complications in terms of sufficient supply of raw materials, genetic variability and product purity, and at the same time to conserve biodiversity, *in vitro* micropropagation methods have been implemented. The current study aimed to identify the effects of plant growth regulators 2, 4-Dichlorophenoxyacetic acid (2, 4-D) and Thidiazuron (TDZ) supplemented in Murashige and Skoog (MS) medium at different concentrations (0.2, 0.4 and 0.6 mg/l respectively) on the induction of callus from leaves and petioles of neem. Based on observations, TDZ induced regeneration via both callogenesis and indirect organogenesis by forming de novo shoots, while 2, 4-D promotes only callogenesis and had an inhibitory effect on chlorophyll formation. The results also showed that TDZ (when applied singly) promotes efficient calli formations in both explant types and as early on 5th day of culture in petioles. Petiole was found to be the most responsive explant type for callus induction, and showed the highest percentage (100%) of explant producing callus when the explant was cultured on both MS media supplemented with TDZ and 2, 4-D (in all concentrations). Among all treatments, leaf explants cultured on 0.6 mg/l TDZ produced the highest fresh weight of callus (3.16 ± 1.03 g). The different colored callus produced in these treatments which are green, brown and cream were subjected to pigment analysis, antioxidant and cytotoxicity assays. Among the colored calluses analyzed, green colored callus showed promising level of pigments and bioactive compounds which are chlorophyll, carotenoids, anthocyanin, phenolic content, flavonoids, alkaloids, tannins, terpenoids and higher antioxidant properties. Application of abiotic stresses on the neem callus and comparison on the resulting proteome and metabolome are also ongoing as part of this study. The protocol established in this study can be used as an approach to substitute the supply of raw materials of neem with higher purity and less genetic variability; therefore, contributes in conserving endangered biodiversity.**