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Earthworm As a Model Organism to Identify Mitogenic and Antimitotic Compounds from Plants

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Initiation and development of AR seedlings is a crucial stage during the vegetative propagation of commercially important plant seedlings. Moreover, initiation of root primordia from callus or explants is crucial in plant transformation. Many medicinal plants and horticulture crops are vegetatively propagated with stem cuttings and it depends on initiation and development of AR. Commercially available plant root inducing hormones such as IAA, IBA or other auxin derivatives are being used in plant tissue culture medium or in other controlled environments to initiate AR.

Usage of synthetic plant hormones in the fields are restricted in few countries, hence an alternative natural organic root inducers are preferred. Coconut water, honey, humic acid and vermicompost-related products have been suggested as alternatives for synthetic plant hormones to promote the vegetative growth of the plants in the field. Vermiculture-related products such as vermicompost, vermicompost-tea and vermiwash had been shown to promote the growth of plants. Microbes such as bacteria or fungi isolated from these products have been shown to produce plant hormones like auxin or cytokinin [119 & 120]. Moreover, coelomic fluid (CF) of earthworms had been shown to increase the growth of not only plant cells but also animal cells. The CF of earthworms had been used in plant tissue culture medium along with vermiwash or vermicompost extract and shown to promote the callus or vegetative growth of plants.

Here we describe the effect of CF from earthworm, E. eugeniae, which is free from other vermicompost-related products, along with plant tissue culture medium by using the model plant N. tabacum L. stem explants. This fluid induced rapid and robust AR in tobacco stem explants when compared to other synthetic root inducing hormones such as IAA or IBA. Moreover, our study predicted a new putative indole-derivative compound from the CF of earthworms. Additionally, significance of indole-derived compound in induction of AR from stem explants has been demonstrated by using amino acid tryptophan.

Mitotic cell division in eukaryotic organisms involves a complicated series of events. Our understanding of how fatty acids contribute or induce mitosis is quite limited. Fatty acids and their derivatives, such as fatty aldehydes and fatty alcohols, were mainly identified in the EA (Ethanol fraction partitioned from Aqueous extract of rhizome) extract of A. calamus rhizomes. This EA induced cell division in onion root tips and blastema development in amputated earthworms. These results suggested that the compounds present in the EA extract may significantly contribute to stimulating cell division in both plant and animal (earthworm) tissues by supplying energy and facilitating the synthesis of structural membrane lipids or acting as signalling molecules. However, it is essential to conduct studies using purified fatty acids or various combinations of fatty acids to grasp their role in promoting rapid cell division. In all our experiments, amputated earthworms retained clitellum region which possess stem cells. The regenerative capacity of earthworms offers a remarkable opportunity to investigate the molecular components necessary for cell division by using the stem cells. In addition, research involving mammalian model organisms could highlight the significance of these fatty acids in the process of repairing damaged cells. It is essential to thoroughly

examine the active compounds or their combinations, of this medicinal plant to gain insights into the mechanisms behind its various therapeutic effects.

Furthermore, we predicted that the aqueous extract of PSBP (Pseudostem of Banana Plant) has three putative antimitotic compounds, such as LI-ATT (á- Tocotrienoxyl radical), L2-NAT (1,2,4-Nonadecanetriol), and L3-THIF (3,4,7- Trihydroxyisoflavone), based on LC-MS analysis. We observed that these three compounds inhibited the cell division in onion root tips, in breast cancer cell line (MCF-7) and inhibited blastema formation in amputated earthworms, when they are present simultaneously (crude extract). Mode of action of these three compounds in arresting cell division is not known yet. Our results of docking studies demonstrated that these three ligands exhibit robust interactions with tubulin and CDK2 proteins, which are essential proteins involved in cell division. Moreover, studies on molecular dynamics simulations showed a significant binding affinity of these three ligands to tubulin and CDK2 proteins, which provided supportive information on binding of the ligands with the proteins. Banana pseudostem, often discarded as waste, can serve as a valuable resource for producing commercially significant drug compounds (leads), particularly in cases where cell division needs to be suppressed.

We explored other possible benefits of earthworm or their products in the growth of plants. Coelomic fluid (CF) of earthworm has been demonstrated to enhance seed germination and promote the growth of plant seedlings. The effect of CF in the growth of the model plant Nicotiana tabacum was studied in plant tissue culture medium, MS. The CF from the earthworm E. eugeniae can trigger adventitious root formation in stem explants of N. tabacum when it is diluted tenfold with MS medium. In tobacco stem explants, whether they contain the shoot apical meristem or not, root development was noted after 7 DPS (Days Post Subculture). However, synthetic root inducing hormones such as IAA or IBA promoted adventitious roots after 13 DPS. For the first time, a compound related to indole was found in the CF through Salkowski's biochemical test. Gas chromatography-mass spectrometry (GC-MS) examination of the concentrated coelomic fluid indicated the presence of a potential 1H-Indole, 5-methyl-2-phenyl compound. Adding tryptophan, an amino acid with an indole ring, to the MS medium led to adventitious root formation in the tobacco explants after 11 DPS, while leucine (having no indole ring) did not have this effect. We suggest that the newly identified indole derivative whether acting alone or in combination with other compounds of coelomic fluid, facilitates the rapid onset of adventitious root formation and promotes the growth of tobacco explants.

The CF of the earthworm E. eugeniae has been demonstrated to enhance the germination rate and vegetative growth of V. radiata seedlings. To determine the importance of CF in plant tissue culture medium, in vitro propagation of the model plant, N. tabacum was carried out. N. tabacum explants, the stem with shoot apical meristem, were used with CF at three different dilutions such as 10, 20 or 50 times with MS medium. CF diluted 10 times induced AR from the stem explants after 7 days of post sub culturing (DPS) as shown in figure 5.1. Development of AR was observed after 11 DPS when CF was diluted at 20 times and after 18 DPS when CF was diluted at 50 times (data not shown). In control explants, where MS medium was used without CF, AR was initiated at the beginning of the fourth week of sub culturing. This result clearly demonstrated that the roots were induced better when the CF was diluted at 10 times. It is consistent with previous report where similar diluted CF was found to induce the germination and development of V. radiata seedlings.

Induction of AR from the stem cuttings or explants depends on many factors such as endogenous plant hormone levels, cross-talk between them and different environmental factors. [125]. It is a very complex process in plants and it has not yet been studied in model plant N. tabacum L. with natural organic products. Earthworm-based products viz. vermicompost, vermiwash and Vermicompost tea were shown to increase the vegetative growth of many plants. These products are gaining popularity as they are environment-friendly, less expensive and promote soil fertility. Filter-sterilized vermicompost-based products in combination with CF has been used in MS medium for induction of callus and found to induce the growth of Brassica juncea. Here, we sterilized by autoclaving of CF along with MS medium and found its potential in rapid induction of AR in stem explants of tobacco plant.

CF induces adventitious roots when diluted at 10 times with MS medium. CF was diluted at 10, 20 or 50 times with MS medium and autoclaved. Stem explants with shoot apical meristem were used. Photograph

was taken after 7 DPS. A ruler with measurements of centimetre is shown at the right side.

Conclusion

Coelomic fluid of earthworm E. eugeniae has potential to initiate AR in stem explants of N. tabacum when it is diluted at 10 times with MS medium. Tobacco stem explants, either with or without shoot apical meristem, root induction was observed after 7 DPS whereas roots were induced after13 DPS by synthetic hormones, IAA or IBA. As a very first time an indole-related compound is identified in the CF by the biochemical, Salkowski's test. GC-MS analysis of concentrated CF suggested the existence of putative 1H-Indole, 5-methyl-2- phenyl- compound. Supplement of tryptophan, indole ring having amino acid, in the MS medium did induce AR in tobacco explants after 11 DPS whereas leucine did not. We propose that the new putative indole-derivative, either directly or with combination of other compounds present in the CF, support rapid initiation of AR and the growth of tobacco explants.

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