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BIOCHEMICAL AND PHYTOCHEMICAL PROFILING OF *IN VITRO* DEVELOPED AND MOTHER PLANTS

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ABSTRACT

For thousands of years, medicinal plants were an essential part in the treatment of illnesses and other health conditions. Even now, medicinal plants continue to play a significant role in the traditional medical systems of many different countries across the globe. The utilisation of various types of organic extracts or the bioactive chemical ingredients in pure form is required for the preparation of this herbal or traditional medication. According to figures provided by the World Health Organization (WHO), more than 4 billion people, or 80 percent of the entire population of the globe, are now using herbal medicine for some component of their basic medical care. Both in the pharmaceutical sector and among traditional users, the value of medicinal plants is growing at an alarming rate nowadays. Because the chemical synthesis of such compounds is either impossible or not economically viable, approximately forty percent of the compounds used in the pharmaceutical industry are derived either directly or indirectly from plants. This is the case because the chemical synthesis of such compounds cannot be done. Because of this, a significant number of plant species, particularly those that are used for medicinal purposes, are in danger of becoming extinct due to over harvesting. Tissue culture methods for very demanding medicinal plants provide a number of opportunities for method improvement, one of which is the possibility of finding a solution to the problem of these plants' existence in nature. Nevertheless, in vitro tissue culture is a mutagenic procedure that induces cytogenetic, genomic, and epigenetic variation. In this article, we suggest a straightforward and user-friendly method of protein banding that may be used to examine the genetic integrity of various medicinal plants.

Keywords:*Phytochemical Profiling, Vitro Developed, Mother Plants*

INTRODUCTION

As more people in both developed and developing nations come to realise that herbal medicines are natural goods that are free of addictive substances and can be purchased without difficulty at reasonable rates, there has been an uptick in the demand for medications derived from plants. In addition, the drugs are a source of health care that is made accessible to the general public. In addition to the rising need for plant-based medicines, there is also a growing demand in the worldwide market for other types of health goods, such as pharmaceuticals, nutraceuticals, cosmetics, and so on. It is predicted that close to 60,000 tonnes of herbal materials are required each year to meet demand. Even in modern times, the majority of therapeutic plants are gathered from the environments in which they are naturally found. The deleterious consequences of such meddling on the natural ecosystem have led to a drop in the natural gene pool as well as vegetation, which in turn threatens the survival of certain species. The establishment of a tissue culture approach for the cultivation of these medicinal plant species has been shown to be a viable and long-term solution to this issue. Now that we have the protocols, we can produce a large number of plants in a very short amount of time and in a very little amount of area. Yet every progress comes some new challenge. Several chemotypes within a species

have been identified by researchers, and these chemotypes exhibit variability in the amount of active principle they contain. Because of this, it is highly vital to have a continual regeneration process in tissue culture that maintains clonal homogeneity. In vitro produced variety, also known as somaclonal variation (Larkin and Scowcroft, 1981), is a fact that, although it does impact the genetic fidelity of the regenerated plants, it simultaneously provides as a rich source of genetic diversity for the introduction of new features into the species. However, whether it be to keep the uniformity in the somaclones or to screen and select novel lines from the somaclonal variants, it is necessary to thoroughly assess the cause and levels of such variations. This is the case whether the goal is to maintain the uniformity in the somaclones or to create novel lines from the somaclonal variants. It has been determined, through the use of DNA-based techniques such as RAPD, RFLP, AFLP, and ISSR, how extensive the somaclonal variation is in tissue culture and primary regenerants of numerous economically significant plant species, including cereals, ornamental, medicinal, and tree species. Nevertheless, these methods are complicated, time-consuming, and provide varying outcomes depending on the number of times they are repeated.

From a review of the relevant published material, it is apparent that some researchers have made use of protein gel methods, namely SDS-PAGE analysis, in order to investigate multiple variations or forms of a species that are morphologically identical (Cooke, 1984; Gardiner, 1988; Gilliland, 1989; Stegmann, 1983; Sammour, 1991). In our experiment, we attempted to utilise this method to examine the clonal homogeneity of plants that were created by the use of tissue culture. In light of all of these considerations, the current study reports on the simple, cost-effective, and user-friendly approach of protein banding to verify the clonal homogeneity of various medicinal plants that have been created by tissue culture.

MATERIALS AND METHODS

Tissue culture studies

In vivo plants were used to develop tissue culture generated plantlets of four medicinal plant species, as previously described by Pramanick et al., 2007 and Pradhan 2005. These medicinal plant species were *Withania somnifera* (L.) Dunal, *Centella asiatica* L., The MS (Murashige and Skoog, 1962) medium was fortified with varying amounts of phytohormones, and the plants were cultivated on this medium. The cultures were grown in an environment with a temperature of 25 2 degrees Celsius, a photoperiod of 16 hours of light and 8 hours of darkness, a light intensity of 50 mol m-2 s-1 provided by cool white fluorescent lamps (2 tubes 40 W, Philips, India), and a relative humidity of 60-65%.

Biochemicalanalysis

To analyse the electrophoretic pattern of the protein, a technique known as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out. The following is the procedure that was utilised to extract the protein: fresh young leaves (200 mg) were taken from the second and third nodes of plantlets that were 6 months old and were grown using TC. Before being centrifuged at 15,000 rpm for 20 minutes to remove cell debris, the explants were first mashed using a mortar and pestle using 2 mL of ice-cold 1.5 M Tris-HCl (PH 8.8), 3.5 (M) sucrose, and a few drops of 2-mercaptoetnanol. SDS-PAGE analysis was performed on the supernatant in accordance with the protocol developed by Laemmli (1970). In a nutshell, the proteins were separated on a polyacrylamide gel containing 10%. Each well was filled with 50 g of proteins in a volume of 25 l, and a current of 2 mA was supplied to each well during the whole experiment.

After running the gel for a certain amount of time, the bromophenol blue was allowed to reach its lowest point. After the completion of the run, the gel was stained with 0.1% Coomassie brilliant blue for an entire night, after which it was carefully cleaned and photographed. The usage of standard marker proteins with a range of molecular weights from 29 to 66 KD was carried out. The experiment was carried out a total of five times.

After the staining and disstaining of the gels, the similarity index based on Nie and Li's was determined. This was done dependent on whether or not polypeptide bands were present on the gels. We determined how many monomorphic and polymorphic protein bands each sample had by counting the total number of protein bands. In order to do cluster analysis, the presence or absence of polypeptide bands was recorded in a binary data matrix as either one (1) or zero (0).

Molecularanalysis

RAPD was used to do a second analysis on the result for *Withania somnifera* (L.) Dunal. Using the procedures outlined in Murray and Thompson's protocol (1980), genomic DNA was isolating and extracted from the young, fresh leaves of in vitro and tissue culture created plants that were allowed to become rigid (both plants were of same age). In order to carry out RAPD finger printing, PCR amplification of 100 ng of genomic DNA was carried out using 10 arbitrary 10 mer-oligonuleotide primers that were acquired from Genuine Chemical Company, India. Amplification reactions were carried out in a volume of 25 L containing 10X PCR buffer, 200 mM dNTP mix, 2.0 mM MgCl2, 1.0 M of each primer, 1.0 unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India), and 25 ng of template DNA. The reactions were carried out in an incubator at 50 degrees Celsius. Amplification was performed using a MJ Research Mini Thermal Cycler in the United States for a total of 42 cycles, the first of which was carried out at a temperature of 940 degrees Celsius for three minutes. This was followed by 40 cycles in which the temperatures were maintained at 940 degrees Celsius for one minute, 360 degrees Celsius for one minute, and 720 degrees Celsius for two minutes respectively. After this, there was one cycle carried out at 720 degrees Celsius for seven minutes. The PCR products were separated using electrophoresis on an agarose gel at a concentration of 1.5% (m/v) in a 1x Tris Acetate DTA buffer. The gel was then stained with ethidium bromide at a concentration of 0.5 g L-1 before being seen using the Gel Documentation System (Uvitec, UK). The size of the amplification products was determined with the use of a DNA marker that had been digested with EcoRI and HindIII (Bangalore Genei Pvt. Ltd., India)

RESULTSANDDISCUSSION

Tissue culture studies

The development of plants was carried out on MS medium that had varying quantities of phytohormones added to it. In a very short amount of time, each of the four plants was grown in MS media that had varying quantities of phytohormones added to it. The considerably largest number of microshoots (15.50) was found in *Withania* when it was cultivated on MS medium supplemented with 1 mg/l of BAP. After callus development in 4 mg/l of NAA, the nodal area of the *Centella* plant exhibited the considerably maximum number of shoots (27.87) when the plant was moved into MS media that was supplemented with 0.5 mg/l of KIN. The greatest number of microshoots was seen in *Tylophora* shoot tips grown on MS media that had been supplemented with 8 mg/l of KIN and 0.2 mg/l of NAA (9.30). The substantial largest number of microshoots was seen in *Bacopa* nodal explants grown in MS media that had been supplemented with 1 mg/l BAP and 0.5

mg/l NAA (42.63).

SDS-PAGE analysis

The SDS-PAGE study of the total Tris-HCL soluble leaf protein of *Withania somnifera* exhibited 24 bands, which were the same in both in vivo and TC produced plants; the acquired bands were similar in number, but some of them had shown a variation in density (Fig.1A). In the instance of *Centella asiatica* L., six bands were created, all of which were identical in in vivo and TC produced plants (Fig.2B). Nevertheless, the banding pattern of the other two medicinal plants exhibited some distinct distinctions. There were 20 bands on the Bacopa monareii L. in vivo plant, while there were only 17 bands on the TC-generated plants (Fig. 2C). On the other hand, *Tylophora indica* (Burm.f.) Merrill exhibited 17 bands in in vivo whereas TC produced plants exhibited only 13 bands (Fig.2D). In the species Withania somnifera, Pramanick et al. (2007) found no evidence of genetic variation when they looked at morphological and biochemical characteristics. When Pradhan (2006) investigated the clonal fidelity of *Centella asiatica* using morphological and biochemical studies, he reported findings that were comparable to those described here. Since it is recognised that micropropagation may generate somaclonal variation in plants, it has always been advised that several markers be used in order to conduct a more accurate investigation of the genetic homogeneity of plants (Lakshmanan et al., 2007). SDS-PAGE examination revealed that plants generated from *Pilosocereus robinii* tissue culture exhibited genetic homogeneity, which is consistent with the results of our investigation (Khattab et al., 2014). In contrast, the genetic analysis of the other two species that were investigated in this research, *Bacopa monareii* (L.) and *Tylophora indica,* revealed genetic differences. It's possible that this is because the genetic make-up of the other two species is different. Narayani et al., (2016) obtained findings that were comparable, and they also reported the same banding pattern in *Orthosipho stamineus* (Benth.). Variations that occur during in vitro propagation are caused by a number of factors, including the source of the explants, the technique of regeneration, the ploidy level, and the age of the in vitro culture. Based on the findings presented above, we have drawn the conclusion that when there is a variation in banding pattern between an in vivo created plant and a plant generated by TC, there is also a difference in morphological and biochemical features. Yet, when there is no discernible change in banding pattern, there is also no discernible difference in the morphological or biochemical characteristics. SDS-PAGE has been used extensively in a great number of research in order to find variation in the protein pattern of a great number of different species (Kamikouchi et al., 2004; Khattab and El Sherif, 2011; Lortal et al., 1997).

RAPD analysis

When using micropropagation for the purpose of producing plants that are genetically identical to their parents, one of the most important steps is determining the genetic integrity of the clones that were created in vitro (Saha et al., 2016). The ability of micropropagated plants to maintain their genetic integrity has significant implications for both industry and agriculture. In light of this information, we used the RAPD marker to conduct fingerprinting analyses on the culture regenerants and the donor plants that were associated with each of them. Our goal was to determine whether or not the plantlets were genetically stable. As a consequence, the SDS-PAGE analysis findings for the case of *Withania somnifera*(L.) Dunal. were re-analyzed by RAPD marker at our laboratory for this research. During the first screening of 10 primers, only 5 primers met the criteria for selection due to their high repeatability (Table 1). A selection of five RAPD primers resulted in the production of seventeen unique and scoreable bands. The number of bands that might be scored for each primer ranged from two (in the case of P4) to five (in the case of P3), with an average of three and a half bands for each RAPD

primer (Table 1; Fig. 2). Primer P3 yielded the greatest number of bands (5), whereas primers P4 produced the fewest number of bands possible. Similar to the findings of Senapati et al. (2013) and Saha et al. (2013), all of the banding profiles were of a monomorphic nature, meaning that they were identical to those of the mother plants. This was an indication of homogeneity among the regenerated culture and genetic uniformity with that of the donor plants (2014). The findings of an SDS-PAGE study performed on the same plant are supported by this result (Fig. 2A).

The previous work makes it abundantly evident that SDS-PAGE examination of total protein may be used in the investigation of homogeneity between plants created in vivo and those generated in tissue culture. Workers who used SDS-PAGE study of Tris-HCl soluble leaf proteins for varietal studies in the past (Cooke, 1984; Gardiner, 1988; Gilliland, 1989; Stegmann, 1983; Sammour, 1991) have mentioned some advantages of the techniques, which are also true for using this technique in study of homogeneity of in vivo and tissue culture generated medicinal plants of our study. The universal distribution of proteins, which means that there are no theoretical constraints to making use of electrophoretic markers, is the first of them. 2. The electrophoretic markers are less sensitive to the effects of the oscillations in their surroundings. 3. The discriminatory power of electrophoresis is often quite strong, and it is frequently possible to differentiate between plant species with less work and the participation of fewer persons than is possible with a method that is based on morphology. 4. The operational expenses are not very high, and consistent findings are obtained from repeated trials. The above explanation leads us to the possible conclusion that the SDS-PAGE protein banding technique is a straightforward method that can be used in the laboratory to verify the clonal homogeneity of medicinal plants that have been created by tissue culture.

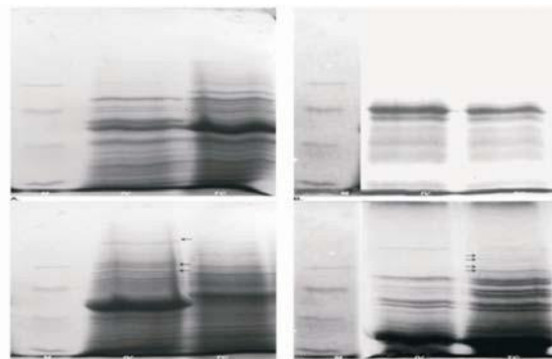

Fig. 1: SDS-PAGE A. *Withania Somnifera* (L.) Dunal and B. *Centella Asiatica*both show protein banding. L. C. *Bacopa Monareii Tylophora Indica* (Burm.f.) Merrill's scientific name is L. D. *Tylophora*. The locations of the band differences are shown by the arrow markings. M stands for marker protein, IV for in vivo, and TC for plants grown in tissue culture.

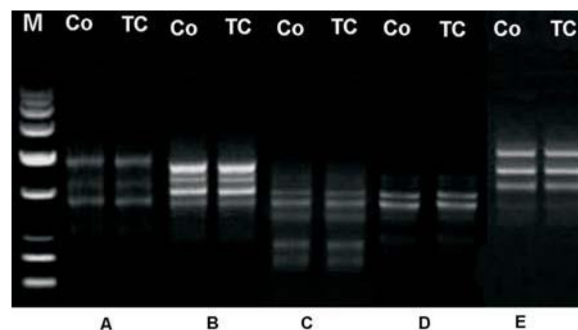

Fig.2:RAPD study of tissue culture produced and in vivo (CO) samples (TC) *Withania somnifera* (L.) Dunal.

Table 1:The following is a list of primers, their sequences, and the sizes of the amplified fragments that were created using randomly amplified polymorphic DNA (RAPD) markers for the purpose of DNA fingerprinting of micropropagated plants of *Withania somnifera* (L.) Dunal.

SL. No.	Primer code	Primer sequence (51-31)	No. of scorable bands per
1.	P_1	GTGACGTAGG	3
2.	P_2	GTCCACACGG	4
3.	P_3	TOGOGGACTC	5
4.	P_4	GGTCCCTGAC	2
5.	P_5	TGCTCTGCCC	3
Total	P_6		17

Conclusion

According to the findings of the current research, an ethanolic extract of *Ficus vasta* Forssk. may include bioactive phytochemicals that are responsible for both therapeutic and pharmacological activity. *F. vasta* had a favourable TPC, TFC, and antioxidant potential, and it possessed a favourable antidiabetic potential for the inhibition of -amylase and -glucosidase. The extract demonstrated effective antimicrobial, antifungal, and antiviral properties when tested against the strains. The amount of *F. vasta* extract used was directly proportional to the degree to which it inhibited the development of HepG2 cells. The GCMS analysis of the ethanol extract suggested the possible existence of significant phytocompounds, which supported the biological activities of the extract's components. Because of this, it is possible to draw the conclusion that the in vitro examination of *F. vasta* demonstrated the plant's therapeutic promise in terms of its antioxidant, antidiabetic, thrombolytic, enzyme inhibition, cytotoxic, anti-viral, antifungal, and antibacterial potential. The enzyme inhibitory activity was better understood thanks to the investigations including in silico molecular docking. According to the results of in vitro and in silico docking investigations, more research is necessary to determine the toxicity profile and clinical trials of this substance. In conclusion, the results of this study might be of assistance to researchers who are continually working towards the creation of innovative and efficient medications derived from natural ingredients. Based on the phytochemical and biological potential that was identified in this plant, it seemed that it may be useful for the further separation of bioactive chemicals.

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