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RESEARCH **P**APER

Genetic variations in *Ocimum americanum* L. (Tulsi) grown in *in vitro* and *in vivo* conditions

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Ocimum americanum has been used extensively in the traditional system of medicine in many countries. It is widely used in folk medicine as antimalarial and anticonvulsant drug. Its juices are used in pain, cough, cold, malaria, etc. Oils extracted from its leaves have antifungal, antibacterial and antiseptic properties. Plants grown *in vitro* may have difference in some aspects with those grown *in vivo.* The study done here showed the difference between the DNA and protein contents of the plant grown *in vitro* and *in vivo* using the technique, agarose gel electrophoresis. The difference in the size of DNA segments obtained shows that the plants grown *in vitro* have smaller DNA segments, some segment might also be broken. This could largely affect the proteins produced after translation which may be useful or even harmful in many ways. Maximum shoot multiplication was observed when concentration of BAP was 2000µl and NAA 20 µl and maximum root formation was observed when NAA concentration was 2000 µl and BAP concentration was 100 µl. Using Lowry's method, the total protein content found in plant grown in *in vivo* conditions was 16.20 per cent whereas plant grown in *in vitro* condition had 10.00 per cent protein content, it was found that DNA of *in vitro* plant was smaller in size than *in vivo* plant.This study can prove to be a major threshold for further studies on the plant. Difference in DNA and protein contents can make large changes in its properties.

Key words : Plant tissue culture, DNA, *In vitro*, *In vivo* medicinal plants

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INTRODUCTION

Plants have been an integral part of traditional medicine across the continents since time immemorial (Solecki and Shanidar, 1975). Plants produce a host of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Cox and Balick, 1994). The use of medicinal plants is not just a custom of the distant past. Approximately 90 per cent of the world's population still depends on medicinal extracts (Duke, 1985). According to a survey, 23 per cent of Canadians use herbal medicines. In addition, as much as 25 per cent of modern pharmaceutical drugs contain plant ingredients (Duke, 1997). Indian subcontinent is a vast repository of medicinal plants that are used in traditional medicinal treatments. Around 20,000 medicinal plant species have been recorded (Dev, 1997), about 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous system of medicine as potential source of bioactive compounds (Prusti *et al.*, 2008).Numerous medicines in use today are extracted from plants. About 50 per cent to 60 per cent of pharmaceutical drugs are either of natural origin or obtained through use of natural products as starting points in their synthesis (Balandrin *et al.*, 1993). In Ayurveda, tulis plant pacifies vitiated vata, kapha, helminthiasis, anorexia, dyspepsia, pruritis, leprosy,

vomiting, poison, migraine and fever. Seeds are good remedy for hyperdipsia, fever, migraine and emaciation. And other uses include, essential oil of the leaves and inflorescences possesses strong antifungal and antibacterial properties (Begum *et al*., 1993). Dried plant is burnt as mosquito repellant (Yusuf *et al.*, 2009). Hepatoprotective activity was exhibited by the aqueous extract of *O. americanum* leaves against paracetamol – induced liver damage in rats. Hepatic damage was induced by paracetamol (Aluko *et al.*, 2013). The present study was conducted for genetic variations in *Ocimum americanum* (tulsi) grown in *in vitro* and *in vivo* conditions.

RESEARCH METHODOLOGY

All the experiments were carried out at the Department of Vaccinology, Institute of Biotechnology (IBT) campus at Patwadangar, Govind Ballabh Pant (G.B. Pant) University of Agriculture and Technology, Pantnagar, Nainital, Uttarakhand.

Plant material :

The plant material used for the present study was grown in the green house of IBT campus. Explants were collected from healthy plants without any disease symptoms.

Culture medium, chemicals and reagents :

In all the experiments, MS (Murashige and Skoog, 1962) medium was used. The medium was supplemented with 3 per cent sucrose, 0.1g inositol and 0.8 per cent agar. All the chemicals were of analytical grade (Sigma Chemical Co. USA, Merck Hi-media and Qualligens India Ltd). The pH for all media was adjusted to 5.8 using 1N NaOH or 1N HCl before autoclaving the medium at 121°C and 108 kPa for 20 minutes.

Explant preparation :

Nodal segments were used as explants in the present study. The explants were cut and brought into the lab in water. The leaves were removed and the nodal segments were made into appropriate size.

Sterilization of explants :

The nodal segments were first washed with tap water 2- 3 times. They were washed with tween- 20 (3 drops) for 5 minutes followed by thorough rinsing with double distilled water. The explants were then transferred into the laminar air flow chamber where they were treated with 0.1 per cent $HgCl_2$ for different time durations (5, 7, 9, 11, 13 and 15 minutes) for obtaining aseptic explants. These explants were then thoroughly rinsed with autoclaved double distilled water (4-5times) and kept for incubation. Both the ends of explants exposed to sterilant were trimmed and placed aseptically in 75 x 125 mm bottles containing 15 ml MS medium supplemented with 3 per cent sucrose as a carbon source, 0.1g inositol and different concentrations of BAP and NAA combination.

Inoculation :

All aseptic manipulations were carried out in the clean laminar air flow chamber (Cleinzoid, Mumbai). Prior to working in the laminar the hood was surface sterilised with 70 per cent ethanol and applying UV light for 30 minutes. The forceps, Petri plate, etc. used for inoculation were autoclaved and later flame sterilized prior to each inoculation. A single explant was placed in each culture bottle containing 15 ml medium and plugged with guaze- wrapped cotton plugs.

Incubation condition :

All cultures were incubated at 25±2°C and 75 per cent relative humidity under 16 hour photoperiod with a light intensity of 40 $\mu Em^{-2}s^{-1}$ provided by cool white fluorescent tubes.

Shoot formation :

In shoot culture, apical meristem (the region of shoot apex laying distal to leaf primordium) is cultured, this technique is also known as meristem culture, meristeming and mericlones (Murashige, 1974). The excised shoot tip cultured on agar solidified simple nutrient medium under appropriate conditions. For shoot culturing, high concentration of cytokinin (BAP) was added, which is responsible for shoot growth formation and low concentration of auxin (NAA) was added in agar nutrient media for different concentrations.

Root formation :

Rooting was initiated by using the various plant growth regulators.

Isolation of high molecular weight genomic DNA from leaf of *O. ameicanum* :

Genomic DNA was isolated by CTAB (cetyl tri-

methly ammonium bromide) method.

Qualitative and quantitative analysis of DNA of *in vitro* and *in vivo grown* plants of *O. americanum* :

Gel electrophoresis was performed for quantative analysis of DNA of *O. americanum*.

Extraction of protein by Lowry's method :

The protein content was estimated by Lowry *et al.* (1951) method.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Culture establishment :

The explants obtained from mature *Ocimum americanum* showed high degree of contamination. All the explants contaminated when inoculated in MS media without any sterilant. Therefore, mercuric chloride ($HgCl_2$) was used as sterilant. Treatment of mercuric chloride was given for different time intervals. At 0 min. there was 100 per cent contamination, and when explant was treated with mercuric chloride for 11 min, 13 min. and 15 minutes, the contamination rate was decreased and the establishment rate was above 80 per cent (Fig. 1). It shows when we increase the time interval; there was decrease in contamination rate. Though environmentally toxic $HgCl_2$ is a widely accepted surface sterilant in plant tissue culture, best results are achieved when used judicially and carefully (Zryd, 1988).

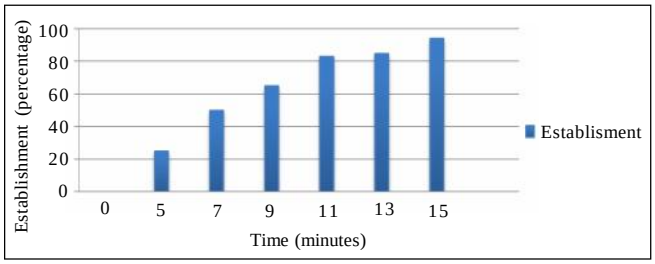


Fig. 1: Effect of 0.1% $HgCl_2$ for culture establishment

Shoot multiplication :

For shoot multiplication, different concentrations of auxin/ cytokinin ratio were used. As the concentration of cytokinin was increased and kept auxin concentration low, maximum shoot multiplication was observed. When BAP concentration was 2000µl and NAA was 20 µl maximum shoot multiplication was observed (Fig. 2). It shows that shoot multiplication took place when BAP concentration was high and NAA concentration was low. This is in accordance with the findings of Dhale *et al.* (2010) who reported that in different concentration of 200 µl NAA and 5000 µl BAP for 45 days gave the highest efficiency of shoot formation per explant (66. 7%) and a higher number of shoots per explant in micro-propagation of *O. basilicum* L.

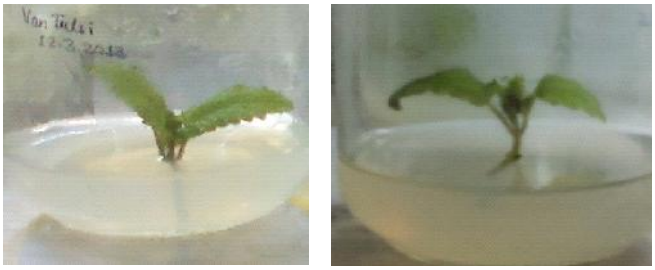


Fig. 2: Shoot multiplication observed in MS+BAP (2000«l)+ NAA (20«l)

Root formation :

Different concentration of auxin/ cytokinin ratio was used for root formation. When NAA concentration was increased and BAP concentration decreased, root formation took place. When NAA concentration was 2000 µl and BAP concentration was 100 µl, maximum root formation was observed (Fig.3). When NAA concentration was 2000 µl and concentration of BAP was 200 µl root formation was observed in lesser amount as compared to above concentrations.

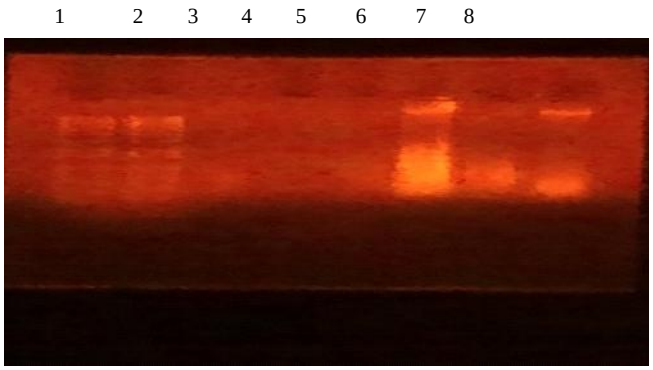
Fig. 3: Root formation in *O. americanum*

Quality and quantity of DNA using agrose gel electrophoresis :

DNA samples of leaves of *O. americanum* grown

in *in vitro* and *in vivo* conditions were extracted and its quality and quantity was checked.

The bands observed in UV showed that the DNA of both *O. americanum* grown *in vivo* and *in vitro* was larger than 1 kb (Fig. 4). The *in vitro O. americanum* has smaller DNA segments than *in vivo O. americanum*. There were also some larger segments in *O. americanum* grown *in vitro*. Some segments might be broken in *in vitro* plant. Quereshi *et al.* (2011) has applied same process based on quantitative observation of three species of *Ocimum*, while this study has been carried out on qualitative measures.



(1-2) 1 kb marker (3-5) *in vivo O. americanum* and (6-8) *in vitro O. americanum*

Fig. 4: DNA bands visualized in UV after agarose gel electrophoresis

Estimation of total protein by Lowery's method :

The leaf samples of *O. americanum* grown *in vitro* and *in vivo* were collected and then estimation of total protein was done by Lowry's method. Fig. 5 shows that absorbance of working standard protein (BSA) was directly proportional to its concentration. Therefore, we got a linear curve when absorbance of BSA was plotted against its concentration (Fig. 5).

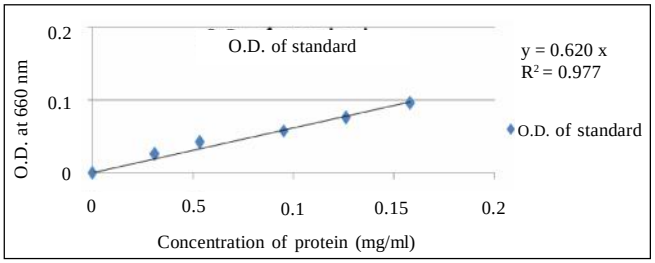


Fig. 5: Standard curve of BSA

Total protein content in plant grown in *in vivo* conditions was 16.20 per cent whereas plant grown in *in vitro* condition had 10.00 per cent protein content. Same process of estimation of protein was applied by Sharma *et al.* (2011), in which the variation of composition of metabolites was studied in various plant parts (stem, leaves and root) and *in vitro* callus of *O. tenuiflorum* and recorded highest amount of protein 3.6±0.65 mg/gfw in the leaf of *O. tenuiflorum.*

The present study was conducted on *O. americanum* plant grown in *in vitro* and *in vivo* conditions. A comparison of molecular properties (DNA and Protein) was carried out and it was observed that there were significant changes in DNA and protein contents of plants. As plant grown in *in vitro* condition showed more DNA segments smaller in size than the plant grown in *in vivo* condition as well as total protein content in plant grown in *in vivo* conditions was 16.20 per cent whereas plant grown in *in vitro* condition had 10.00 per cent protein content. This comparative study was done in view of adding a threshold for further research. Difference in DNA and protein contents can make large changes in its properties. It may reduce the production of some harmful metabolites and may also induce the production of some useful metabolites not found naturally.

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