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Impact of 2, 4 Dichlorophenoxyacetic Acid (2, 4 D) Plant Growth Regulator on *in vitro* Callus Induction of *Ocimum gratissimum*

Chaudhary Priyanka[1], Sharma Vikas[2]

[1]*Department of Biotechnology and Microbiology, Arni School of Basic Sciences, Arni University, Kathgarh, Himachal Pradesh (India)*

[2]*Department of Biotechnology, DAV University, Jalandhar (India)*

ABSTRACT

Ocimum gratissimum is an important medicinal perennial herb, also known as shrubby basil belonging to the family Lamiaceae and has enormous potential in cosmetic and drug industry due to occurrence of several Phytoconstituents. The present examination was embraced to evaluate the most appropriate concentration of 2,4 D plant growth regulator(PGRs) for in vitro callus induction of O. gratissimum. Cultures were kept at 28±2°C, 16 hour light photoperiod (48µmolm^{-2}s$^{-2)}$ for 45 days. For in vitro callogenesis, an initial phase of dark period was given for seven days. Callus induction from leaf explants of O. gratissimum was conducted by incubating leaf explants on MS medium supplemented with different concentrations of 2,4 D. It was observed that PGRs at different concentration brought out significant variation in callogenesis. The best callus induction (90.4 ± 0.50) was obtained in MS medium supplemented with 0.5mg/l 2,4 D from leaf explants.

Keywords: Callogenesis, Herb, Industry, Lamiaceae, Medicinal, Phytoconstituents.

I. INTRODUCTION

The genus Ocimum is ranked high among some of the astonishing herbs for having enormous medicinal potentialities (1). *Ocimum gratissimum* commonly known as Ram Tulsi (Hindi), Shrubby Basil, Fever Leaf (English) and belongs to Lamiaceae family. It is found throughout the tropical and subtropical regions and its maximum variability occur in Tropical Africa and India (2). Ayurveda illustrate the uses of leaves of *O. gratissimum* in the treatment of several ailments such as respiratory tract infections, tooth and gum disorder, diarrhea, headache, ophthalmic, skin diseases, pneumonia, cough, rheumatism, sunstroke, influenza, gonorrhea, mental illness (3) and malaria (4). The entire plant and the volatile oil are utilized as a part of conventional medication particularly in Africa and India (5, 6). The phytochemical investigation confirmed the presence of alkaloids, tannins, phenolics, saponins, glycoside, resins, steroids and terpenoids (7, 8).

O. gratissimum is a globally imperative monetary crop since it has a trade value of US$ 15 million every year (9) but unfortunately with the quick increment in total populace, outrageous weight on the accessible cultivable land and speedy vanishing of herbal habitats for restorative plants it is astoundingly difficult to secure plant-derived compounds (10). *In vitro* culture technique offers a magnificent alternative for the conservation of medicinal plants and is a proficient source for rapid multiplication of species therefore enthusiasm for utilizing

these procedures has been altogether expanded (11). By *in vitro* technique, one of the strategies to build up a reproducible of plantlet was through callus since it was the most appropriate material utilized for hereditary change in plant (12), expanding secondary metabolites production, to determine the culture conditions required by the explants to survive and develop, study cell development and get cell suspension in propagation (13, 14, 15) and induction of somatic embryogenesis. Therefore in the present study we revealed the impact of 2, 4D at different concentrations for *in vitro* callus induction of *Ocimum gratissimum*.

II. MATERIALS AND METHODS

2.1 COLLECTION OF EXPLANTS

Healthy and profusely growing plantlets of *Ocimum gratissimum* were collected from Medicinal and Aromatic Plant Garden of Arni University, Himachal Pradesh. The plants were dried and prepared herbarium was submitted to The National Institute of Science Communication and Information Resources (NISCAIR), located at New Delhi, India for identification of species and plants were recognized as *Ocimum gratissimum.*

2.2 Surface Sterilization of Explants

Nodal portions of *Ocimum gratissimum* were excised from plants of *Ocimum gratissimum*. The explants were excised and the contaminants were washed under running tap water for 30 minutes to evacuate foreign contaminants. During washing surfactant (Tween-80) was added and explants in the solution were agitated constantly for 3 minutes. Thereafter, the detergent was completely drained out from the explants by washing it under running tap water for 30 minutes to expel the microbial load and dust particles. Consequently the explants were taken to laminar airflow cabinet for further sterilization. Then explants were transferred to laminar airflow chamber for further sterilization with aqueous mercuric chloride (0.1% $HgCl_2$) for five minutes simultaneously. After this treatment, explants were thoroughly washed 3-4 times with autoclaved distilled water to evacuate any trace of the surface sterilants under aseptic conditions (16).

2.3 Culture Medium and Conditions

The culture medium used was Murashige and Skoog's (1962) basal medium supplemented with 3% (w/v) sucrose The pH of the medium was adjusted to 5.8 with 1N NaOH or HCl before gelling with 0.8% (w/v) agar and then sterilized by autoclaving for 20 min under 1.1kg/cm^2 pressure at 121^{0}C. All the cultures were incubated in a culture room at 25±2^{0}C, under photoperiod of 16 hours light, 8 h dark provided by white fluorescent tubes with the intensity of 1000lux.

2.4 Callus induction

The leaf explants were cultured on semisolid MS basal medium supplemented with different concentrations of concentrations of 2, 4-dichlorophenoxyacetic acid (2,4-D) for callus induction. MS medium devoid of plant growth regulator was used as the control. The day of initial callus formation, the morphology and color of the callus were recorded. At the end the observation period, percentage of explants forming callus as well as the degree of callus formation was measured.

2.5 Statistical analysis

Data were analyzed by analysis of variance (ANOVA) to detect significances between means. Means differing significantly were compared using Duncan's multiple range test (DMRT) at a 5% probability level. Data were recorded on the percentage of explants forming callus. Means and Standard errors were carried out for each treatment.

Callus induction percentage (CI %) = $\frac{\text{No. of explants with callus}}{\text{Total number of Explants}} \times 100$

III. RESULT AND DISCUSSION

Table1: Effects of different concentrations of plant growth regulators for callus induction from young leaf explants of *Ocimum gratissimum*

Concentration of 2,4 –D(mg/l)	Percentage of callus induction (Mean ± S.E)	Callus nature		
		Degree of callusing	Callus Color and Morphology	Texture
Control	–	–	–	–
0.5	90.4 ± 0.50[a]	+++++	Creamy White, light greenish	Compact
1	85.0 ± 0.31[b]	++++	Creamy White, light greenish	Compact
1.5	75.4 ± 0.24[c]	+++	White, light greenish	Compact
2	70.2 ± 0.37[d]	++	White, light greenish	Compact
2.5	60.8 ± 0.37[e]	++	White, light greenish	Compact
3	55.0 ± 0.31[f]	+	White, light greenish	Compact

Medium: MS+ PGRs; mean± SD, n= 5 replicates, Means having the same letter in each Column do not different significantly at $P< 0.05$.

– = no callus formed, += poor callus formation, ++=minor callus formation, +++ average callus formation, ++++=moderate callus formation, +++++= profuse callus formation.

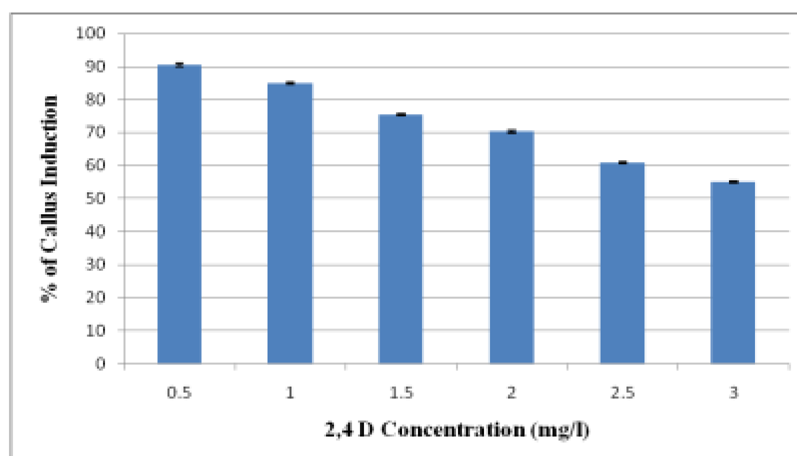

Figure1: Percentage of callus induction (CI) obtained when MS medium was supplemented with different concentrations of 2, 4 D.

Selection of explants was exceptionally urgent in callus induction because explants response is highly genotype dependent, so that for any given species, not all different types of explants were appropriate to prompt embryogenic callus (17). Frequency of callus induction is influenced by many factors such as media composition, explants source, genotype and environment. Subsequently accomplishment of micropropagation to a great extent depends on the determination of appropriate plant part, which is to be utilized as the beginning material for the experiment. Callus is an unorganized mass of cells started from the cut portions of the explants in which cells undergoing mitosis, which leads to the formation of callus and its formation is controlled by growth regulating substances present in the medium (18). Ideal explants must contain a lot of competent cells, for example, young leaf since it could be effectively adjusted in *in vitro* conditions (19). Therefore in the present investigation leaves of *O. gratissimum* excised from natural habitat were used as explants. The smaller size of explants were chosen due to fact that smaller size of explants provide less chance of contamination, and in addition longer leaves showed total loss of morphogenic potential (20).

Leaves of *O. gratissimum* were cultured on MS basal medium supplemented with various concentrations of 2,4-D (0.5, 1, 1.5, 2, 2.5, 3 mg/l) for the induction of callus. It has been observed that leaves on each medium containing 2, 4-D in the range of 0.5 - 3.0 mg/l responded differently. Among the different concentrations of 2,4-D, highest response of callus induction was observed on MS medium supplemented with 0.5mg/l 2,4-D and 90% of explants responded at this concentration and callus formation was observed after 15 days of culture (Table 1). During initiation the explants did not show any leaching or browning of tissues. It has been discovered that callus formation on the explants occurred at wounding site of major veins increased significantly and covered the entire surface of the explants within 3 weeks. The formed calli were compact with creamy white and light greenish color (Figure 1).

At highest concentration (3 mg/l) of 2, 4-D, callus induction was declined. However, the percentage of callus induction decreases with increase in hormonal concentration and this corroborate the findings on *Achyranthes aspera* (21) and *Brucea mollis* (22). It has been observed that basal media without plant growth regulators did not induce any callus growth (control). Frequent sub culturing of callus was done to fresh medium to avoid browning and to increase the survival rate. Brown exudates promote dying of cells by interfering with the metabolic activities of cells. However, the percentage of callus induction decreases with increase in hormonal concentration.

For the induction of callus concentration of 2, 4-D varies from species to species and even depends on the source of explants (23). Callus induction is important for large scale production of plant materials which play a pivotal role in producing secondary metabolites and bioactive compounds. 2, 4-Di-chlorophenoxy-acetic acid (2,4-D) is applied to induce callus growth since it can revert cells in the explants to a dedifferentiated state and begin to divide (24). Effectiveness of 2, 4-D in callus formation has been reported in several therapeutic herbs, for example, *Ocimum sanctum* (25), *Ipomaea obscura* (26), *Ionidium suffruticosum* (27), *Vitex negundo* (28), *Aquilaria agallocha* (29), *Solanum trilobatum* (30), *Achyranthes aspera* (21), *Chlorophytum borivilianum* (31), *Centella asiatica* (32).

IV. CONCLUSION

The present investigation has built up the callogenic capability of leaf explants of *Ocimum gratissimum*. It can be inferred that improvement for quick callus enlistment is essential in tissue culture strategy. Callus generation from *O. gratissimum* is utilized to gather secondary metabolites and diminish the over misuse of plants from their *in situ* natural surroundings. Consequently, this convention is essential for mass proliferation of *O. gratissimum* and furthermore opens another approach to encourage secondary metabolites production and isolation of prescribed drugs from callus rather than harvesting the plant itself.

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