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In Vitro Germination and Tissue Culture Protocol Establishment For *Canabbis sativa L.*

Poonam, Aaditya, Akshay Kharyal, Vimlendu Bhushan Sinha*

Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, Uttar Pradesh, India-201306

*Corresponding author : Dr. Vimlendu Bhushan Sinha,Assistant Professor,
Department of Biotechnology,School of Engineering & Technology, Sharda University
Email: vimlendusinha@gmail.com,

ABSTRACT

Tissue culture is important for the mass propagation of plants and help us to save many endangered species. The cultivation of *Cannabis* is restricted due to which the utilization of cannabinoids remains limited despite its immense potential in therapeutic utilities. This problem can be solved by mass propagation of C*annabis* using tissue culture protocols which remains the prime objective of the work. Germination protocol using water-agar and half MS medium was established and callus was regenerated from leaf explants in the current work.

Key words: *Cannabis;* Hemp; Tissue culture; mass propagation, MS medium

1.INTRODUCTION

Cannabis is a genus of flowering plants of the Moraceae family and includes *Cannabis indica, Cannabis sativa,* and *Cannabis ruderalis* which exhibit morphological differences like height of the plant, branch number, bushy growth etc. [1]. Most of the *Cannabis* is short day plants, with *C. ruderalis* as exception which is commonly described as auto-flowering and day neutral plant [2]. Secondary metabolites like cannabinoids, terpenoids etc. are secreted by glandular trichomes found on floral calyxes and bracts of the female plants. *Cannabis* is believed to made its appearance in Central Asia about 5000 BC, found in various habitats ranging from sea level to the temperate and alpine foothills of the Himalayas and contains a number of medicinally important compounds [3]. As far as geographical distribution of *Cannabis* is concerned, it extends from north of latitude 30°N and south of latitude 60°N [4]. The main psychoactive compound isolated from *Cannabis* is Δ 9 - tetrahydrocannabinol (THC) and is well known for its medicinal potentials [5]. The propagation of *Cannabis* plants are usually through seed or by vegetative propagation, using stem cuttings [6]. As a plant, it is valued for its hallucinogenic and medicinal properties and efforts has been made to evaluate its potentials for pain, glaucoma, nausea, asthma, depression, insomnia, and neuralgia [7]. Plant tissue culture is the regenerative ability which is relatively and readily expressed by the explants comprising of living plant tissues and cells. Whenever explants are isolated from the parent plant and cultured *in vitro* under appropriate chemical and environmental stimuli, the cells which were quiescent resume division and often lead to developmental pathways for the production of new cells, tissues, organs and/or complete entire plants. Recent advances in biotechnology including the one utilized for the direct integration of foreign genes into same / different germplasm paves the way for developing improved genotypes encompassing difficulties of crossing different taxa. The development /success of any such technologies essentially depends on correct and directed plant regeneration protocols. *Cannabis* is cultivated worldwide, and China is the largest producer of hemp fiber [8]. The cultivation of *Cannabis* is prohibited in most of the countries and is allowed only for research and pharmaceutical utilities [9].Considering all these in mind, *in vitro* germination of *Cannabis* seeds attempted in order to obtain young and surface sterile plant material for the subsequent establishment of regeneration protocol using leaf explants in the current work.

2. MATERIALS AND METHODS

2.1 Procurement of seeds

For the experiment, the seeds of *Cannabis sativa* L. were collected from Pithoragarh, Uttarakhand. The seeds were brought down to Department of Biotechnology, Sharda University, Greater Noida, India and stored at 4ºC in order to maintain the viability of the seeds.

2.2 Viability checking of the seeds

Cannabis seeds (hundred seeds) were germinated under controlled condition on wet blotting paper kept in petri dish. This helped in verifying the viability of the experimental set of seeds.

2.3 Sterilization of the seeds and germination of seeds on artificial media

The seeds were sterilized by washing the seeds with tap water for removing any contaminants attached to it. The seeds were exposed to 70 % ethanol for 30 s, washed thrice with autoclaved distilled water followed by treatment with 0.1% $HgCl_2$ 30 s and washed again thrice with autoclaved sterile water. The sterilized seeds were blot dried and then transferred to half MS medium and water + agar (0.8 %) medium for germination. The tube containing the seeds were kept in dark for 48 h and once the radical emergence was observed, tubes were transferred to were maintained at 2000 lux light intensity and 25 ºC [10].

2.4 Emergence of callus from the leaf explants of the *in vitro* germinated seeds

The germinated seedlings were allowed to grow till cotyledonary stages and the leaf explants was used for regeneration protocol establishment. The seedling was taken out from the experimental tube and the tip of the leaf was cut with sterilized scalpel. With the help of scalpel and forceps, rectangular blocks of leaf explants was placed on callusing medium which was MS medium + IAA+ BAP (IAA and BAP were used in the ratio of 1:5). Sucrose at 3% (w/v) was used as the carbon source while agar at 0.8% (w/v) was used to provide solid support to the explants. The explants were incubated at 25 ±1 ºC under 16/8 h photo period. Each treatment was tested in three replicates and monitored regularly to record morphological changes. Observations were recorded in each replicate for callus induction and sub- cultured after 15 days.

3. RESULTS

3.1 Viability testing of seeds

The viability of seeds is an important parameter for checking the likelihood of germination potential. If the seeds are more than 90% germinating under controlled condition, then it represents good quality seeds and can be used for tissue culture protocols [11,12] . In our experiment the seeds were 93 % viable which represents a good plant material to work with.

3.2 Germination of seeds on artificial medium and induction of callus

Seeds were kept for germination on half MS medium and water Agar medium (**Fig 1**). Germination was observed on both the medium but water agar showed better germination rate and faster growth of seedlings under *in vitro* conditions (**Fig 2**). This may be due to the effects of various salts present in the MS medium [13]. After three weeks callus was induced in the tested artificial medium (**Fig 3**). The obtained callus varied in their characters and ranged from friable, compact and color ranging from pale yellow to green.

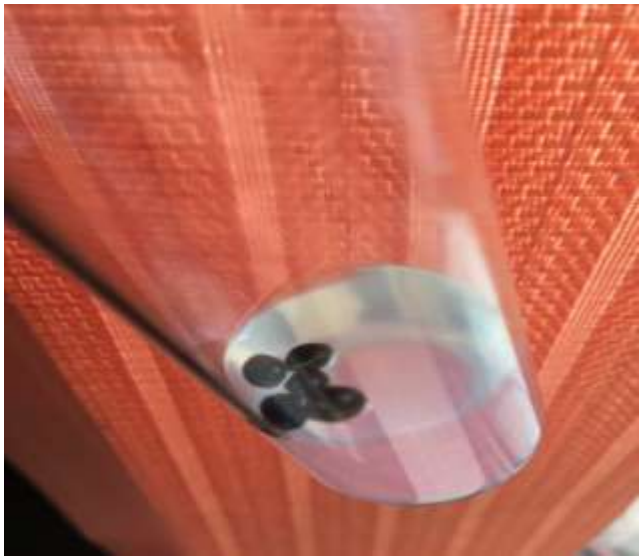

Fig. 1: Seeds placed in Agar/MS medium for germination

Fig 2: Growth of seedling under artificial medium

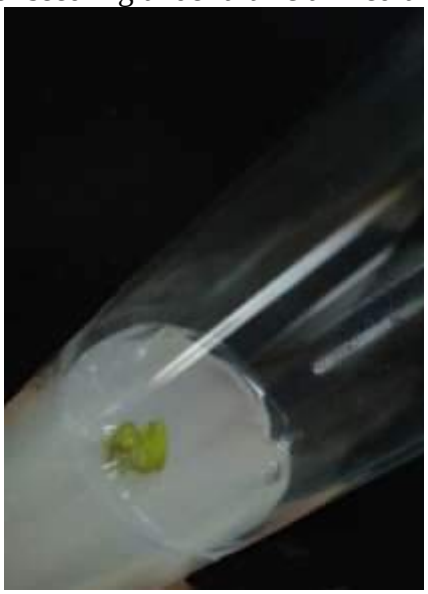

Fig.3: Induction of callus in artificial medium after three weeks of culture

4. DISCUSSION

In vitro seedlings are of great importance as they can be directly used in any tissue culture related experiments without the need of sterilization. *In vitro* tissue culture offers high rates of multiplication from meristematic segments of tissue and can be regarded as an efficient tool for obtaining large

numbers of individuals free from contaminating sources [14]. In this study, we have studied the regeneration of callus from leaf explants of *Cannabis sativa* L. Callus was obtained from the explants which is in agreement with the previous study [15]. It has been reported that cotyledon and root explants are not capable of producing callus well [16]. Similar reports of callus induction has also been reported on Acacia, Tomato and other plants [17]. The work may further be extended to test the complete regeneration potentials of *Cannabis.*

5. CONFLICT OF INTEREST

Authors declare none

6. ACKNOWLEDGEMENT

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