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In vitro shoot regeneration of *Populus nigra*

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Abstract

Populus nigra is very useful forest species used as timber production and very ease of vegetative propagation. It is mainly cultivated in forest as woody biomass and reforestation because have good tolerance and has several medicinal properties also. However, long generation time, seed dormancy and long period for evolution of mature traits are the limitations. Micro-propagation is a practice used to propagate plants under sterile conditions often to produce clones of plant, to production of exact copies of plant, produce good flowers and fruits. It is very useful to quickly produce mature plants, production of multiples of plants in the absence of seeds or necessary pollination to produce seeds. In present study nodal segment of *Populus nigra* were collected and after sterilization were grown in basal MS medium with different ratio of benzylaminopurin as MS+0.1 BAP and MS+0.5 BAP. And we got that initiation percentage on MS + 0.5 BAP give better result.

Keywords: *BAP, Plant hormone, MS medium*

1. Introduction

Poplar is an opportunistic species as ease of colonize and good tolerance capacity. black poplar (*Populus nigra*) is a species of poplar in the cotton wood (Aegiros) section of the genus Populus, it is native to Europe, southwest, Central Asia and Northwest Africa, cultivated in North-Western Himalaya at 900 – 3700 m. There are about 60 species distributed all over the world of poplar. Black poplar from silicaceae family can live for over 250 years and there are historical records of trees approaching 300 years of age[1]. It is needs only 30 to 50 years to grow as a majestic tree with around 30-35 m in height and 2m in trunk diameter, *Populus nigra* is a fast growing and easily propagated in flourish soil and reach maximum height when it is close to water. It has a wide rounded crown and dark gray, fissured bark with many swelling on its large trunk. The buds are close together, sticky and brownish in color. Leaves are 2 to 5 inches long which twist around their stems are diamond shaped, nearly triangular with long drawn out points. The genus *Populus* L. (Salicaceae), in addition to its value for wood products, provides a range of ecological services, including carbon sequestration, bioremediation, nutrient cycling, and biofiltration[2,3]. Black poplar is now one of the most threatened tree species in Europe[4] and is close to extinction in a large part of Western Europe[5]. It has good ability to tolerate high water level and high temperature, the tree extracts have a good antioxidant and anti-inflammatory effects[6,7]. Both tree breeders and conservationists are aware of the importance of black poplar and propagate black poplars by means of cuttings or grafting. Factors that are affecting on shoot and root morphogenesis in explants has been applied extensively for micro propagation[8]. *Populus nigra* cultivated as woody biomass for forest industries used as reforestation and has many medicinal and antibiotic properties as well, but due to some limitations like as seed dormancy and long generation time. However, there is only little information available on the response of *Populus nigra* therefore it is necessary to develop and evaluate micro propagation method for *Populus nigra*. This study aim was evaluate the effect of application of growth hormones with basal MS media on sprouting behavior of the *Populus nigra.*

2. Materials and Methods

Healthy and dieses free plants were evaluated for this experiment and new, fresh, young twigs were collected from plants for further processing. Cut the collect explants from internodes. Immediately

deep into savlon water, explants were washed with tap water (three times). Then washed with liquid soap for three times and washed with sterile distilled water. Then explants were treated with wide range fungicide (Bavisteen) for 30 minutes and washed with sterile distilled water. For culturing the explants in Laminar air flow. Explants were washed thoroughly with sterile distilled water (2 times) and were treated with 70% alcohol for 90 seconds again washed with sterile distilled water (2 times). Then the explants were treated with 0.1% $HgCl_2$ for 180 seconds and finally washed with sterile distilled water (2 times). And it is now ready for culturing

1. After aseptically sterilization explants were transferred in test tubes which contained MS medium. Test tubes were tightly packed with cotton plug. In which 9 test tubes of 0.1 BAP and 9 test tubes of 0.5 BAP.
2. Test tubes were incubated in standard culture room where temperature (22 + 2°C) and light (1000 lux) were maintained.

Here we were taken two media MS +0.1 BAP (media-1) and MS+ 0.5 BAP (media-2). After 7 days in MS+0.1BAP only one explant was initiated out of nine, where 4 were contaminated and MS+0.5 BAP two explants were initiated out of nine, where only one was contaminated. After 14 days in MS+0.1BAP in one ex plant plantlet was developed and in MS+0.5BAP in two explants plantlet were developed, three were initiated and two were contaminated. After 21 days in MS+0.1BAP two were initiated and only one plantlet in MS+0.5BAP in four explants plantlets were developed and two were initiated. After 28 days no changes in MS+0.1BAP and in MS+0.5BAP two were initiated; only two plantlets observed and other were contaminated. And After 35 days in MS+0.1BAP two explants were initiated and others were contaminated. MS+0.5BAP gives better result in compare to MS 0.1 BAP shown in table.

3. Results and Discussion

Mostly nodal segment as a explants used for the in vitro propagation as the cells of meristem present in nodal segment and undergo continuous multiplication through mitotic division due to which there is very less chance to genetic change and infection also. The poplars species is very susceptible about inter and intra specific hybrid formation and heterosis is frequently seen it have very good timber quality and alleviate for vegetative propagation. Species of Poplars were fist used for cell culture initiation and regenerated from the callus[9,10]. Parsons *et al.* in 1986 performed successfully an experiment on genetic transformation that was timber producing poplar[11]. An easy and efficient protocol was developed by Biswas KK *et al.* 2012 experiment was carried out on *Populus nigra* with different ratio plant hormones with basal MS medium as indole-3-acetic acid, benzylaminopurine, indole-3-butyric acid, and 1-naphthylacetic with 80-88% successful result[12]. In present investigation we preferred nodal segment for *in vitro* multiplication which is transferred on Murashige and Skoog basal Medium (MS) with containing two different ratio of cytokine (6-Benzylaminopurine) as MS+0.1BAP and MS+0.5BAP **Table 1**. Proper maintenance and time to time evaluate culture and initiation data were record **Fig. 2**. After 28 days of our experiment we found that there is 0.5BAP showing good result of initiation with compare to 0.1BAP as here concluded that an optimum ratio of plant hormones is effective for regeneration **Fig. 1**.

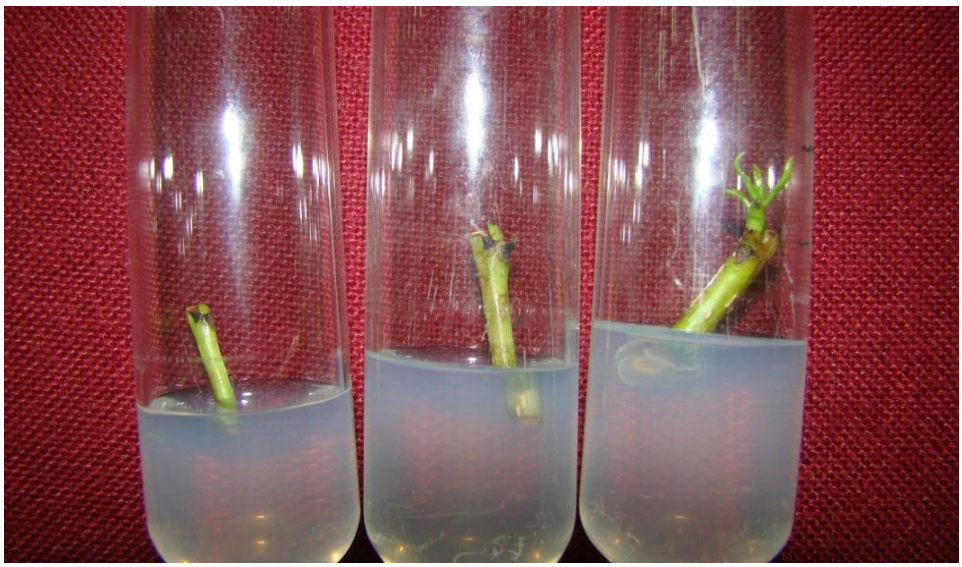

Fig 1: Initiation of Populus nigra after 21 days.

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Table 1: Growth of plantlets in different days

Test tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Media	MS+0.1BAP									MS+0.5BAP								
7 Days	-	-	C	-	-	0.5	-	0.7	-	-	-	-	-	-	-	0.7	0.9	-
14 Days	-	-	-	-	C	0.5	-	1.0	-	-	-	-	0.6	1.3	-	0.7	1.2	-
21 Days	-	-	-	-	-	0.7	-	1.2	-	-	-	-	0.8	C	-	C	1.2	C
28 Days	-	-	-	-	C	0.7	C	1.2	-	-	-	-	1.4	C		C	C	-

Where is (-) = Plantlet not developed, C = Contamination and plantlet height in cm

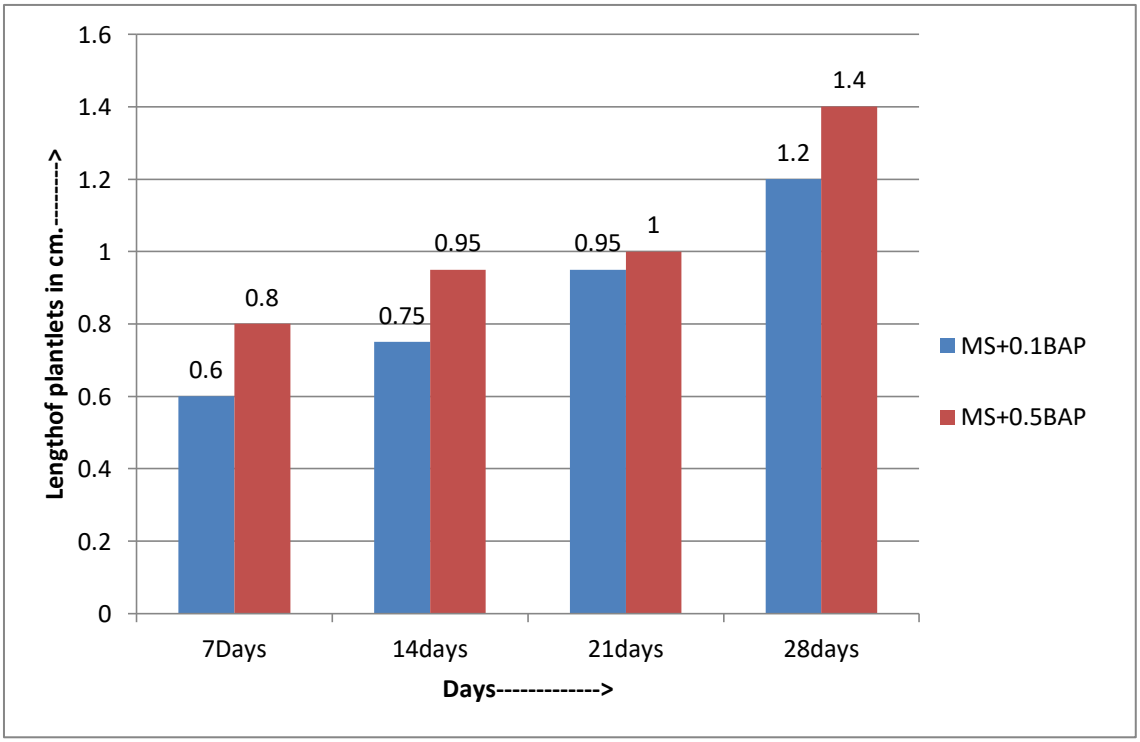

Fig 2: Graph showing comparison within MS+0.1BAP and MS+0.5BAP

4. Conclusion

We found that shoot regeneration from poplar twigs segments can be achieved by a simple procedure, in which pre-culture in CIM is not necessary. No callus induction in this method might be another advantage, because it is well known that plants regenerated via a callus phase may differ from the mother plant due to somaclonal variations. The present protocol is also economical, because it results high frequency (87%) direct shoot regeneration without trans-zeatin, an expensive substance that have used in the previous

protocols of *Populus nigra* regeneration. Our method is able to produce and perpetuate a large number of disease-free Lombardy poplar plants, and will thus benefit physiological and genetic studies of hardwood plants by providing a constant supply of competent and efficient plant materials.

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