

PROPAGATION OF *JUNIPERUS POLYCARPOS* C. KOCH THROUGH TISSUE CULTURE I. INDUCTION OF CALLUS

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Abstract. Induction and growth of callus on shoot segments of *Juniperus polycarpus* was studied. Basal medium used was that of Murashige and Skoog's. The effect of auxins, kinetin, coconut milk and casein hydrolysate was studied on induction and further growth of callus. Auxins were NAA (0.05, 0.1, 0.5 and 1.0 mg/l), 2,4-D (0.1, 0.5 and 1.0 mg/l). Cytokinin used was BAP (1.0 mg/l). Our results indicated that size of the explant, presence or absence of apical bud, controlled conditions of light and temperature are important factors in callus formation. A successful callus could only be raised on MS medium supplemented with 1.0 mg/l of 2,4-D, CM 10%, sucrose 2% in diffused light at $25 \pm 2^\circ$ C in shoot segments having apical bud. For further growth of callus, addition of 1.0 mg/l of BAP to this medium was beneficial.

Introduction. *Juniperus polycarpus* C. Koch (Syn. *J. macropoda* Bioss) is a moderate sized evergreen tree, distributed in the inner arid ranges of the Himalayas from Nepal westwards at 5,000-14,000 ft. In Pakistan juniper forests occur in Baluchistan, where they are estimated to cover an area of 65,000 to 1,00,000 sq. ft. For a variety of reasons the natural regeneration of juniper is very slow. The timber and fuel requirements of the local population are increasing so much that the existence of juniper is threatened. Preservation and improvement of these forests is possible only through artificial regeneration. Recent investigations on germination of juniper seed have shown that only 1-3% of the berries contain viable seeds and the seeds exhibit delayed germination.

In recent years there has been increasing interest in tissue cultures as an alternative in a-sexual propagation of plants, which otherwise cannot be easily propagated by seeds or vegetative methods. In addition to orchids (Morel, 1966), tissue culture techniques have proved to be commercially practical in many horticultural species (Murashige, 1978), *Asparagus* (Dore, 1975), *Chrysanthemum* (Ben-Jaacov and Langhans, 1972) and *Gerbera* (Murashige *et al.*, 1974).

Gymnosperms tissue is still in its infancy as compared to its angiosperm counterpart. Success in maintaining a continuous culture of coniferous tissue was first reported by Ball (1950). Later Reinert and White (1956) cultured the normal and tumorous tissue of *Picea glauca*. On the other hand, callus has been established for many species of Pinaceae, representing the genera *Pinus*, *Abies*, *Picea* and *Larix* (Chenkova, 1977). Mott *et al.*, 1977) have presented tissue culture methods for the production of multiple propagules from

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single seeds of *Pinus taeda*. Differentiation and subsequent plantlet formation has been achieved from cotyledons of *P. palustris* (Brown and Sommer, 1977). Adventitious bud formation has been achieved in cultures of Douglas fir (*Pseudotsuga menziesii*) (Cheng, 1975). Plantlets have been developed from *in vitro* cultures of *Picea glauca* (Campbell and Durzan 1976) and *Thuja plicata* (Coleman and Thorpe, 1977).

The aim of the present work is to propagate *Juniperus polycarpus* by tissue culture techniques. So far success has been achieved in induction of callus in shoot segments of *J. polycarpus*. In these studies the effect of auxins, coconut milk (CM), casein hydrolysate (CH) and Benzyl amino purine (BAP) was studied on induction and further growth of callus.

Materials and Methods. Two year old plants of *Juniperus polycarpus*, were obtained from the Silviculture Garden of the Pakistan Forest Institute, Peshawar.

Murashige and Skoog (MS) medium was employed in this work. MS medium was prepared according to Gamborg and Wetter (1975) and supplemented with 2% sucrose. Difco-Bacto agar was used at 0.8% concentration and pH of the medium adjusted to 5.8. The medium was sterilized by autoclaving at 15 psi pressure and 120° C temperature for 15 minutes.

Shoot cuttings of *Juniperus polycarpus* were washed thoroughly with tap water. They were surface sterilized with 1% solution of mercuric chloride for about 8 minutes and rinsed three times with sterile distilled water. Shoot cuttings were then cut into pieces and inoculated on the solidified agar medium in a sterile chamber.

Experiments were carried out in full day light and diffused light. Cultures were kept in a room with temperature regulated at $25 \pm 2^\circ\text{C}$. The effect of the following growth substances (mg/l) were studied on callus induction and its growth; Naphthalene acetic acid (NAA) 0.05, 0.1, 0.5, 1.0; 2,4-Dichlorophenoxyacetic acid (2,4-D) 0.05, 0.1, 0.5, 1.0; Benzyl amino purine (BAP) 1.0; Coconut milk (CM) 10% v/v, Casein hydrolysate (CH) 500 mg/l.

Results. Excised shoot pieces of two types were used, a decapitated shoot with needles and shoot with needles and apical bud. Both types of excised shoot pieces were inoculated on the basal medium (BM). Callus formation was not observed and both types of shoot pieces died after 4 weeks of culture. Signs of desiccation and change of colour from green to brown were noted a bit earlier in decapitated explants. In further experiments the effect of various auxin (NAA and 2,4-D), CM and CH were studied on callus induction and growth.

Effect of 2, 4-D: Excised shoot pieces of both types were inoculated on BM supplemented with 0.05, 0.1, 0.5 and 1.0 mg/l of 2,4-D. Experiments were carried out both in diffused and full day light. Callus formation was not observed and the explants died after 4 weeks of culture.

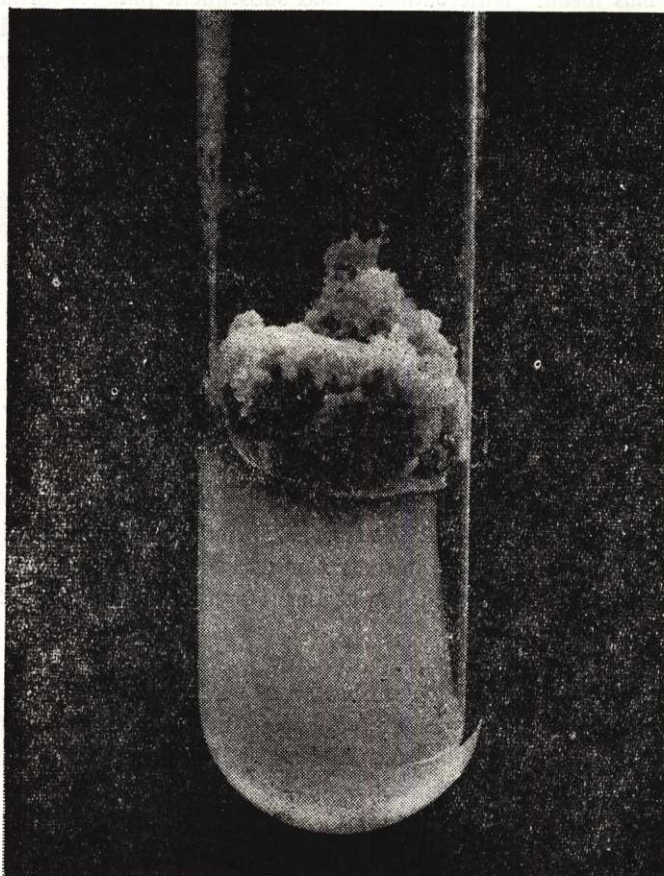


Fig 1. Callus formation on shoot explants with apex on BM supplemented with 1 mg/l of 2, 4-D-CM 10% in diffused light after a) 5 months of culture.
b) 3 months

Effect of 2,4-D and CM: Excised shoot pieces of both types were inoculated on BM supplemented with 2,4-D (0.05, 0.1, 0.5 and 1.0 mg/l) and CM 10% (v/v). Callus formation was observed on 0.5 and 1.0 mg/l of 2,4-D in shoot pieces with apical bud, after 6 weeks of culture, but 1.0 mg/l 2,4-D gave better results. (Fig-1). Moreover callus formed in diffused light at 1.0 mg/l of 2,4-D showed vigorous growth as compared to callus formed in full day light. The callus formed was yellowish in colour, soft and nodular in appearance. Later on the colour changed from yellowish to light brown. In decapitated shoot segments no callus formation was observed at the above mentioned concentrations of 2,4-D and CM. It was also observed that callus could not be induced in shoot segments less than 1.5 cm in length with apical bud.

Callus formed on medium containing 1 mg/l of 2,4-D was transferred to the medium containing 1.0 mg/l of 2,4-D alone, 1.0 mg/l of 2,4-D and CM and 1.0 mg/l of 2,4-D and CM and BAP (1.0 mg/l). At 1.0 mg/l of 2,4-D alone callus growth was slow. Addition of CM to this medium enhanced callus growth. Callus growth on the medium containing either CM 10% or BAP alone was very slow and ceased after some time. However, the medium supplemented with 2,4-D (1.0 mg/l) and CM (10%) and BAP (1.0 mg/l) exhibited the best growth of callus, when compared to above mentioned media. (Fig-1).

Effect of 2,4-D, CM and CH: Excised shoot pieces, with or without apical buds were inoculated on BM supplemented with 2,4-D (0.05, 0.1, 0.5, and 1.0 mg/l), CM (10%) and CH (500 mg/l). Callus formation was observed at 1.0 mg/l of 2,4-D in the shoots with an apex. In decapitated shoots no callus formation was observed. It was noticed that addition of CH to the medium had no additive effect on callus initiation and growth.

Effect of NAA: Excised stem pieces with or without apical buds did not develop at any concentration of NAA (i.e., 0.05, 0.1, 0.5 and 1.0 mg/l) alone or in combination with 10% (v.v.) CM. After 4 weeks of culture such shoot segments became brown and died.

Callus initiated on the medium supplemented with 2,4-D and CM was transferred to the medium supplemented with 1.0 mg/l of NAA alone or in combination with either CM (10%) or BAP (1.0 mg/l). Callus growth on the medium supplemented with 1.0 mg/l of NAA alone or in addition to either CM or BAP was slow as compared to callus growing on the media supplemented with 2,4-D (1 mg/l) with either CM or BAP 10 mg/l.

Discussion. From the results obtained in the preceding section, it was evident that callus could not be induced in shoot segments on BM (MS) supplemented with 2% sucrose. Even the addition of 2,4-D (0.05, 0.1, 0.5 and 1.0 mg/l) to this BM was not effective. However, callus was induced on the medium containing 0.5 and 1.0 mg/l of 2,4-D, when supplemented with 10% CM. When this callus was excised and transferred to the medium lacking CM, it grew slowly, while the callus transferred to the medium containing either CM or BAP in addition to 2,4-D (1.0 mg/l) exhibited better growth. The callus growing on the medium supplemented with both CM and BAP in addition to 2,4-D, showed still better results. Das *et al.*, (1956) found that culture of pith cells of tobacco showed marked cell division in the presence of IAA and Kinetin. IAA or Kinetin alone could not produce such an effect. In the present work also, 2,4-D (1 mg/l) alone could not induce callus in shoot

segments. Callus was induced in shoot segments by the supplementation of CM in addition to 2,4-D. Moreover addition of BAP to this medium enhanced further growth of callus. The results indicate that for callus induction and its further growth supplementation of cytokinin in addition to 2,4-D is necessary.

Konar (1974) reported the requirement of CM and CH in addition to 2,4-D to the medium for *Pinus gerardiana*. In our studies the addition of CH to the medium containing 2,4-D and CM had no beneficial effect on callus growth.

In the present studies callus could not be induced in decapitated segments. This might be due to the synthesis of some hormone or metabolite in the apical bud. Probably such a factor is required to keep up the level of the regulatory substances or this could be critical for the initiation and growth of the callus.

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