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IN-VITRO CULTURE OF ETHNOMEDICINAL ENDANGERED PLANT *CALLIGONUM POLYGONOIDES* (PHOG) LINN.

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ABSTRACT: This study mainly focusses on survey, conservation and awareness of ethno-medicinal plants in the Thar desert area of Barmer. *Calligonum polygonoides* Linn was collected from Thar Desert region of Malana village of Barmer district, locally known as "PHOG" and "PHOGRA" and belongs to Polygonaceae family. The rareness of the plant showed need for its micropropagation. Survey was conducted in specific region of Thar Desert of Barmer District. Ethnomedicinal values of maximum plants were seen and *Calligonum* was selected for mass multiplication. *In-vitro* culture was done in *Calligonum polygonoides* with different hormonal concentration in MS media with BAP (6-Benzylaminopurine) as cytokinin and IAA (Indole-3-acetic acid), IBA (Indole-3-butyric acid) 2,4-D (2,4-Dichlorophenoxyacetic acid), NAA(1-naphthaleneacetic acid) as auxin. In results good amount of callus induced in NAA 4mgL, shooting and callus in 2mg/L NAA with 1mg/L BAP, and rooting in IAA 1.5mg/L. This plant requires conservation via *ex-situ* or *in-situ* protocols, but in our study, in-vitro micro-propagation was performed for its conservation and their further research. The enhanced deforestation, with huge demand for wood and fruit, has lead to requirement of more amounts of trees adapted to present climatic conditions and, thus, improvement of genetic traits and quality of plants.

INTRODUCTION: India is a rich diversity region of the world and contains medicinal plants and plant drugs under the popular heritage [1]. Overexploitation of medicinal plants (used in pharmaceutical industries) results into destruction of natural populations of medicinal flora. Medicines consumed from plants are used in different fields *i.e.* Ayurveda, Allopathy, Unani, and Homoeopathy and in various other systems [1].

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This region contains number of endangered and vulnerable medicinal plants used as drugs and medicines in pharmaceutical industries. Ethnomedicinal plants of this area are used to treat and cure various diseases. We have collected the information via survey in region of Barmer.

Local communities and villagers of Barmer District *Kalbelia, Lohar, Jogi, Ojha, Nath, Bheel, Bhopa, Baba, Bhagda, Mangniyaar, Langa, Banjara* and *Raika* are belonging to scheduled caste or others. Diminishing of plant species of Thar region is because of anthropogenic activities. *Calligonum* genus belongs to family Polygonaceae and it has over 80 species in the world but species *polygonoides* is endangered in category which is found in Pakistan and near Pakistan border in India

(Rajasthan). *C. polygonoides* Linn, is commonly called Phog, grows on dry sandy soil of desert part of Barmer district. This is a leafless shrub plant and has smooth branches and stems, small succulent fruits which have hair like outgrowth and bearing abortive flowers. Joint of branches are reddish in colour because of anthocynine secretion. Branches are green in colour and perform photosynthesis due to absence of leaves. *Calligonum polygonoides* Linn. is locally known as Phog or Phogra, their Buds locally known as Lasson, stem with green branches locally known as lassoan, vegetable made of flowers is known as Fogeseae. Local population feed Fogeseae in February and March for their good health [2]. Flowers contain high amount of proteins [11]. *Calligonum* is abiotic stress tolerant shrub and energy rich fuel wood.

The paste of shoot, stem and lassoan with water is applied on eyes against scorpion sting, powder of roots and catechu mixer is used for gargling sore throat, latex used in treating eczema, dogs bite and abortion. The flowers have tonic and digestive properties due to presence of some chemical Compounds and used against cough, asthma and cold. Local people use this shrub as food at the time of famines [2]. Methanol extract of the *C. polygonoides* showed strong toxicity in brine-shrimp. Lethality test on brine-shrimp is performed by Yawer *et al.,* 2007 via methanol extract of *Calligonum polydonoides* [2].

The roots of *Calligonum polygonoides* with methanolic extract that act against the two fungi *Candida albicans* and *Aspergillus niger* [3]. The vegetables of Barmer contain good amount of oil yielding plants which is income source of the localities [4]. *C. polygonoides* provides potential soil ameliorating factor as well as a facilitative herbaceous plant in India and Iran [5]. This plant contains many chemical phenolics, alkaloid compounds like calligonolides, butanolides, steroidal ester, tetracosan-4-olide, b-sitosterol and its glucoside, and ursolic acid [6]. *Cistanche tubulosa* (Schenk) R. Wight (Orobanchaceae) is found as root parasite on *Calotropis procera* L. in desert habitats of Western Rajasthan, especially in Barmer desert region [7]. Fruits of *Calligonum polygonoides* were cultured in liquid MS media supplemented with 1 mg/L indole-3-butyric acid (IBA) in the dark at 25°C which resulted into root formation after 14 days [7]. Plant *Calligonum polygonoides* is categorised endangered plant via Red Data Book of the International Union for Conservation of Nature and Natural Resources [8]. This plant requires conservation *via in-situ* and *ex-situ* techniques. Micro-propagation technique is found to be good and alternative approach for conservation of the endangered and threatened plants, their propagation for future bio-chemical reactions, new drug discoveries and in pharmaceutical field [8].

Phenolic compounds and antioxidant activity highly observed in winter and summer season [9]. Plant cell and tissue culture is strong and healthy method to enhance secondary metabolite production and endogenous phytohormone metabolism signaling in several plants [10-13].

In the review "Micropropagation of *Eucalyptus"* by Abiri *et al.* summarized and performs the practice on Eucalyptus and examine the most important physiological and molecular aspects and identified the bottlenecks hampering and production of bioactive compounds [10]. Many research expose many chemical, physiological factors mainly temperature which directly affect the plant development are sensitive therefore tissue culture is more beneficiary for practical level in research fields [14].

Study Area: Floristic survey of Barmer District was carried out from year 2020 to 2022. *Calligonum polygonoides* Linn was collected during the survey from different localities, in the present investigation **Fig. 1, 2.**

METHODOLOGY:

Survey: Survey was conducted in selected regions of Thar area in Barmer district to collect information about ethno-medicinal plants. Micro-propagation technique was applied on *Calligonum polygonoides* plant which required conservation due to Exploitation by human activities. Survey was conducted in Thar Desert of Barmer district of Rajasthan during June 2020 to December 2021 during different time periods. Information was collected about ethno-medicinal plants through interaction with tribal and local people. Survey was done in selected regions like Jaisindhar ganv, Jaisindhar station, Bhure ki Basti, Khalife ki Bawri, Malana, Khadin, Viratara Mata Mandir-Viratra

(dhok), Keradu temple from Keradu. The collection of information about ethno-medicinal plants in curing various health ailments was on the basis of interview and structured questionnaire.

FIG. 1: INTERACTION WITH INDIGENOUS PEOPLE DURING SURVEY IN JAISINDHAR GANV, JAISINDHAR, KHADIN, MALANA, KHALIFA KI BAWRI

FIG. 2: HABITAT OF *CALLIGONUM POLYGONOIDES* AND ITS FLOWERING STAGE

RESULTS: During the survey around 30 plants were identified for their ethno-medicinal potential in curing various health ailments. The plants were collected and were further identified in Herbarium, Department of Botany, University of Rajasthan, Jaipur. The explants viz stem having node and internodes were subjected on MS medium supplemented with various auxin and cytokinin added singly or in combination, maximum regeneration was seen on MS medium supplemented with 3mg/L NAA and 1mg/L BAP **Table 1.** *In-vitro* culture practice was performed on *Calligonum polygonoides* Linn. on MS medium supplemented with different hormone concentrations. In previous studies most used explants were fruit, but in current work not only fruits but also stem with node and internodes were used as explants. In our work especially stem with node and internodes were cultured on MS Media. In-vitro culture of plant *Calligonum polygonoides* was subjected to shoot proliferation in presence of BAP(6-benzylaminopurine) 4mg/L, 5mg/L, 6mg/L with IBA(indole-3-butyric acid) 1mg/L from 1mg/L stock. Callus induced by the same concentration of IBA(indole-3-butyric acid) and BAP(6-benzylaminopurine) 1mg/L and 400µL/L from stock 1mg/L. Callus was fragile and soft with hair like structure on tips. Rooting was observed on MS medium supplemented with 1.5 mg/L IAA. Cultures were kept in aseptic culture chamber with 26°C and 16 hours light and 8 hours darkness.

TABLE 1: SHOOTING, ROOTING AND CALLUS INDUCTION ON MS MEDIUM SUPPLEMENTED WITH VARIOUS GROWTH HORMONES

Sr. no.	Hormone concentration		Shooting	Rooting	Callus
	Auxin (mg per L)	Cytokinin (mg per L)			
	NAA	BAP			
1(a)	0.5	4	++	-	-
(b)	0.5	3	+	-	-
(c)	0.5	1.5	++	-	-
(d)	0.5	0.30	+	-	-

(e)	0.5	0.35	+	-	-
(f)	0.5	0.50	+	-	-
2(a)	2	1	++	-	+
(b)	3	1	++	-	+
(c)	4	1	+	-	+
	IAA				
3(a).	0.5	5	++	-	-
	NAA				
4(a)	0.5	1.5	+	-	++
(b)	0.4	0.4	-	-	+
(c)	0.5	1.5	-	-	+
5(a)	1	1	-	-	++
(b)	2	1	-	-	+
(c)	3	1	-	-	+
6(a)	1.5	0.5	-	-	++
(b)	0.5	1.5	-	-	+
7(a)	1	Nil	-	-	+
	2	Nil	-	-	++
	3	Nil	-	-	++
	4	Nil	-	-	+++
	IAA				
8	1.5	-	-	+	-

+ Response positive, ++ more response, - response negative, ++ more positive response, HC hormone concentrations, nil (no amount), HC hormone concentration, IAA- (Indole-3- acetic acid, IBA(indole-3-butyric acid), NAA(1-Naphthaleneacetic acid), BAP(6- benzylaminopurine).

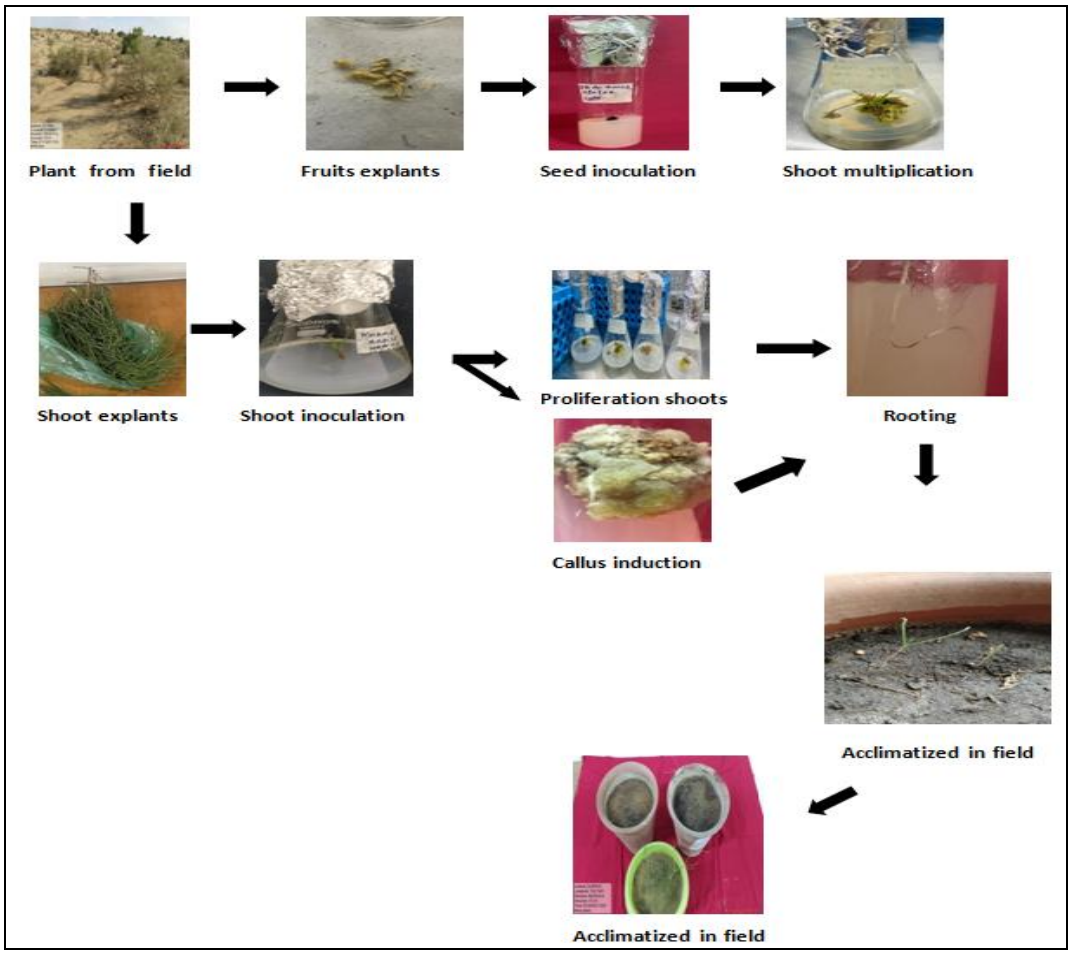
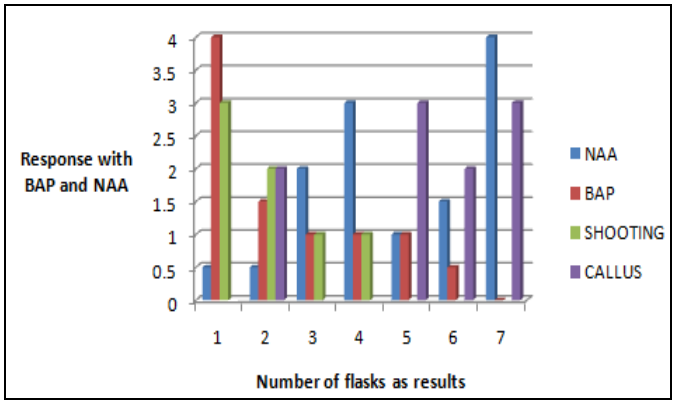
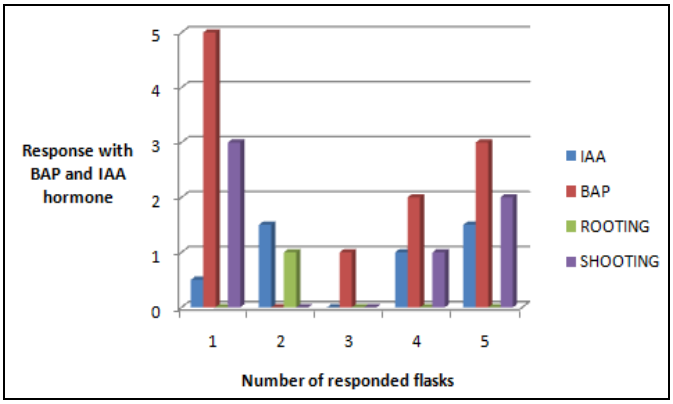


FIG. 3: OVERVIEW OF TISSUE CULTURE OF *CALLIGONUM POLYGONOIDES*



GRAPH: 1 SHOOTING AND CALLUS RESPONSE WITH COMBINATION OF NAA AND BAP HORMONE



GRAPH: 2 SHOOTING AND ROOTING RESPONSE WITH COMBINATION OF IAA AND BAP

Shooting Results:

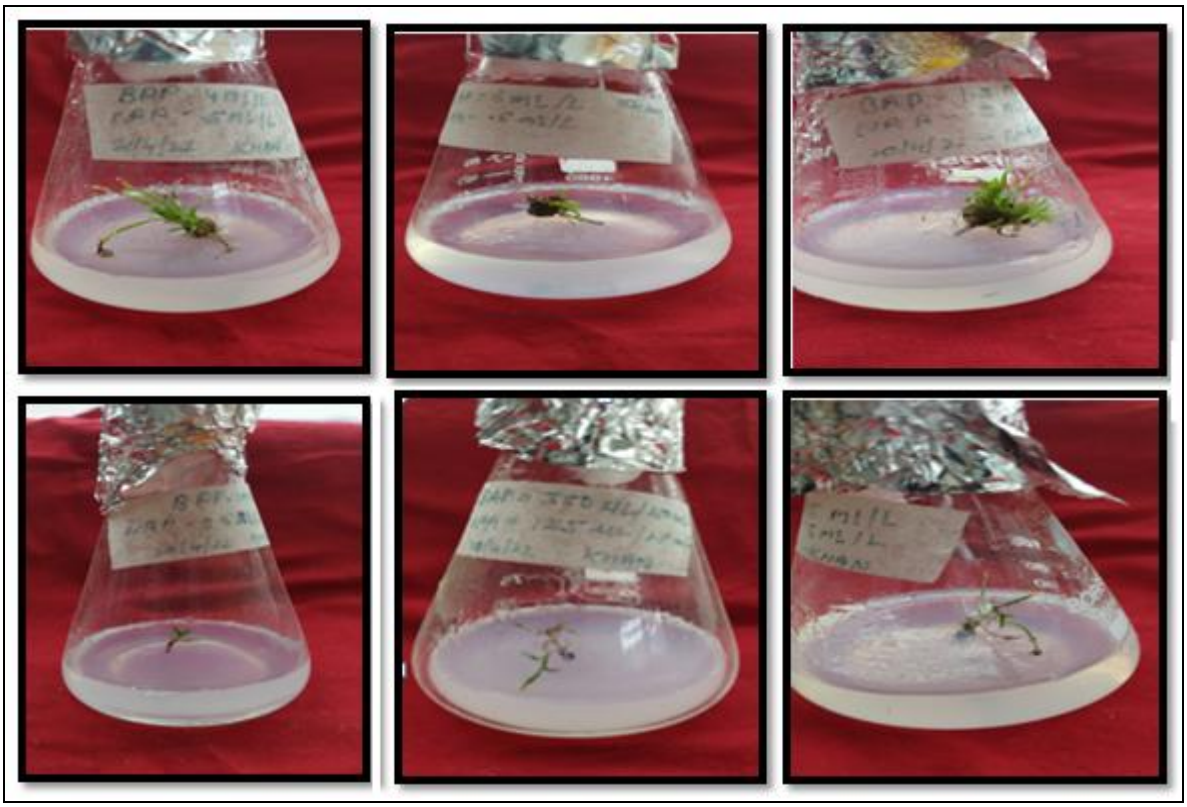


FIG. 4: PLANT REGENERATION VIA SHOOT, NODE AND INTERNODE EXPLANTS ON MS MEDIA

Plant regeneration was seen on MS medium supplemented with hormonal combination;

1. BAP 4.0mg/L and NAA 0.5mg/L,
2. BAP 3mg/L and NAA 0.5 mg/L, and
3. BAP 1.5 mg/L and NAA 0.5 mg/L.
4. BAP 300µg/L and NAA 0.5mg/L,
5. 350µg/L and NAA 0.5mg/L,
6. BAP 0.5 mg/L and NAA 0.5mg/L. Cultures were placed at 26°C with 16 hours light, 8 hours darkness.

Shoot formation was seen on MS medium supplemented with hormonal combinations;

1. BAP 1mg/L and NAA 4mg/L,
2. BAP 1mg/L and NAA 1mg/L, and
3. BAP 1mg/L and NAA 3mg/L. Callus were seen at the lower ends of explants and on upper side multiple shoots were seen on callus. Flask containing 1mg/L BAP and 4mg/L NAA showed more shooting response than the callus, other showed more callus than shoots, in some

more callus induced than the previous two. Maximum shoot formation was seen on MS medium supplemented with BAP and NAA hormonal combination **Fig. 4.**

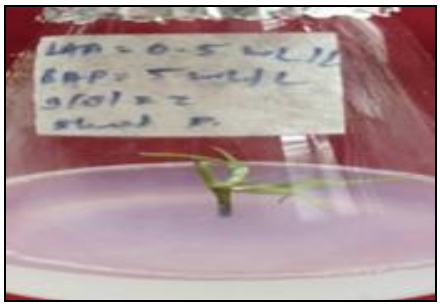


FIG. 5: PLANT REGENERATION FROM STEM EXPLANTS

Plant regeneration from shoot explants on MS medium supplemented with combination of plant growth regulators IAA (0.5 mg/L) and BAP (5mg/L) on MS media. Presence of IAA and BAP 1:10 are effective for shoot proliferation **Fig. 5.** Shoot multiplication was seen on MS medium supplemented with hormone combination of BAP (6-benzylaminopurine) 5mg/L with IAA (indole-3-acetic acid) 0.5mg/L

Formation of Callus on MS Medium Supplemented with Auxin and Cytokinin Hormone: Callus induction was seen from shoot with node and internodes explants on MS medium supplemented with BAP (1.5 mg/L) and NAA (0.5mg/L) and 0.4mg/L BAP and 0.4mg/L NAA hormone concentration **Fig. 6.** Callus was induced from stem explants on BAP and NAA in the ratio 1:1, 1:2 and 1:3 **Fig. 7.**

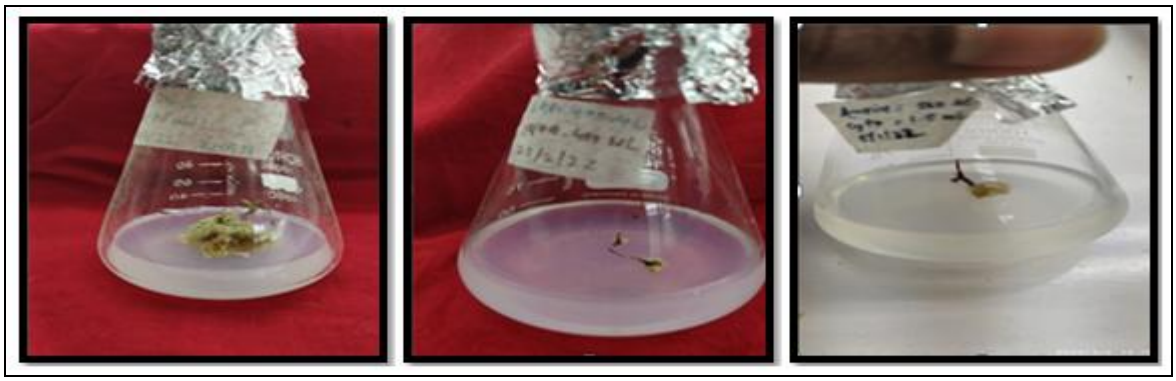


FIG. 6: CALLUS INDUCTION

FIG. 7: FORMATION OF CALLUS FROM STEM EXPLANTS

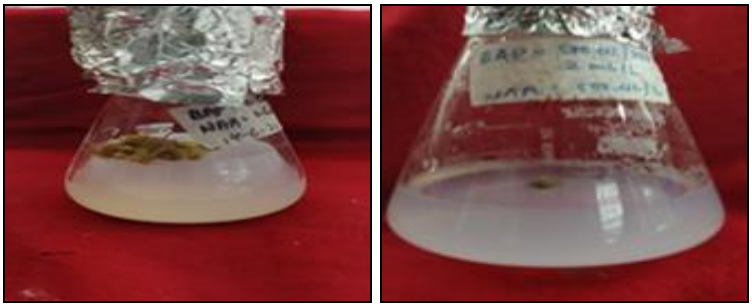


FIG. 8: CALLUS INDUCTION ON MODIFIED MS MEDIUM SUPPLEMENTED WITH VARIOUS COMBINATIONS OF BAP AND NAA

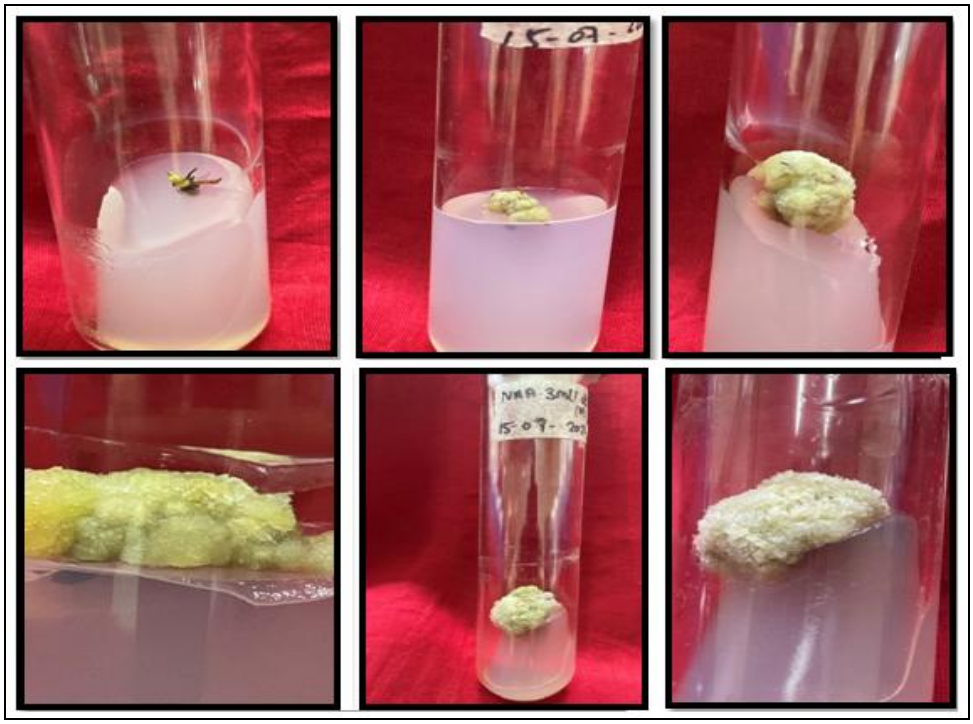


FIG. 9: CALLUS INDUCTION ON MS MEDIUM SUPPLEMENTED WITH ROOTING HORMONE (NAA)

Callus was induced instead of rooting, with different concentration of NAA 1, 2, 3, 4 mg/L. Cultures were kept at 26°C temperature in culture chamber with 16 hour light followed by 8 hour darkness **Fig. 9.**

Rooting of *In-vitro* Cultured Shoot: *In-vitro* cultured shoots were kept for rooting. Culture media was modified MS medium supplemented with IAA.

Cultures were placed in 26 °C with 16 hours light and 8 hours darkness controlled by autotimer. Rooting was induced on MS medium supplemented with 1.5 mg/L IAA **Fig. 10.**

FIG. 10: ROOTING ON MODIFIED MS MEDIUM SUPPLEMENTED WITH IAA HORMONES IN DIFFERENT CONCENTRATION

Acclimatization of *In-vitro* Cultured Plantlet: The rooted plants were transferred in pots under field condition where they acclimatized successfully.

FIG. 11: ACCLIMATIZATION OF *IN-VITRO* CULTURED PLANTLETS

DISCUSSION AND CONCLUSION: Various ethnomedicinal plants were identified for their use in curing health ailments. The present study provides means for *in-vitro* conservation of endangered medicinal plant species *C. polygonoides* using various explants. The kind and concentration of plant growth regulators in the medium were important for production of shoot and further differentiation. Shoot proliferation was induced with BAP (5mg/L) on MS media. Callus induction was seen on MS medium supplemented with NAA (1mg/L, 2mg/L, 4mg/L) and BAP (1mg/L) hormones. Hormone NAA responded more than IAA, IBA, 2, 4-D. Rooting was induced on MS medium supplemented with IAA 1.5mg/L.

The rooted shoots were transferred to field conditions where gradual acclimatization was successfully done. To conclude, the experiment with *C. polygonoides* is an example for in-vitro technique to offer an alternative and renewable source for multiplication of endangered valuable medicinal plants which provide a good chance to improve secondary metabolite yields.

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CONFLICTS OF INTEREST: Nil

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