***J. Adv. Sci. Edu. Res.* 2021: 2: 147-156**, ISSN: 2583-0155 (ONLINE)

https://doi.org/10.56253/JASER.2.1.2021.151-162
Pubished: 25.12.2021(http://jaser.rkmvccrahara.org/)

Cytologically stable micropropagation and *in vitro* conservation of *Artemisia vulgaris* L. (mugwort)

Tuhin Chatterjee

Swami Vivekananda Centre for Multidisciplinary Research in Basic Sciences and Social Sciences
Ramakrishna Mission Vivekananda Centenary College
Rahara, Kolkata- 700118, West Bengal, India

****For Correspondence:*** *tuhinchatterjee15@gmail.com*

Abstract

A simple effective practice of *Artemisia vulgaris* L was established for *in vitro* propagation through node culture, which is an important indigenous medicinal plants belongs to Asteraceae family. Nodal segments obtained from *in vitro* proliferated shoots of *A. vulgaris* for large- scale propagation. MS medium supplemented with 1.0 mg/l BAP individually proved for best shoot multiplication (95± 3.0%) and a high percentage of root initiation (85± 0.3%) was observed in MS medium supplemented with 0.5 mg/l IAA. Maximum 90± 8.23% response was succeeded by using MS medium with 2% sorbitol and 2% mannitol at 10℃ temperature and the plant materials can store up to 8 months without any subculture. The well-developed plantlets were regenerated from nodal segments were hardened- off effectively with 95± 0.2% survival frequency. In this study, *in vitro* short term preservation done by using different osmotic agents and an efficacious *in vitro* propagation system were established from morphologically as well as cytologically stable (2n= 36) plant of *A. vulgaris*.

Key words: *Artemisia vulgaris*, micropropagation, *in vitro* conservation, somatic chromosome

Introduction

The genus *Artemisia* is an elevated aromatic perennial herb of the Asteraceae family, consisting of more than 800 species which are common over the world [9]. Being a traditional herbal medicine, this plant is being widely used for the cure of diabetes, the aerial

parts remain being used as antihelminth, antiseptic, antispasmodic and various disorders including hypotheses and also exposed antibacterial activity [7]. The essential oil is also used as mosquito repellent against *Aedes aegypti*, which transmit yellow fever [3]. Medicinal plants are significant modules of plant biodiversity [1]. The IUCN (International Union for the Conservation of Nature and Natural Resources) were included 8321 number of Vulnerable plants species during the period of 1996 to 2004 in the Red List [4]. The wild collection of medicinal plants from the natural population, inhabitants growing, suburbanization is producing in a mistreatment of wild plant resources and therefore it become a stock of urgency [6, 10]. To manage with upsetting situation, there is an urgent solution for preservation of inherited variety, agronomy and defensible practice of crucial therapeutic herbal species for forthcoming consumption [6]. Through plant biotechnological aspect, supplementary protection approaches have been exploited for considerate esteemed germplasm in the *in vitro* condition [2]. A continuous supply of medicinal plants based medicines permits their qualitative and quantitative propagation through tissue culture method. *In vitro* culture method is convincing and has released wide areas of investigation for biodiversity conservation [6].

In this study, a short term preservation technique and effective regeneration system were recognized of morphologically and cytologically (2n= 36) stable plant of *A. vulgaris*.

Materials and Methods

Plant material

Artemisia vulgaris L. (mugwort) from several localities in West Bengal, India were collected.

Surface sterilization of explants

Surface disinfection of *A. vulgaris* explants were completed by a general fungicide like 2% bavistin solution (w/v) for 20 minutes and then re- sterilization done by a liquid soap solution of 5% Tween- 20 (v/v) for 10 minutes. Final surface sterilization was done with 0.1% (w/v) mercuric chloride solution for 5 minutes and then explants were carefully washed with sterile distill water in advance to inoculation.

Culture media

For *in vitro* shoot multiplication of *A. vulgaris* the culture medium was augmented with altered concentrations of cytokinins and auxins viz., BAP, Kn, 2iP, NAA (0.5, 1.0, 2.0, 3.0

and 5.0 mg/l) individually or in combination and in various concentrations of auxins viz., IAA, IBA, NAA (0.2, 0.5, 1.0, 2.0 mg/l) separately were used for root initiation.

Culture Conditions

The culture were nursed at 25± 2°C temperature and 55- 60% relative humidity with a photoperiod of 16 hrs. per day with photon flux density for nearby 45 µ Mol $m^{-2}s^{-1}$ on circumstance that by white shining light.

***In Vitro* Conservation**

For *in vitro* preservation experiments of *A. vulgaris*, slow growth methods were adopted. MS media were complemented with mannitol (1–3% w/v), sorbitol (1–3% w/v) with 3% sucrose (w/v) and 0.8% (w/v) agar were used on the survival and re-growth of the *in vitro* cultures of *A. vulgaris*.

Acclimatization

For acclimatization, the well rooted regenerated saplings of *A. vulgaris* were placed at room temperature for 7 to 10 days.

Mitotic chromosome study

Young and healthy root tips of *A. vulgaris* were removed from the field growing mother plant as well as tissue culture derived plants in order to analyses the chromosome numbers. Fixed root tips were hydrolysed with 45% acetic acid for 20 min at room temperature and stained with 2.0% aceto-orcein: 1 (N) HCl (9:1, v/v) mixture and incubating for 2 h at room temperature and squashed in 45% acetic acid to obtained well spread metaphase plates on glass slides were done for somatic chromosome analysis.

Result and Discussion

Role of plant growth regulators on *in vitro* shoot proliferation and multiplication:

Among different explants, nodal segments showed the best shoot multiplication result and it were collected from physiological healthy mother plant from our experimental garden. Multiple shoot buds originated when MS medium [5] having different types of cytokinins (BAP, Kn and 2ip) and one auxin (NAA). But the result showed that MS media supplemented with BAP was most effective in shoot initiation and proliferation (Table:1). A maximum response of 95± 3.0% was recorded in MS media were complemented with 1.0 mg/ l BAP alone (Fig. A), where the number of shoot per explants was highest (28.67± 0.43)

with a usual length of 4.97± 0.03 cm. Similar result was studied of *Eclipta alba* (L.) Hassk. (Kashif and Anis, 2006).

Fig. A: Sh

Table-1: Effect of different cytokines on axillary bud proliferation and shoot multiplication from nodal explants of *A.vulgaris* (Data collected after 30 days of culture)

Cytokinin	Concentration (mgl^{-1})	% of explants forming axillary buds	Mean No. of Shoots/explants	Mean shoot length (cm)
BAP	0.5	87.0± 2.0^{b}	22.66±0.33^{de}	4.86±0.08^{f}
	1.0	95.0± 3.0^{a}	28.67±0.43^{b}	4.97±0.03^{b}
	2.0	75.0± 1.1^{c}	17.0±0.57^{a}	3.13±0.03^{a}
	3.0	74.0± 2.0^{b}	16.01±0.50^{c}	2.23±0.08^{d}
	5.0	72.0± 3.0^{a}	15.67±0.34^{hi}	1.56±0.03^{gh}
Kinetin	0.5	70.0± 1.0^{b}	10.34±0.32^{fghi}	3.36±0.06^{i}
	1.0	67.0± 1.2^{d}	7.66±0.30^{d}	3.30±0.03^{g}
	2.0	65.0± 0.5^{cd}	6.66±0.35^{c}	2.33±0.05^{d}
	3.0	58.0± 1.0^{c}	3.34±0.58^{def}	2.03±0.03^{f}
	5.0	45.0± 1.0^{bc}	3.00±0.32^{ghi}	1.60±0.05^{g}
2iP	0.5	47.0± 1.3^{c}	3.67±0.34^{efgh}	1.33±0.03^{ih}
	1.0	57.0± 1.5^{cd}	6.32±0.33^{c}	3.83±0.03^{c}
	2.0	50.0± 0.1^{f}	5.34±0.56^{d}	2.83±0.03^{e}
	3.0	30.0± 2.0^{c}	4.00±0.58^{efg}	1.53±0.02^{gh}
	5.0	25.0± 1.3^{bc}	1.32±0.32^{i}	1.23±0.04^{i}
NAA	0.5	25.0± 1.0^{bc}	1.33±0.44^{ef}	1.03±0.14^{f}
	1.0	20.0± 1.0^{c}	1.02±04^{gh}	1.00±0.2^{i}
	2.0	Callus	Callus	Callus
	3.0	Callus	Callus	Callus
	5.0	Callus	Callus	Callus
BAP+Kn	0.5+0.5	75.0± 1.0^{ef}	18.6±0.23^{a}	3.68±0.69^{g}
	1.0+0.5	82.0± 1.3^{c}	24.2±0.37^{ij}	4.50±0.58^{cd}
	3.0+0.5	68.0± 1.1^{e}	13.36±1.02^{c}	4.02±0.51^{bc}
BAP+2iP	0.5+0.5	75.0± 0.3^{f}	15.3±0.65^{ab}	3.60±0.57^{i}
	1.0+0.5	79.0± 0.5^{fg}	19.30±0.26^{gh}	4.00±1.03^{e}
	3.0+0.5	75.0± 1.0^{gh}	10.25±0.95^{fg}	3.40±0.87^{a}
Kn+2iP	0.5+0.5	62.0± 1.2^{ghi}	10.20±0.91^{ghi}	3.30±0.44^{fg}
	1.0+0.5	70.0± 1.3^{cd}	11.23±0.65^{ab}	3.80±0.51^{e}
	3.0+0.5	60.0± 0.1^{cd}	8.20±1.20^{i}	3.70±1.02^{fg}

BAP + NAA	0.5+1.0	50.0± 1.0^{ghi}	5.0±1.2^{c}	3.12±1.3^{b}
	2.0+1.0	31.0± 1.0^{f}	3.12± 0.4^{d}	2.0±1.90^{cd}
	3.0+1.0	Callus	Callus	Callus
Kn + NAA	0.5+1.0	29.0± 1.0f^{g}	3.10±0.32^{cd}	1.79±2.1.2^{bc}
	2.0+1.0	20.0± 1.1^{gh}	1.17±0.12^{ef}	1.45±2.1^{cd}
	3.0+1.0	Callus	Callus	Callus
2iP + NAA	0.5+1.0	23.0± 0.2^{cd}	2.0±1.0^{def}	1.20±0.32^{def}
	2.0+1.0	16.0± 1.0^{gh}	1.0± 0.2^{efg}	0.07±0.21^{ghi}
	3.0+1.0	Callus	Callus	Callus

(Each value represents the mean ± SD of 10 replicates and each experiment was repeated thrice)

Shoot elongation

Shoots of *A. vulgaris* were removed with a moderate size and were cultured in MS medium having 1.0 mg/l BAP. In the presence of various concentrations of GA_3, the culture was evaluated for maximum shoot length for 2 weeks. The inspiring for shoot elongation of *A. vulgaris*, the result put forward that lesser quantities of GA_3(0.6 mg/l) in combination with 1.0 mg/l BAP were more effective. Shoots were achieved a height of 11.5± 1.5 cm during GA_3 treatments.

Initiation of roots from *in vitro* developed micro shoots

For *in vitro* rooting experiments of *A. vulgaris*, among the three different auxins were verified and the root length as well as number of roots were varied. *In vitro* saplings suggestively were established lengthy roots and root initiation was strength within 25 to 30 days of culture (Table:2). MS- medium having various concentrations of IAA, IBA and NAA (0.2, 0.5, 1.0, 2.0 mg/l) alone were used and 0.5 mg/l IAA, exhibited well result than the other concentration. The average number of roots per shoot was 5.8± 3.5 and maximum root length was 6.3± 2.2 cm. (Fig. B).

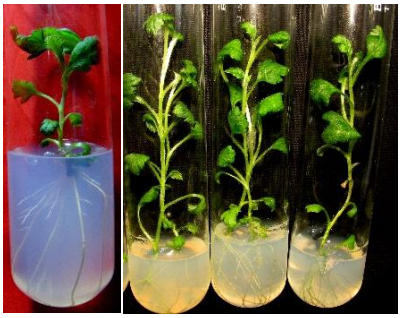

Fig. B: Root induction of *A. vulgaris*

Table -2: *In vitro* rooting of *A. vulgaris* by using different types of auxins. (Data collected after 30 days of culture).

MSO + Auxins	% of rooting	No. of root/ shoot	Root length (cm)
Control	-	-	-
IAA			
0.2	67.0± 0.7^{c}	3.9± 1.2^{c}	4.3± 0.2^{b}
0.5	85.0± 0.3^{a}	6.4± 2.5^{a}	6.3± 2.2^{a}
1.0	78.0± 1.1^{b}	4.3± 0.7^{c}	4.9± 1.1^{c}
2.0	45.0± 0.2^{c}	2.8± 1.2^{e}	3.2± 0.4^{bc}
IBA			
0.2	53.0± 3.2^{f}	3.8± 1.8^{cd}	3.9± 2.3^{b}
0.5	65.0± 2.1^{cd}	4.8± 3.0^{a}	4.8± 2.0^{a}
1.0	48.0± 0.7^{e}	4.0± 2.5^{b}	3.1± 1.5^{c}
2.0	32.0± 0.4^{c}	2.3± 0.5^{e}	2.1± 0.5^{bc}
NAA			
0.2	38.0± 0.3^{fg}	3.5± 0.5^{f}	3.2± 0.2^{f}

0.5	31.0± 0.1^{h}	2.7± 1.0^{e}	2.1± 0.7^{e}
1.0	23.0± 0.1^{g}	1.8± 0.7^{c}	1.2± 0.1^{e}
2.0	callus	callus	callus

(For each value represents the mean ± SD of 10 replicates and each experimentation was repeated thrice)

In Vitro Conservation

For *in vitro* conservation practice of *A. vulgaris*, the cultures were keep up in control medium *i.e.*, MS medium with 3% sucrose, without any osmotic agents. The culture was maintained in healthy condition with 100 % survival rate up to 90 to 115 days without any subculture. But after 3 to 4 months, the control medium did not help to survive and survival rate of the culture was gradually deteriorated. But when mannitol and sorbitol were included in the culture then the survival rate was increased up to 8 months without any subculture.

By using different osmotic agents, the growth of the *in vitro* culture can be controlled. The results showed that 2% sorbitol (w/v) and 2% mannitol (w/v) with MS medium has augmented height survival rate 90± 8.23% at 10°C. Our outcomes also showing that during slow growth *in vitro* preservation techniques of *A. vulgaris*, 10°C temperature was better than 4°C temperature. After 8 months' storage condition, the culture was moved into fresh MS medium augmented with various combinations and concentration of cytokinins and auxins and no any remarkable change of *in vitro* shoot and root growth was observed during *in vitro* slow growth preservation techniques.

Acclimatization

For acclimatization, the well rooted regenerated saplings of *A. vulgaris* were moved to a small earthen pot having soilrite and covered with apparent polytheen bags. Lastly, the regenerated saplings were shifted to Poly House and maintained 90 to 120 days and then plants were moved to the experimental field condition (Fig. C). 95% survival rate was observed in the field condition.

Fig. C: Field growing *A. vulgaris*

Mitotic Chromosomal study:

Mitotic chromosomal study of *A. vulgaris* in our present experiments from indiscriminately selected root tips from the field growing mother plant and presented 2n= 36 number of chromosomes. Cytological preparations from the *in vitro* regenerated plantlets of *A. vulgaris* was also indicated mainly the diploid number (2n= 36) of chromosomes (Fig. D).

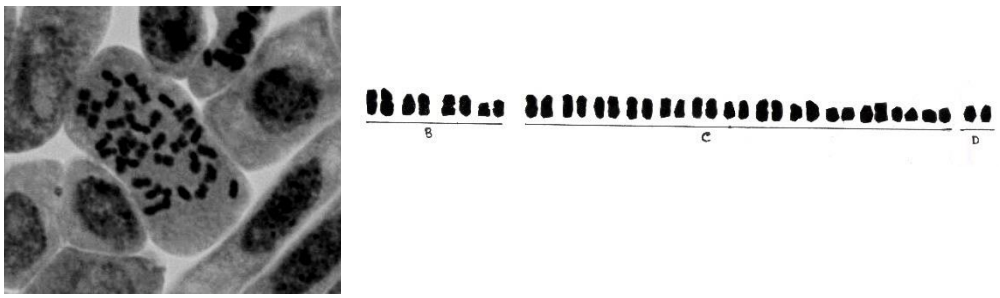

Fig. D: Mitotic Chromosome (2n= 36) with karyotype

Conclusion:

Our studies offer a modest and efficient procedure of micropropagation method for *in vitro* conservation of *A. vulgaris* under *in vitro* slow growth techniques and germplasm can be preserved effectively for 8 months without any subcultures and also allowing for the observing effort in the research laboratory. The method also suggested here has the benefits of both simplicity and effectiveness. In connection with this study, we also established a simple protocol of chromosomal status of conventional plant as well as regenerated plants and showed stable chromosome number.

Acknowledgement

Author is acknowledged Dr. Swami Kamalasthananda, Principal, Ramakrishna Mission Vivekananda Centenary College, Rahara, Kolkata (India) and Dr. Biswajit Ghosh, Plant

Biotechnology Lab., Ramakrishna Mission Vivekananda Centenary College, Rahara, for the facilities on condition that in the present study. Also, DST-FIST program for infrastructural amenities is acknowledged.

References

1. Balaraju, K., Agastin P., Lgnacimuthu, S. 2009. Micropropagation of *Swertia chirata* Buch- Hams. Ex Wall: a critically endangered medicinal herb. Acta physiologia plantarum. **31**:487-494.

2. Bhojwani, S.S., Razdan, M.K. 1996. Plant Tissue Culture, Theory and Practice, a Revised Edition. Elsevier, Amsterdam. 483-536.

3. Chatterjee, T., Ghosh, B., 2012. Mass propagation and *in vitro* conservation of Indian ginseng – *Withania somnifera* (L.) Dunal. GJRMI **10**: 529-538.

4. Hwang, YSWuKH, Kumamoto, J, Axelo, H., Mulla, M.S. 1985. Isolation and identification of mosquito repellents in *Artemisia vulgaris*. J. Chem. Ecol. **11**:1297-1306.

5. IUCN. 2004. IUCN Red List of Threatened Species. www.iucnredlist.org.

6. Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**:473–497.

7. Sharma, S., Rathi, N., Kamal, B., Pundir, D., Baljinder, K., Arya, S. 2010. Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology- a review. Agric. Biol.J.N.Am. **1**:827-833.

8. Sujatha, G., Kumari, R.D.B. 2007. High- frequency shoot multiplication in *Artemisia vulgaris* L. using thidiazuron. Plant Biotechnology Rep. **1**:149-154.

9. Sujatha G., Ranjitha Kumari B.D. 2007. Effect of phytohormones on micropropagation of Artemisia vulgaris L. Acta Physiologiae Plantarum. 29: 189-195.

10. Kashif Husain M., Anis M. 2006. Rapid *in vitro* propagation of *Eclipta alba* (L.) Hassk. Through high frequency axillary shoot proliferation. Acta Physiologiae Plantarum.28: 325-330.