

A New Cytotype/Polytypydi of *Artemisia Vulgaris* L.(Mugwort) From West Bengal: Cytologically Stable in Vitro Propagation for Their Genetic Conservation

Tuhin Chatterjee

Ramakrishna Mission Vivekananda Centenary College Rahara, Kolkata- 700118, West Bengal, India

DOI: <https://doi.org/10.51583/IJLTEMAS.2026.150800148>

Received: 14 September 2026; Accepted: 19 September 2026; Published: 26 September 2026

ABSTRACT

A simple effective protocol was developed for in vitro propagation through node culture of *Artemisia vulgaris* L., is an important medicinal plants in India 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 ($95 \pm 3.0\%$) and a high percentage of root initiation ($85 \pm 0.3\%$) was observed in MS medium supplemented with 0.5 mg/l IAA. Maximum $90 \pm 8.23\%$ response was achieved using 2% sorbitol and 2% mannitol in combination with MS medium at 10°C temperature up to 8 months. The well-developed plantlets were regenerated from nodal segments were hardened- off successfully with $95 \pm 0.2\%$ survival frequency. In this study, short term conservation through reduced growth conditions and a successful regeneration of morphologically stable plants with stable chromosome numbers ($2n = 36$) from *A. vulgaris*, were established.

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

INTRODUCTION

The genus *Artemisia* is one of the largest and most widely distributed genera within the Asteraceae family and is a tall aromatic herb. *Artemisia* species are well known for their volatile oil that is used in the food and pharmaceutical industries and in folk medicine to treat gastrointestinal disease (Turi et al., 2014). *A. vulgaris* known as common mugwort, is a pesty weed frequent over the entire country. The plant is perennial, 50- 150 cm in height, with dark green, segmented, pubescent leaves on dark red/ purple stem. Flowering in August and setting seeds by September. The plant body is densely hairy, with leaves pinnatifid to pinnatisect, displaying a wide range of body sizes (Gupta et al., 2014). This species grows throughout the India in areas up to 2,400 m elevation and also grown in open fields, roadsides and waste ground, often forming dense colonies [14]. Mugwort is commonly used in traditional European medicine as choleric and for amenorrhea and dysmenorrhea (Teixiera da Silva, 2004, for the treatment of diabetes and epilepsy, and in combination for psychoneurosis, depression, irritability, insomnia and anxiety stress (Walter et al., 2003). Infusion of the leaves is given as a vermifuge. It is used in traditional herbal medicine and this plant is being widely used for the treatment of diabetes, the aerial parts are being used as antihelminth, antiseptic, antispasmodic and various disorders including hypothesises, also showed antibacterial activity and a tonic for vital organs and for various disorders including hepatitis (3). Several medicinally active components of *A. vulgaris* have been identified including coumarins, sesquiterpene lactones, volatile oils and inulin (USDA-ARS-NGRL, 2004). The essential oil of *A. vulgaris* was reported to exhibit 90% repellency against *Aedes aegypti*, a mosquito that transmit yellow fever [Hwang et al., 1985, 12]. The essential oil of the plant was also reported to exhibit repellent and fumigant activity against *Musca domestica* and the stored product insect pest *Tribolium castanum* [15, Judzientence and Buzelyte, 2006; Wang et al., 2006].

Cytologically, this species is quite diverse by earlier reports and showing a number of cytotypes, of which the present chromosome count ($2n = 2x = 36$) represent the most common cytotype and confirms the previous reports of Mehra & Ramanandan (1974), Khoshoo & Sobti (1958), Koul (1964b). Further cytotypes

reported include, $2n=24$ (Koul 1964b), $2n=36, 40, 54$ (Nirmala & Rao 1984) and $2n=90$ (Mehra & Ramanandan 1974). Bakshi (1982) also reported B- chromosomes in diploid cytotype.

Out of 500 taxonomically known species, about 373 are chromosomally characterized (including 21 from India). $x=9$ is the most common base number of the genus and mostly dominated by diploids with $2n=18$ (181 species). Polyploids, more specifically tetraploids ($2n=36$) are also of high incidence with 117 species. Base number $x=8$ is less frequent, with diploids of $2n=16$ being only available in 36 species. A third base number $x=17$ is also reported, and diploid chromosome number of $2n=34$ occurs in just 14 species. Aneuploidy is of significant incidence in the genus, being present in 39 species. The highest ploidy level found to date in *Artemisia* is $2n=144$ in the species *A. medioxima* (Pellicer et al., 2007).

Despite the presence of rich diversity, the wild relative complexes have not been the subject of intense studies, which is essential for identification, classification and management of the genetic resources. The information related to chromosome numbers and comparative karyotype is fundamental to overall understanding of the genome in different species or in morphologically diverse populations within a species. Karyomorphological analysis is a useful method for characterizing plant chromosomes and genome organization. The structure and morphology of the chromosome is of vital importance when studying the origin, evolution and classification of taxa. The differences and similarities in the karyotype are regarded as basis of genetic variation, as well as distance or relatedness among diverse genomes. Chromosome studies are important when attempting to evaluate relationships and deduce phylogenetic distances, have played a major role in creation of the genome concept of wild and cultivated species. Crop improvement, conservation and utilization of plant genetic resources has benefited from chromosomal studies of germplasms. Chromosome analysis can reveal whether the plant is stable at the chromosomal level and whether additional variation originates over a long period of time against climatic, biotic and abiotic stresses. Chromosome study has been considered to have a great potential in resolving taxonomic affinities and phylogenetic relationships. Even chromosome number counts provide indispensable information on genetic discontinuities within and among species (Semple et al., 1989).

Medicinal plants are important components of biodiversity [1]. During the period 1996 to 2004, a total of 8321 plant species were added to The International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species [13]. To cope up with alarming situation, there is a strong need for preservation of genetic diversity, cultivation and sustainable usage of important medicinal plant species for future use [21]. With the advent of plant biotechnology, additional conservation strategies have been utilized for protecting valuable germplasm in the in vitro condition [2]. Conservation of plant genetic resources via tissue culture had an immense effort in research work in the last three decades [18]. Storage of meristem derived explants under the slow rate of growth has a significant use in the international germplasm resource units [25]. Slow growth is usually achieved by reducing the culture temperature, by modifying a culture medium with supplements of osmotic agents, growth inhibitors, or by removing growth promoters [17]. Several types of plant materials have been used for in vitro preservation of in vitro propagated crops. In vitro conservation can be performed by slow growth respectively. The major advantage of this methodology is the reduction of space requirements and consequently, of labor costs for the maintenance of collections [6].

In order to the flow of loss of biodiversity, an attempt to conserve through in vitro technique of *A. vulgaris*, has been made in this study. Nodal explants were introduced for establishing in vitro culture, induced to multiply. The objective of the present study based on to develop a simple, efficient protocol via node culture for large scale uniform plant production, in vitro conservation using a slow growth technique of *A. vulgaris* and the regner

MATERIALS AND METHODS

Plant Material

Artemisia vulgaris L. (mugwort) were collected from various locations in West Bengal, India, were grown in

the Field Gene Bank at RKMVC College campus under identical environmental conditions of the experimental garden of the RKMVC College, Rahara, Kolkata, W.B., India. In the present study node explants were used which were collected for six months old disease free healthy plant.

Surface Sterilization of Explants

Collected explants from the mother plant were kept under running tap water for 20 minutes and then immersed in 2% bavistin solution (w/v, a systemic fungicide) for 20 minutes and dipped it on to the 5% Tween- 20 (v/v, a liquid soap solution) for 10 minutes and washed with distilled water to make the exploits free from detergents. Finally, surface sterilization done with 0.1% (w/v) mercuric chloride solution for 5 minutes and washed thoroughly with sterile distill water before inoculation onto sterilized nutrient agar media pre distributed in culture vessels. Disinfection was performed under aseptic conditions in a laminar air flow cabinet.

Culture Media

Nodal explants were inoculated on to solid MS medium [19] containing 3% sucrose (w/v). The medium solidified with 0.8% agar (w/v, Merck, India). The medium enriched for multiplication and shoot proliferation with different concentrations of cytokinin and auxin viz., BAP, Kn, 2iP, NAA (0.5, 1.0, 2.0, 3.0 and 5.0 mg/l) individually or in combination and for root initiation different concentrations of auxins viz., IAA, IBA, NAA (0.2, 0.5, 1.0, 2.0 mg/l) individually were used. The pH of the medium was adjusted to 5.6 ± 0.2 with 1(N) KOH and 1(N) HCl before gelling with agar. Media were steam- sterilized at 121°C temperature and 1.1 kg cm^2 pressure for 18 min.

Culture Conditions

The cultures were incubated at a temperature of $25 \pm 2^{\circ}\text{C}$, 55- 60% relative humidity with a photoperiod of 16 hrs per day with photon flux density for about $45 \mu \text{ Mol m}^{-2}\text{s}^{-1}$ provided by white fluorescent light.

Multiple Shoots Induction and Plantlet Regeneration

Nodal segments (8-12 mm) excised from mother plant were inoculated vertically on to MS medium containing 3% (w/v) sucrose (w/v, Merck, India), 0.8% agar (Merck, India) supplemented with different concentration of BAP, Kn, 2iP, NAA individually and in combination and replicates thrice of each experiment. The multiple shoots were sub- cultured at every two weeks for 60 days. A control group was maintained (basal medium without hormones) to record the frequency of response.

Shoot Elongation

After 30 days, multiple shoots induced were excised individually and subcultured on to MS medium supplemented with BAP (1.0 mg/l) and varying concentrations of gibberellic acid (GA_3) (0.2, 0.4, 0.6, 0.8, and 1.0 mg/l) for elongation of shoots. A control group (basal medium without GA_3) was also maintained.

Induction of Rooting

Micro shoots (1-3 cm) were excised from the culture and transferred to half- strength MS medium augmented with different concentrations of auxins viz., IAA, IBA and NAA (0.2, 0.5, 1.0 and 2.0 mg/l) and 2% sucrose (w/v, Merck, India) for root initiation. Micro- shoots were cultured in one tube each under the same culture conditions as described above. After 4 weeks the percentage of shoots forming roots, the number of roots per shoot and root length was assessed. One culture set was inoculated in basal MS medium without any plant growth regulator and considered as control.

In Vitro Conservation

For in vitro conservation, slow growth techniques were used. The effect of osmotic agents and temperature

on the survival and re-growth of the in vitro cultures of *A. vulgaris*, MS media were supplemented with mannitol (1–3% w/v), sorbitol (1–3% w/v) with 3% sucrose (w/v) and 0.8% (w/v) agar. Shoot tips and nodes were dissected from aseptically grown cultures and inoculated onto the slow growing media in order to increase sub-cultural intervals. Cultures were maintained at 4°C, 10°C and 18°C into growth chambers under a 16 h photoperiod with fluorescent light. After conservation period, the plants were a sub –culture of normal MS medium for their re-growth. Cultures were monitored during and after storage for survival and subsequently transferring on to the shoot multiplication medium (MS media with PGR) under culture room conditions at 25°C. The number of new buds and shoots induced on multiplication media was counted 30 days after transfer. Collected data reflect the rate of plant conservation from storage buds to proliferating buds, shoots and plantlets. Proliferated shoots were rooted on MS medium containing IBA.

Acclimatization and Field Experiments

For hardening, at first the tissue culture derived healthy rooted plantlets were placed at room temperature for 7 to 10 days. Then the plantlets were removed from the agar medium, washed thoroughly under running tap water and transferred to earthen pots containing sterilized soilrite (Keltech Energies Ltd, Bangalore, India). To preserve moisture, the potted platelets were covered with transparent polythene bag and the pots were placed in a plastic tray containing water under diffused light (16 h, photoperiod) in the poly-house for 25-30 days. After 30 days, these plants were transferred to the earthen tub (8 inch diameter) containing the mixer of garden soil, sand and vermicompost (2:1:1) and placed in the greenhouse. After 30 days, surviving plants were transferred to the field.

Mitotic Chromosome Study

Young and healthy root tips of *A. vulgaris* were excised of the field growing mother plant as well regenerated plants in order to analyse the chromosome numbers. The root tips were pre- treated with saturated solution of paradichlorobenzene for 7 hrs at 14°C and kept in aceto ethanol solution (1:3, acetic acid: ethanol, v/v) for overnight at 4°C temperature. For somatic chromosome counts, fixed root tips were hydrolysed with 45% acetic acid for 20 min at room temperature and stained with 2.0% aceto-orcin: 1 (N) HCl (9:1, v/v) mixture followed by incubating for 2 h at room temperature and squashed in 45% acetic acid to obtained well scattered metaphase plates on glass slides. Chromosome plates were observed in Leica DM750 microscope and photographed with Leica DFC 295 camera. Minimum of 5 metaphase plates from each root tip were analyzed to determine the somatic chromosome number at the metaphase stage. At the same time, Camera Lucida drawing of metaphase plates was drawn by using 100x × 15x under the microscope of 2000 magnification. Magnification of the microscope was measure by Camera Lucida drawing of stage micrometer using 100x × 15x. Lengths of short and long arms of each chromosome were calculated. These values were then used to calculated total chromatin length (TCL) and centromeric index (CI). Arrangement of chromosomes in grouping of each karyotype were made on the basic of centromeric index, and ordering them by decreasing length with in each group. The mean centromeric index value (TF%) was calculated by using Huziwara formula: $TF\% = \frac{\text{total sum of short arm length}}{\text{total sum of chromosome length}} \times 100$ [11]. The mean chromatin length and total arm ratio calculated by using Levan et al. formula [10]. Chromosome were classified as median (m) submedian (Sm) and subterminal (St) according to the position of the centromere (Hirahara and Tatuno 1967) and on the basis of chromosome length. Morphologically the chromosomes were graded as type A: Secondary constriction, type B: median chromosome, type C: sub median chromosome and type D: sub telocentric chromosome.

Statistical Analysis

All the experiments were conducted under controlled conditions with three replications. Means and standard errors were carried out for each experiment and data was analyzed using one way Analysis of Variance (ANOVA) to detect significant differences between means. Data analysis was performed using SPSS v 16.0 software.

RESULT AND DISCUSSION

Role of Plant Growth Regulators on in Vitro Shoot Induction and Multiplication

It was observed that multiple shoot buds originated from nodal explants collected from physiological healthy source plant, when MS medium [19] containing cytokinins (BAP, Kn and 2iP) and one auxin (NAA). But MS media supplemented with BAP was most effective in shoot induction and proliferation (Fig. 1A). Within 7 days of inoculation, explants swelling were observed. First appearance of buds was observed within 3 to 5 days. Multiple shoots commenced to emerge from the cut ends in case of BAP and Kn supplemented media. MS media supplemented with NAA, poor multiplication was observed and a callus like structure was formed. A maximum response of $95 \pm 3.0\%$ was recorded in MS media supplemented with 1.0 mg/l BAP alone, where the number of shoot per explants was highest (28.67 ± 0.43) with an average length of 4.97 ± 0.03 cm (Table- 1). It was reported that BAP is the best cytokinin for *Aegle marmelos* [20]. MS medium supplemented with Kn and 2iP showed comparatively less response. MS medium containing with 0.5 mg/l Kn showed maximum response as compared the five concentrations of Kn (0.5, 1.0, 2.0, 3.0 and 5.0 mg/l) assessed and showed maximum $70 \pm 1.0\%$ sprouting frequency and an average of 10.34 ± 0.32 number of shoots per nodal segment. Shoot length was maximum 3.36 ± 0.06 cm. MS medium containing with 1.0 mg/l 2iP showed maximum response $50 \pm 0.1\%$ as compared the five concentrations of 2iP (0.5, 1.0, 2.0, 3.0 and 5.0 mg/l) was recorded. Sub- culturing of the resultant shoot buds of *A. vulgaris* on to the MS medium supplemented with BAP+ Kn, BAP+ 2iP, Kn+2iP, BAP+NAA, Kn+ NAA, 2iP +NAA, at different concentrations and in combination (0.5, 1.0, 3.0 mg/l) separately with the MS media. But the resulted in stunted growth of the shoots and multiplication rate also less than others.

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

Cytokinin	Concentration (mg ⁻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 ± 0.4^{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± 1.0 ^{fg}	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

Although a maximum of 28.67± 0.43 shoots per explants was observed (Table- 2), it was necessary to establish a shoot elongation protocol. Excised shoots that were cultured in medium consisting of 1.0 mg/l BAP and in the presence of GA₃ at different concentrations for 2 weeks was evaluated for shoot length. The result suggest that small amounts of GA₃ (0.6 mg/l) in combination with 1.0 mg/l BAP were effective in stimulating *A. vulgaris* shoot elongation (Fig. 1B). Shoots attained a maximum height of 11.5± 1.5 cm during GA₃ treatments. The same result showed for shoot elongation for the effect of GA₃ in *Withania somnifera* cultures [3] and *A. vulgaris* [23].

Table 2: Effect of GA₃ on shoot elongation of *A. vulgaris* from regenerated shoots cultured on MS medium supplemented with GA₃ and BAP (1.0 mg/l). (Data collected after 30 days of culture.)

BAP (mg/l)	GA ₃ (mg/l)	Shoot elongation response (%)	Mean Shoot length (cm)
Control (MSO)	-	-	3.0± 1.2 ^f
1.0	0.2	85.0± 2.5 ^c	6.2± 1.0 ^c
1.0	0.4	90.0± 2.0 ^b	9.8± 0.5 ^b
1.0	0.6	98.0± 1.6 ^a	11.5± 1.5 ^a

1.0	0.8	75.0± 0.3 ^c	7.0± 1.2 ^{ab}
1.0	1.0	65.0± 1.2 ^b	5.0± 0.2 ^{ef}

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

Induction of Roots From in Vitro Grown Micro Shoots

To induced rooting, elongated shoots (2.0 cm) were cultured on to MS medium supplemented with different types of auxins in different concentration viz., IBA (0.2, 0.5, 1.0, 2.0 mg/l), IAA (0.2, 0.5, 1.0, 2.0 mg/l) and NAA (0.2, 0.5, 1.0, 2.0 mg/l) individually. A control group was also maintained (MS medium without any auxins). Among the three different auxins tested, root length and number of roots varied. Plantlets significantly developed lengthy roots and root induction was strength within 25 to 30 days of culture.

For rooting experiment of *A. vulgaris*, IAA was found to be more adequate than IBA and NAA (Table- 3). Roots were visible within 6 to 8 days following transfer of elongated shoots to the rooting medium. After 15 days, plantlets developed primary and secondary root system (Fig. 1C). Increasing the concentration of IAA gradually led to a decrease in the frequency of root regeneration. MS medium containing different concentrations of IAA, IBA and NAA (0.2, 0.5, 1.0, 2.0 mg/l) individually used and 0.5 mg/l IAA, showed better result than the other concentration the average number of roots per shoot was 5.8± 3.5 and maximum root length was 6.3± 2.2cm. Similar response was observed in *A. villosa* [24] and *Sesbandia drummondii* [5]. MS medium fortified with 0.5 mg/l IBA showed the maximum number of roots per shoots 4.8± 3.0 and average length of 4.8± 2.0 cm. MS medium containing NAA, found slender roots and a callus like structure was formed on the base of the shoots.

Table -3: 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	5.8± 3.5 ^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

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

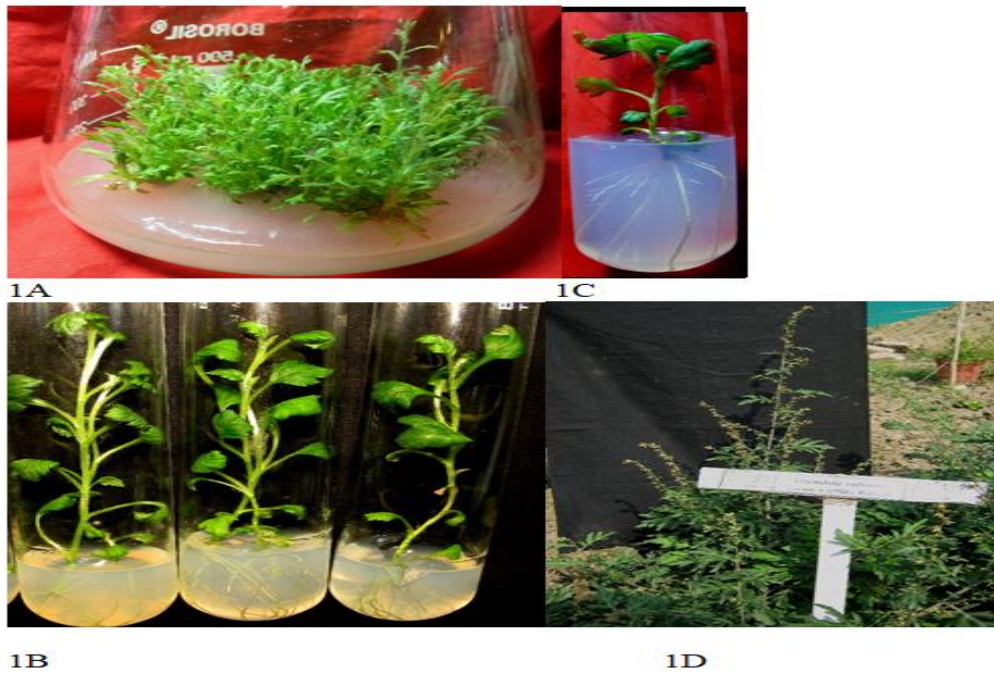


Figure legends:

Fig.-1A; showing in vitro multiplication of *Artemisia vulgaris* L.

Fig. -1B; showing in vitro elongated shootlet of *Artemisia vulgaris* L.

Fig. -1C; showing in vitro complete plants with roots of *Artemisia vulgaris* L.

Fig.-1D; showing in vitro raised plants in field condition of *Artemisia vulgaris* L.

In Vitro Conservation

The cultures maintained in good health conditions in controlled medium (MS medium with 3% sucrose, without osmotic agents) with 100 % survival rate up to 3 to 4 months without subculture, but thereafter survival rate was gradually decrease and finally the control treatment did not help to survive after 4 months of conservation. But when cultures were maintained in manitol and sorbitol survived up to 8 months without subculture.

At low temperature regime (4°C), all the cultures showed poor performance as the shoots degenerated and become whitish in colour after 35 to 40 days and culture did not survive as in case of control at 25°C. In moderate temperature regime (18°C) the cultures grew healthy with reduced growth in comparisons to control. This experiment suggests that cultures growth could be reduced at 10°C, but storage period could not be increased to maintain healthy cultures. This aspect needs further experimentation to prolong the sub-culture period. It also suggests that low temperature (4°C) is not suitable for in vitro storage of *A. vulgaris*. The growth suppression approach using osmotic agents was attempted in this study and proved to be very useful. The effect of MS medium in combination with different concentration of sorbitol and mannitol, on growth of *A. vulgaris* cultures were studied (Table-5). The influences of various osmotic agents on this species showed different results. The growth of shoots cultured in MS medium supplemented with 3% sucrose (without osmotic agents) was controlled for our experiments. The results showed that the addition of sorbitol (w/v) and mannitol (w/v) in MS media, at different concentrations, was more effective for in vitro storage of these important medicinal plants than the storage at low temperature (4°C). The addition of osmotic agents 2% sorbitol (w/v) and 2% mannitol (w/v) in combination with MS medium, has increased survival rate $90 \pm 8.23\%$ at 10°C. A declination in survival rate and re-growth occurred when the cultures were stored also at higher concentrations of osmotic agents i.e., with 3% mannitol (w/v) and with 3% sorbitol (w/v) each and with a combination of 2% sorbitol (w/v) and 2% mannitol (w/v). Our results also

showed that 10°C and 16 h photoperiod were better than 4°C during slow-growth storage condition for this plant species (Table- 4). The shoots survived after slow-growth storage had longer shoot height than those not maintained in slow-growth condition. In case of 4°C temperature, the leaves of some plants were curled and withered. After 8 months, these in vitro shoots of all the plant species were transferred onto fresh MS medium supplemented with different concentration and combinations of cytokinins like BAP, Kn, 2iP and NAA (0.5 to 5.0 mg/l) and different auxins like IAA, IBA and NAA (0.2, 0.5, 1.0 and 2.0 mg/l) for in vitro multiplication and in vitro rooting and cultured for 6 to 8 weeks. Growth suppression had positively reduced the labor during culture maintenance in the tissue culture laboratory and also promoted uniformity of growth among the converted plantlets. No signs of shoot or root growth was noticed during the 8 months of storage. Adding sucrose to the media has prevented dehydration in storage but did not improve shelf-life of germplasm, while frequency of plantlet conservation was higher on shoot tips stored on 2% (w/v) sucrose/agar support with rootsbreaking during low temperature (4°C) storagecondition. All the cultures in storage condition were able to form roots during re-growth andsuccessfully acclimatized in soil rite. After lowtemperature storage, plantlets improved their survival during acclimatization and more vigorous in field plantings was observed. Similar reports have documented post-storage beneficial effect in *Prunus armeniaca* [16] and *Withania somnifera* [3]. Our studies provided an effective protocol for storage of this medicinal plant under slow growth conditions. Germplasm can be stored effectively for 8 month without subcultures, alleviating maintenance labor in the laboratory.

Table: 4: Effect of low temperature on in vitro slow growth storage conditions of *A. vulgaris*

Storage condition	Re- growth±SE after storage 4 mo	Survival (%)	Re- growth±SE after storage 6 mo	Survival (%)	Re- growth±SE after storage 8 mo	Survival (%)
Low temperature						
4°C	2.1± 1.5 ^b	77.0± 1.5 ^b ^c	1.7± 1.0 ^c	50.0± 0.3 ^{cd}	1.7± 1.7 ^c	52.0± 0.5 ^{cd}
10°C	15. ± 0.5 ^a	92.0± 2.3 ^a	21.1± 1.5 ^a	92.0± 1.8 ^a	20.1± 3.9 ^a	95.0± 1.2 ^a
18°C	6.3± 1.0 ^c	82.0± 0.7 ^b	4.5± 1.0 ^c	65.0± 0.4 ^b	3.2± 1.6 ^c	60.0± 0.1 ^c

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

Table: 5: Effect of different osmotic on in vitro slow growth storage conditions of *A. vulgaris* at 24°C.

Medium	Plant Height (cm)	Survival%	Storage Period (month)
Full MS	9.3± 0.46 ^a	65.0± 0.43 ^b	4
	11.7± 0.73 ^c	50.0± 0.67 ^{ef}	6
	11.4± 1.02 ^c	40.0± 0.50 ^f	8
MS+1% Sorbitol	6.3± 0.36 ^b	75.0± 2.0 ^{ab}	4
	8.4± 0.33 ^d	68.0± 0.7 ^{bc}	6
	8.5± 0.02 ^{cd}	55.0± 0.45 ^b	8
MS+2% Sorbitol	6.3± 0.40 ^a	85.0± 0.72 ^a	4
	8.3± 0.43 ^c	79.0± 0.25 ^b ^c	6
	8.4± 0.37 ^{bc}	65.0± 0.12 ^e	8
MS+3% Sorbitol	5.3± 0.87 ^b	70.0± 2.5 ^{bc}	4
	5.7± 0.73 ^{bc}	61.0± 0.71 ^c	6
	5.8± 0.02 ^c	52.0± 2.4 ^{ef}	8
MS+1% Manitol	5.3± 0.56 ^b	77.0± 0.91 ^b	4

	5.7± 0.70 ^c	71.0± 0.63 ^{bc}	6
	5.7± 1.12 ^{cd}	63.0± 0.12 ^{fgh}	8
MS+2% Manitol	4.3± 0.16 ^b	82.0± 0.54 ^b	4
	5.3± 0.73 ^c	77.0± 0.23 ^c	6
	5.4± 0.02 ^b	68.0± 0.70 ^{cd}	8
MS+3% Manitol	4.3± 0.46 ^a	71.0± 1.03 ^b	4
	4.7± 1.73 ^{bc}	62.0± 0.30 ^{cd}	6
	4.9± 0.05 ^c	48.0± 2.1 ^{ef}	8
2% Sorbitol+ 2% Manitol	8.12± 0.26 ^a	90.0± 8.23 ^a	4
	8.42± 0.73 ^{ac}	82.0± 5.1 ^{ab}	6
	8±1.22 ^b	75.0± 0.81 ^{bc}	8

Acclimatization

The successfully rooted plantlets were transfer to a small earthen pot containing soilrite and covered with transerent polytheen bags for hardening. All the plantlets were maintained in the culture room (25± 1°C) conditions initially 6-7 weeks and then transferred to normal laboratory in room temperature conditions and maintained for about 4- 5 weeks. Finally the plantlets were transferred to Poly House and maintained 3- 4 weeks and then it transferred to the experimental field condition (Fig. 1D). There was 95% survival rate and the plantlets that were root in MS medium containing different types of auxins (IAA, IBA) where the plants showed healthy growth. However the following 8- 20 weeks, substantial increase in plant height was observed. Initially 2 to 3 healthy leaves were developed adjacent to the main shoot. The number of leaves increased often 10 to 15 weeks of acclimatization. There was no noticeable variation among the acclimatized plants with respect to morphological and growth characteristics. All the micropropagated plants were free from external disease.

Mitotic Chromosomal Study

Karyomorphological studies of *A. vulgaris* was made. Somatic chromosome of mother mlant as well as tissue culture raised plant were found 2n= 36 (Fig-1e, f). In this study, the chromosomes were grouped into the following types:

Type A: Relatively long to short chromosome with two constrictions primary and secondary, dividing chromosome into three arms.

Type B: Relatively short chromosomes (length- 1.25- 2.25 µm) with a primary constriction at median to median region.

Type C: Relatively short chromosomes (length- 1- 3.5 µm) with a primary constriction at the submedian to nearly submedian region.

Type D: Relatively short chromosomes (length- 1-3 µm) with a primary constriction at the sub terminal region.

Chromosomal analysis in our present studies from randomly selected root tips from the source plant showed 2n= 36 chromosomes; cytological preparations from the in vitro derived plantlets (25 root tips and 50 metaphase/ root tip) showed predominantly diploid number (2n= 36) of chromosomes. All the regenerates so far analysed do not show any visible cytological changes and same number of mitotic chromosome of conventional grown plants (Fig-1E, 1F,1G,1H,1I) and tissue culture raised plants (Fig-1J,1K,1L,1M,1N) was observed 2n=36 remains the same without any marked structural alternations.The karyotype details of

conventional grown plant and tissue culture raised plant were given on Table-6 and Table-7. The karyotype formula of conventional grown plant is $2n=36; 8B_m+26C_{sm}+2D_{st}$ and in tissue culture raised plant is $2n=36, 2B_m+28C_{sm}+6D_{st}$ (Table-6). Although there are some minor difference between percentage of metacentric, sub- metacentric and sub- telocentric chromosome, but no any remarkable change were observed of chromosome constrictions of both conventional grown plants and tissue culture raised plants. All metaphases revealed a constant chromosome number $2n=36$. Thus the somatic chromosome complements of in vitro generated *A. vulgaris* plants remained stable even after passing through three cycles of multiplication. It also confirms that no chromosomal variability was induced from the short term conserved plants among the regenerates. It indicates that reduced growth conditions applied in our studies along with the inherent genotypic potentiality and stability of the species helped to maintain germplasms over such a short period of time in maintained morphological and chromosomal uniformity among the regenerates. Such mode of preservation can be utilized in other medicinal and economically important plants. A similar cytogenetically stable plant was also observed in *Asparagus cooperi* [7, 8]; *Gloriosa superba* [9]; *Plumbago zeylanica* [4]; *Withania somnifera* [22].

Polyploidy is believed to play a significant role in the production of divergent lineages and species (**Sharma and Sharma, 2014**). A diploid *Artemisia vulgaris* ($2n= 18$ and $n= 9$) has been found in North- Western Himalyas (**Koul, 1963**). Naseri et al., 2009 reported two basic chromosome numbers in the genus *Artemisia*. Five of the studied taxa have $x= 9$, the most common basic number in the genus *Artemisia*, the tribe Anthemideae and the family Asteraceae as well (**Solbrig 1977, Schweizer and Ehrendorfer 1983, Oliva and Valle,s 1994, Valle,s and Silja- Yaknovlev, 1997**). Among the considered taxa, *A. vulgaris* and *A. scoparia* have the less common basis number $x= 8$, this is evidence for the descending dysploidy that occurs in the genus (**Fernandez Casas and Susanna 1986, Valle,s and Silja- Yaknovlev 1997, Valles et al. 2001, 2005**). Among Iranian population belong to taxa basic number $x= 8$. Chromosome number in seven Iranian *Artemisia* species counted were listed follows; $2n= 36$ for *A. spicigera*, *A. campsteris* and $2n= 18$ for *A. fragrans*, *A. absinthium*, *A. chamaemelifolia* and finally $2n= 16$ for *A. vulgaris* and *A. scoparia* (**Naseri et al., 2009**).

Our studies provide a simple and effective protocol of in vitro propagation for in vitro storage of *A. vulgaris* under slow growth conditions and germplasm can be stored effectively for 8 months without subcultures, alleviating maintenance labor in the laboratory. The method also suggested here has the advantages of both simplicity and efficiency. In connection with this study we also established a simple protocol of chromosomal status of conventional plant as well as regenerated plants and showed cytologically stable.

Table-6: Karyotype details in *Artemisia vulgaris* (Conventional grown plant)

Sl. No.	Chromosome Types	Short Arm Length (µm)	Long Arm Length (µm)	Total Length (µm)	Arm ratio	F %	Centromeric nature
1	B	2.5	2.5	5.0	1.0	50.0	M
2	B	2.5	2.5	5.0	1.0	50.0	M
3	B	2.5	2.5	5.0	1.0	50.0	M
4	B	1.25	1.25	2.5	1.0	50.0	M
5	C	2.5	3.0	5.5	0.83	45.5	Sm
6	C	1.5	3.5	5.0	0.42	30.0	Sm
7	C	1.5	3.5	5.0	0.42	30.0	Sm
8	C	1.5	3.5	5.0	0.42	30.0	Sm
9	C	1.5	3.0	4.5	0.5	33.3	Sm
10	C	1.5	3.0	4.5	0.5	33.3	Sm

11	C	1.5	3.0	4.5	0.5	33.3	Sm
12	C	1.5	3.0	4.5	0.5	33.3	Sm
13	C	1.5	3.0	4.5	0.6	37.5	Sm
14	C	1.5	2.0	3.5	0.75	42.8	Sm
15	C	1.5	2.0	3.5	0.75	42.8	Sm
16	C	1.0	2.5	3.5	0.4	28.5	Sm
17	C	1.0	2.0	3.0	0.4	33.3	Sm
18	D	1.0	3.0	4.0	0.33	25.0	St

M=Metacentric, Sm=Sub metacentric; St=Sub telocentric

Table-7: Karyotype details in *Artemisia vulgaris* (Tissue culture raised plant)

Sl. No.	Chromosome Types	Short Arm Length (µm)	Long Arm Length (µm)	Total Length (µm)	Arm ratio	F %	Centromeric nature
1	B	2.25	2.25	4.5	1.0	50.0	M
2	B	2.25	2.25	4.5	1.0	50.0	M
3	B	2.0	2.0	4.0	1.0	50.0	M
4	B	2.0	2.0	4.0	1.0	50.0	M
5	C	1.5	3.0	4.5	0.5	33.3	Sm
6	C	1.5	3.0	4.5	0.5	33.3	Sm
7	C	1.5	3.0	4.5	0.5	33.3	Sm
8	C	1.5	2.5	4.0	0.6	37.5	Sm
9	C	1.5	2.5	4.0	0.6	37.5	Sm
10	C	1.5	2.5	4.0	0.6	37.5	Sm
11	C	1.5	2.5	4.0	0.6	37.5	Sm
12	C	1.5	2.5	4.0	0.6	37.5	Sm
13	C	1.25	2.75	4.0	0.45	31.25	Sm
14	C	1.0	2.5	3.5	0.4	28.5	Sm
15	C	1.0	2.5	3.5	0.4	28.5	Sm
16	C	1.0	2.5	3.5	0.4	28.5	Sm
17	C	1.0	2.5	3.5	0.4	28.5	Sm
18	D	1.0	3.5	4.5	0.28	22.2	St

M=Metacentric, Sm=Sub metacentric; St=Sub telocentric

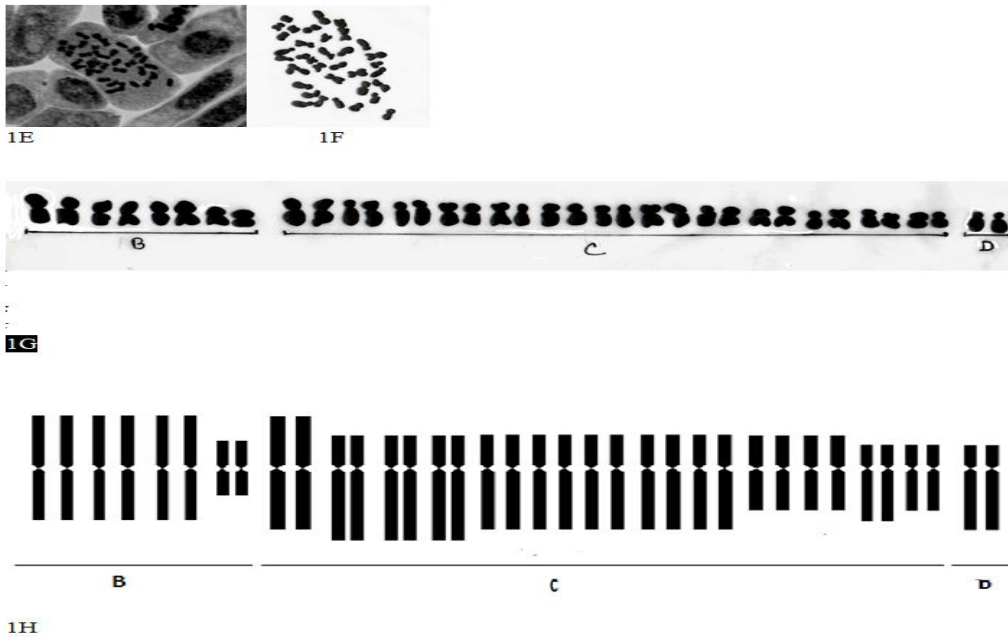


Figure LegendSs:

Fig. –1E; showing metaphase plate with $2n= 36$ chromosome number of conventional grown plant of *Artemisia vulgaris* L.

Fig. –1F; showing hand drawing of metaphase plate with $2n= 36$ chromosome number of conventional grown plant of *Artemisia vulgaris* L.

Fig. –1G; showing hand drawing of karyotype of metaphase plate with $2n= 36$ chromosome number of conventional grown plant of *Artemisia vulgaris* L.

Fig. –1H; showing ideogram of metaphase plate with $2n= 36$ chromosome number of conventional grown plant of *Artemisia vulgaris* L.

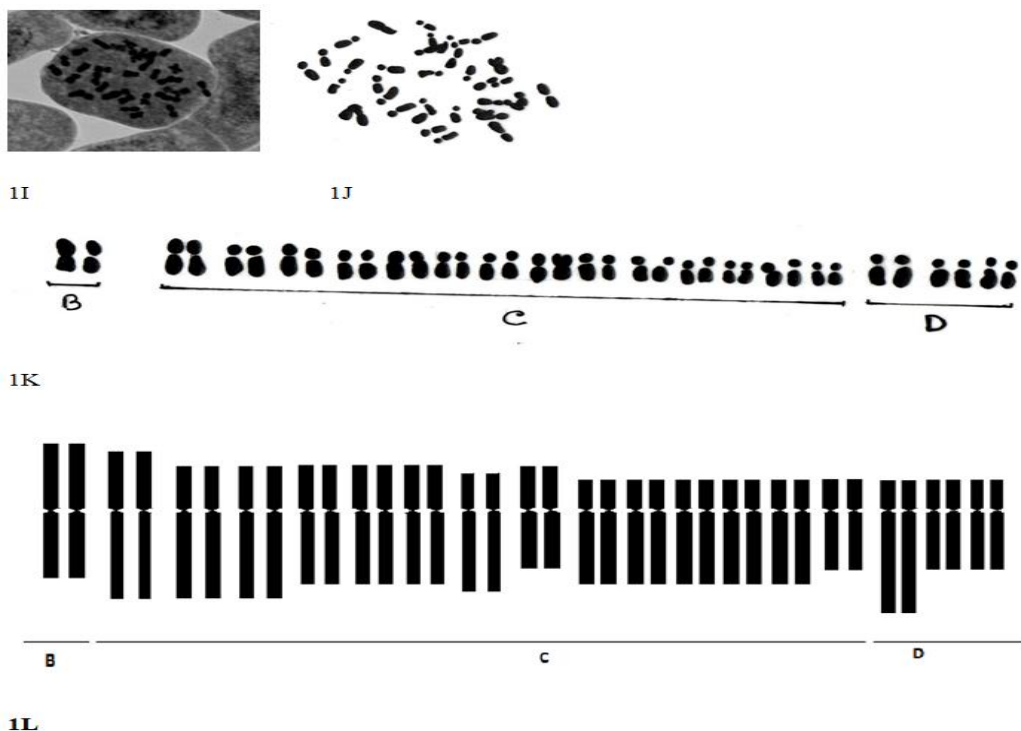


Figure Legends:

Fig. –1I; showing metaphase plate with $2n= 36$ chromosome number of tissue culture raised plant of *Artemisia vulgaris* L.

Fig. –1J; showing hand drawing of metaphase plate with $2n= 36$ chromosome number of tissue culture raised plant of *Artemisia vulgaris* L.

Fig. –1K; showing hand drawing of karyotype of metaphase plate with $2n= 36$ chromosome number of tissue culture raised plant of *Artemisia vulgaris* L.

Fig. –1L; showing ideogram of metaphase plate with $2n= 36$ chromosome number of tissue culture raised plant of *Artemisia vulgaris* L.

ACKNOWLEDGEMENT

Both authors are acknowledged Dr. Swami Kamalasthananda, Principal, Ramakrishna Mission Vivekananda Centenary College, Rahara, Kolkata (India) for the facilities provided during the present study. Also, DST-FIST program for infrastructural facilities is acknowledged.

REFERENCES

1. Balaraju K, Agastin P, Lgnacimuthu S. Micropropagation of *Swertia chirata* Buch- Hams. Ex Wall: a critically endangered medicinal herb. *Acta physiologia plantarum*. 2009;31:487-494.
2. Bhojwani SS, Razdan MK. *Plant Tissue Culture, Theory and Practice*, a Revised Edition. Elsevier, Amsterdam. 1996;483-536.
3. Chatterjee T, Ghosh B. Mass propagation and in vitro conservation of Indian ginseng - *Withania somnifera* (L.) Dunal. *GJRMJ*. 2012;10:529-538.
4. Chatterjee T, Ghosh B. Simple protocol for micropropagation and in vitro conservation of *Plumbago zeylanica* L: an important indigenous medicinal plant. *International Journal of Bio- resource and Stress management*. 2015;6:068-075.
5. Cheepala SB, Shirma NC, Sahi SV. Rapid in vitro regeneration of *Sesbania drummondii*. *Biol. Plant*. 2004;48:13-18.
6. Engelmann F. Plant cryopreservation: progress and prospects. *In vitro Cell Dev Biol Plant*. 2004;40:427-433.
7. Ghosh B, Sen S. Stable regeneration in *Asparagus cooperi* Baker. as controlled by different factors. *Plant Science*. 1992;82:119-124.
8. Ghosh B, Sen S. Micropropagation of *Asparagus cooperi* as affected by growth regulators. *Biologia Plantarum*. 1994;36:527-534.
9. Ghosh S, Ghosh B, Jha S. In vitro tuberisation of *Gloriosa superba* L. on basal medium. *Sci. Hort*. 2007;114:220-223.
10. Levan A, Fregda K, Sandberg AA. Nomenclature on centromeric position on chromosomes. *Heredities*. 1964;52:201-220.
11. Huziwaru Y. Karyotype analysis in some genera of Compositae VIII. Further Studies on the Chromosomes of Aster. *American Journal of Botany*. 1962;49:116-119.
12. Hwang YSWuKH, Kumamoto J, Axelo H, Mulla MS. Isolation and identification of mosquito repellents in *Artemisia vulgaris*. *J. Chem. Ecol*. 1985;11:1297-1306.
13. IUCN. IUCN Red List of Threatened Species. 2004; www.iucnredlist.org
14. Judzentiene A, Buzelyte J. Chemical composition of essential oils of *Artemisia vulgaris* L. (mugwort) from North Lithuania. *Chemija*. 2006;17:12-15.
15. Kapoor LD. *Handbook of Ayurvedic medicinal plants*. CRC Press, Boca Raton. 2000;53.
16. Koubouris G, Vasilakakis M. Improvement of in vitro propagation of apricot cultivar 'Bebecou'. *Plant Cell Tissue Organ Cult*, 2006;85:173–180.
17. Lata H, Moraes MR, Bertoni B. In vitro germplasm conservation of *Podophyllum peltatum* L. under slow growth conditions. *In Vitro Cell.Dev.Biol. Plant*. 2010; 46: 22- 27.

18. Moges AD, Karma NS, Shibli RA. Slow growth in vitro preservation of African violet (*Saintpaulia ionantha* Wendl.) shoot tips. *Advanced Hort Science*, 2010;17:1-8.
19. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 1962;15:473-497.
20. Nayak P, Behera PR, Manikkannan T. High frequency plantlet regeneration from cotyledonary node cultures of *Aegle marmelos* (L.) Corr. *In vitro Cell Derv. Biol.* 2007;43: 231-236.
21. Sharma S, Rath N, Kamal B, Pundir D, Baljinder K, Arya S. Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology- a review. *Agric. Biol. J. N. Am.* 2010;1:827-833.
22. Samaddar T, Nath S, Halder M, Sil B, Roychowdhury D, Sen S, Jha S. Karyotype analysis of three important traditional Indian medicinal plants, *Bacopa monnieri*, *Tylophora indic* and *Withania somnifera*. *Nucleus*. 2012;55:17-20.
23. Sujatha G, Kumari RDB. High- frequency shoot multiplication in *Artemisia vulgaris* L. using thidiazuron. *Plant Biotechnology Rep.* 2007;1:149-154.
24. Vijayalakshmi G, Giri CC. Plant regeneration via organogenesis from shoot based derived callus of *Arachis stenoperma* and *A. villosa*. *Curr Sci.* 2003; 85:1624-1629.
25. Wilkins CP, Dodds JH. Tissue culture conservation of woody species. In: Dodds, J.H. ed. *Tissue Culture of Trees*. Croom Helm, London. 1983;138.