



## Keywords

*Asparagus Officinalis* L.,  
*In Vitro*,  
Callus Culture,  
Clonal Micropropagation,  
Adventive Shoot

Received: November 9, 2015

Revised: December 15, 2015

Accepted: December 17, 2015

# Elaboration of *Asparagus officinalis* L. by *in vitro* and Hydroponic Combined Method

Elmira Sargsyan<sup>1</sup>, Anush Vardanyan<sup>1</sup>, Ali Parsaeimehr<sup>2</sup>, Ani Bekyan<sup>1</sup>

<sup>1</sup>G. S. Davtyan Institute of Hydroponic Problems, National Academy of Sciences Republic of Armenia, Laboratory of "Tissue Culture", Yerevan, Armenia

<sup>2</sup>Institute of Biotechnology, Jiangsu Academy of Agricultural Sciences, Nanjing, China

## Email address

elmira\_sargsyan14@yahoo.com (E. Sargsyan), ann\_vardanyan@yahoo.com (A. Vardanyan), anibekyan@yahoo.com (A. Bekyan), ali.parsaeimehr@gmail.com (A. Parsaeimehr)

## Citation

Elmira Sargsyan, Anush Vardanyan, Ali Parsaeimehr, Ani Bekyan. Elaboration of *Asparagus officinalis* L. by *in vitro* and Hydroponic Combined Method. *American Journal of Microbiology and Biotechnology*. Vol. 2, No. 6, 2015, pp. 82-86.

## Abstract

Main objective of the research is cultivation and propagation of valuable medicinal plant *Asparagus officinalis* L. (*Mary Washington*) by hydroponics and *in vitro* combined method. From seeds of hydroponic plants were obtained *in vitro* plantlets. To obtain callus tissues various explants (hypocotyl, root, apical bud, stem segment) were separated from micro-plantlets. *In vitro* callus cultures were obtained. Biosynthetic possibility and activity of callus tissues in case of various growth regulators and concentrations were determined. All tested explants on MS nutrient medium with hormones (BAP - 1.0 mg/l, 2.4D - 2.0 mg/l and BAP - 1.0 mg/l, 2.4D - 0.5 mg/l,  $\alpha$ -NAA - 0.5 mg/l) induced callus tissues. The replanting of callus was implemented once in a month, on the fresh nutrient medium. Viable callus cultures were obtained and used for induction of rhizogenesis and organogenesis. On MS medium optimum result was in case of callus from hypocotyl explant, with BAP - 1.0 mg/l, 2.4D - 0.5 mg/l and  $\alpha$ -NAA - 0.5 mg/l or Kinetin - 1 mg/l concentration, which provided regeneration of 15 - 20 adventive shoots. Shoot-regenerants multiplied micro-clonally. Among tested phytohormones the best result was 0.5 mg/l IBA concentration, providing 62% rhizogenesis of shoot-regenerants. In spring, the rooted *in vitro* micro-plantlets after acclimatization were planted in the open air gravel hydroponic conditions for propagation.

## 1. Introduction

*Asparagus (A) officinalis* L. is native to Europe, Northern Africa and America, Western Asia, Australia, New Zealand and is widely cultivated as a vegetable crop more than 2000 years. Young, new germinated shoots of some species are widely used in food as delicacy [7]. Major bioactive substances of *A. officinalis* are steroidal saponins [3]. Other primary biochemical substances of roots and young shoots are amino acids (asparagines, lysine, arginine), flavonoids (rutin, quercetin, kaempferol) [15, 9], oligosaccharides [4, 16], tannin, essential oil, folic acid, proteins and vitamins: A, B<sub>1</sub>, B<sub>2</sub>, C, E [9]. Young shoots are more useful due to high content of vitamin C and mineral salts, mainly with K. Seeds of this plant contain 15% fats. Matured fruits contain organic acids (malic acid, citric acid), carbohydrates and alkaloids [7]. Application of this plant contributes to removal of chlorides and phosphates from human organism. *A. officinalis* contains strong antioxidant glutathione which inhibits the early aging [6].

Pharmacological properties of *A. officinalis* stipulate anti-inflammatory [5], antifungal [14], anti-tumor [1, 12], antioxidant [11] and antimutagenic [4, 5] activities. *A. officinalis* fruits as remedy against the sexual weakness are used. *Asparagus* due to low caloric (21 kcal) and high cellulose content is considered as an irreplaceable component for diabetes and people suffering from overweight [6, 7].

**Aim and goal.** Considering multilateral positive qualities of this medicinal plant, elaboration of plant biotechnology of *A. officinalis* by *in vitro* method is actual.

Investigations in *in vitro* culture were carried out in the following directions:

- introduction of *A. officinalis* in *in vitro* and selection growth conditions,
- application of clonal micropropagation methods,
- investigation of callus induction, organogenesis as well as stimulation of shoot regenerants root formation possibility,
- selection of plant growth regulators and their optimal concentration for nutrient mediums.

## 2. Material and Methods

Rhizomes of *A. officinalis* L. (*Mary Washington*) were acquired from the USA and grown in the open air hydroponics conditions.

To introduce in to aseptic *in vitro* conditions intact seeds from hydroponics plants were used. Before sterilization seeds by sterile water 24 hours were wetted, an hour to the influence of ultraviolet rays subjected. Sterilization of seeds was in 96% of ethyl spirit - 1 minute, then in 0.1% solution of Diacid (aqueous solution of 0.6 g/l cetylpyridinium chloride and 0.3 g/l methyl mercury chloride) – 10 minutes and finally were washed three times per 15 minutes in sterile water.

Investigations were done by methods of clonal micropropagation [2, 10] and implemented in the following directions: by seedling, callus tissue induction and organogenesis of adventives shoot.

In *in vitro* culture the main nutrient medium was selected Murashige and Skoog (MS) [8]. For root induction of micro cuttings and shoot-regenerants was tested ½ MS medium with partially reduced macro, micro salts, as well as supplemented with concentrations of indolebutyric acid (IBA) and  $\alpha$ -naphthylacetic acid ( $\alpha$ -NAA) (Table 1).

**Table 1.** Nutrient media tested for root formation of *A. officinalis*.

Nutrient substances	Media, №				
	I	II	III	IV	V
Mineral salts	½ MS	½ MS	½ MS	½ MS	½ MS
Thiamine, mg/l	1.0	1.0	1.0	1.0	1.0
Pyridoxine, mg/l	0.5	0.5	0.5	0.5	0.5
Sucrose, g/l	20.0	20.0	20.0	20.0	20.0
Agar, g/l	7.0	7.0	7.0	7.0	7.0
$\alpha$ -NAA, mg/l	---	---	0.3	---	0.5
IBA, mg/l	0.1	0.3	---	0.5	---
pH	5.5 - 5.6				

Callus tissues regenerated from segments of root (1mm), hypocotyl (3 mm), apical bud and shoot with lateral buds (2 mm) of 25-30 day germinated plants.

For callus formation MS medium with various concentrations of phytohormones was tested: 6-benzylaminopurine (BAP),  $\alpha$ -NAA, 2,4-dichlorophenoxyacetic acid (2,4D) and Kinetin (Table 2).

**Table 2.** Nutrient media for callus formation of *A. officinalis*.

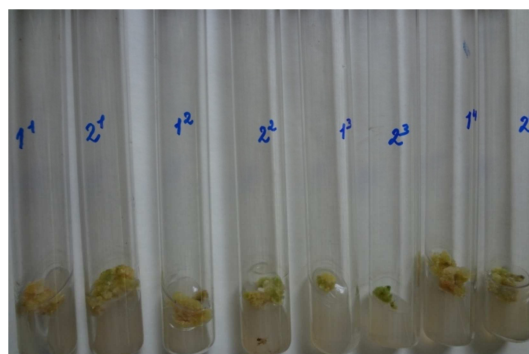
Nutrient substances	Media, №		
	1	2	3
Mineral salts	MS	MS	MS
Thiamine, mg/l	1.0	1.0	1.0
Pyridoxine, mg/l	0.5	0.5	0.5
Sucrose, g/l	30.0	30.0	20.0
Agar, g/l	7.0	7.0	7.0
$\alpha$ -NAA, mg/l	---	0.5	---
BAP, mg/l	1.0	1.0	---
2,4-D, mg/l	2.0	0.5	---
Kinetin, mg/l	---	---	1.0
pH	5.5 – 5.6		

Callus tissues replanting was done once monthly. In artificial climate chamber 22 – 26°C temperature was maintained, photoperiod: 16 hours, air humidity: 60 – 70%. Test-tubes with planted explants were placed under the light (3000 – 8000 Lux).

## 3. Results and Discussion

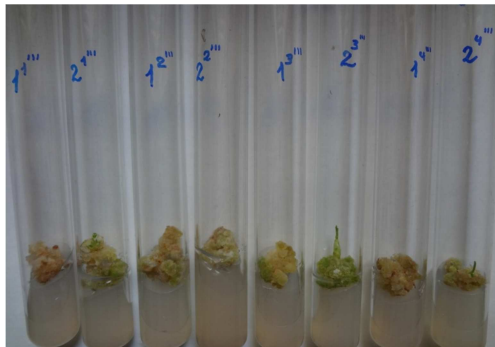
In the open air hydroponic conditions at the end of vegetation period the height of the plants grown form rhizomes was 77cm, with 9 shoots per plant. On one shoot matured 49 fruits, per fruit: 3-6 seeds. *A. officinalis* seeds from hydroponic plants were used for introduction to *in vitro* culture.

Efficiency of seed sterilization in *in vitro* conditions was 100%, which begun to germinate after 9 days of sowing. Seed germination was 88.5%. Test-tube micro-plants were obtained, which vegetative organs subsequently as explants were used.



**Fig. 1.** Primary callus of different explants of *A. officinalis* on N1 (BAP - 1 mg/l; 2,4D - 2 mg/l) and N2 (BAP - 1 mg/l; 2,4 D - 0.5mg/l;  $\alpha$ -NAA - 0.5 mg/l) nutrient media: root, ²- hypocotyl, ³- apical bud, ⁴- shoot segment with lateral buds).

Callus formation of explants was registered after 15 days. Tested explants (root, hypocotyl, apical bud, shoot segment with lateral buds) on media N1 and N2 formed yellow-green callus (Fig. 1). All tested explants generated callus.

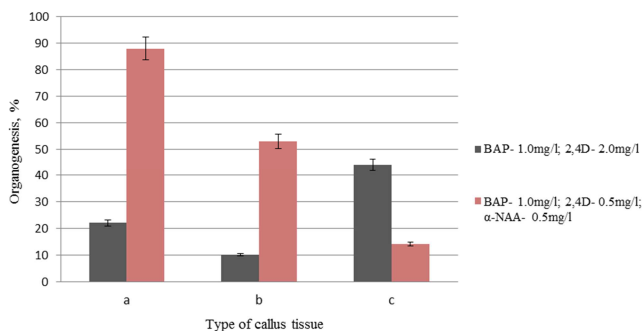


**Fig. 2.** The third replanting of callus tissues: (explants: <sup>1</sup>- root, <sup>2</sup>- hypocotyl, <sup>3</sup>- apical bud, <sup>4</sup>- shoot segment with lateral buds).



**Fig. 3.** Callus culture of *A. officinalis*.

Hypocotyl explants formed white, solid callus mass and during further months cultivation was induced intensive organogenesis. According to data in Fig. 5, after the 11<sup>th</sup> and 12<sup>th</sup> replanting, on the medium N1 was observed 22% adventive shoot regeneration of callus tissues derived from hypocotyl explant (on average 3 – 5 shoots).



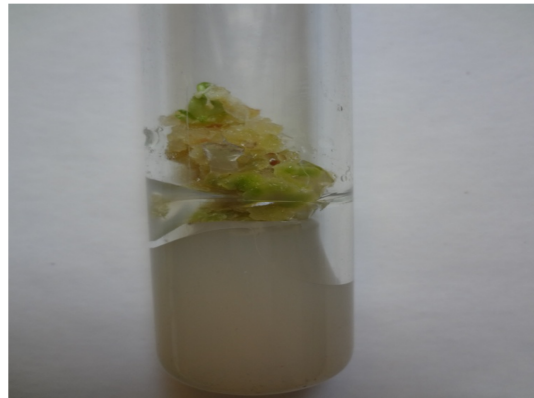
**Fig. 4.** Organogenesis of adventive shoots and roots on the 1/2 MS nutrient medium supplemented with BAP, 2,4D and α-NAA: a - adventive shoot-regeneration on hypocotyl callus; b - rhizogenesis on root callus; c - rhizogenesis on stem callus.

On N2 medium shoot regeneration was 88% (on average 10 – 15 shoots).

On nutrient medium (N3) with 1 mg/l Kinetin

The first replanting of callus tissues after 3,5 months was implemented. The following replanting once monthly on the same fresh medium were done. After the first replanting of callus tissues intensive growth in all variants was observed. Concentration of 0.5 mg/l α-NAA and decrease of 2,4D up to 0.5 mg/l induced organogenesis (1 – 2 shoots) of callus tissues (Fig. 2).

Callus tissues (Fig. 3) during the cultivation were yellowish with green segments, which testify about the content of cells synthesizing chlorophyll. All the tissues were of solid consistency.



**Fig. 5.** Callus tissues with adventive shoot- regenerants from hypocotyl explants on medium N3.

In callus tissues from root and stem explants



organogenesis (rhizogenesis) was observed during further replanting: on the medium N1 – 10% and 44% and on the medium N2 – 53% and 14% respectively (Fig. 4).

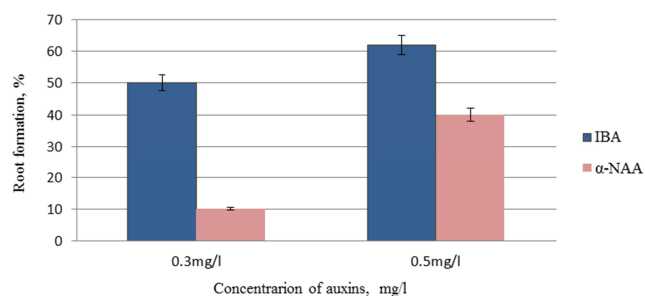


**Fig. 6.** Adventive shoot- regenerants (hypocotyls explants) with callus segments on  $\frac{1}{2}$ MS medium.

As an optimal material for further micro-propagation was tissues derived from hypocotyl. Shoot-regenerants from hypocotyl explant were separated and with callus segment

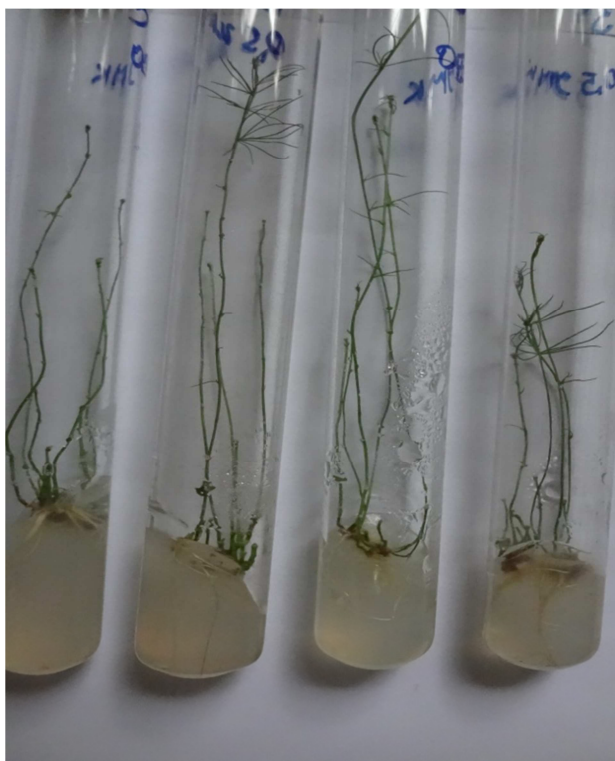
replanted on  $\frac{1}{2}$ MS nutrient medium (Table 1) for rhizogenesis (Fig. 6). Literature data testifies that *in vitro* root initiation of *Asparagus* micro cuttings is difficult [9, 13]. Therefore, the results of our investigation was 3% root-formation of micro cuttings on the medium with 0.1 mg/l IBA.

According to data shown in Fig. 7 optimal concentration for root formation of shoot-regenerants was at 0.5 mg/l content of auxin, and the best phytohormone was IBA.



**Fig. 7.** Root formation of shoot-regenerants on the  $\frac{1}{2}$ MS medium with IBA and  $\alpha$ -NAA.

Therefore, on the medium supplemented with IBA (0.5 mg/l) shoot-regenerants formed well expressed roots (Fig. 8). Rhizogenesis was 62% (Fig. 7).



**Fig. 8.** Shoot-regenerants on the medium with 0.5 mg/l IBA.

Root formation of shoots on the medium with 0.5 mg/l  $\alpha$ -NAA was 40% (Fig. 7 and 9).



Rooted shoot-regenerants after acclimatization were planted and grown in open air gravel hydroponics conditions.



Fig. 9. Shoot-regenerants on the medium supplemented with 0.5 mg/l  $\alpha$ -NAA.

#### 4. Conclusions

The possibility of *Asparagus officinalis* micropropagation was confirmed as the result of the research. In *in vitro* culture 25-30 day germinated seedlings of all explants (hypocotyl, root, stem, apical meristem, rhizome) were obtained viable callus tissues. In case of various growth regulators (BAP, 2,4D,  $\alpha$ -NAA, Kinetin) combinations and concentrations callus tissues induction and organogenesis was induced. Optimal explant for micro-propagation hypocotyl explant was selected. It was found out, on MS nutrient medium for shoot-regenerants formation optimal concentration was BAP - 1.0mg/l, 2,4D - mg/l,  $\alpha$ -NAA - 0.5mg/l or kinetin- 1.0 mg/l. The best phytohormone for root-formation of shoot-regenerants (62%) was IBA- 0.5mg/l concentration.

Accordingly, biotechnology by *in vitro* and hydroponic combined method of *Asparagus officinalis* was elaborated. The possibility of *A. officinalis* introduction to *in vitro* conditions, callus induction, tissues growth, micro-propagation and rhizogenesis and optimal conditions selection was confirmed.

#### References

- [1] Bousserouel S., Le Grandois J., Gosse F., Werner D., Barth S.W., Marchioni E., Marescaux J., Raul F.A. (2013) Methanolic extract of white *Asparagus* shoots activates trail apoptotic death pathway in human cancer cells and inhibits colon carcinogenesis in a preclinical model. *Int. J. of Oncology*, 43 (2), p. 394-404.
- [2] Butenko R. G. (1999) The Biology of cultivated cells of higher plants *in vitro* and biotechnology on its base. Monograph, Moscow, FAC-Press, p.159 (In Russian).
- [3] Huang X., Kong L. (2006) Steroidal saponins from roots of *Asparagus officinalis*. *Steroids*, V.71, Issue 2, p. 171-176.
- [4] Huang X.F., Lin Y.Y., Kong L.Y. (2008) Steroids from the roots of *Asparagus officinalis* and their cytotoxic activity. *J. Integr. Plant Biol.* 50, p.717-722.
- [5] Jang D.S., Cuendet M., Fong H.H., Pezzuto J.M., Kinghorn A.D. (2004) Constituents of *Asparagus officinalis* evaluated for inhibitory activity against cyclooxygenase-2. *J. Agric. Food Chem.*, 52, p. 2218-2222.
- [6] Khorasani A., Sani W., Philip K., Mat Taha R., Ra A. (2010) Antioxidant and antibacterial activities of ethanolic extracts of *Asparagus officinalis* cv. Mary Washington: Comparison of *in vivo* and *in vitro* grown plant bioactivities. *African J. of Biotechnology*, V. 9 (49), p. 8460-8466.
- [7] Lebeda A.F., Drujenko N.I., Isayko A.P., Sobko V.G. (2005) Medicinal plants. p.722 (In Russian).
- [8] Murashige T., Skoog F.A. (1962) Revised Medium for Rapid Growing and Bioassay with Tobacco Tissue Culture. *Physical Plantarium*, V. 1 (15), p. 473-497.
- [9] Negi J.S., Singh P., Joshi G.P., Rawat M.S., Bisht V.K. (2010) Chemical constituents of *Asparagus*. *Pharmacognosy Review*, 4(8), p. 215-220.
- [10] Petrus-Vancea A. (2011) New techniques in *Asparagus officinalis* L. micro-propagation. /Scientific Papers, UASVM Bucharest, Series - A, V. L IV, p. 431-438.
- [11] Rodriguez R., Jaramillo S., Rodriguez G., Espejo J.A., Guillen R., Fernandez-Bolanos J., Heredia A., Jimenez A. (2005) Antioxidant activity of ethanolic extracts from several asparagus cultivars. *J. of Agric. Food Chemistry*, V.53, p. 5212-5217.
- [12] Shao Y., Chin C.K., Ho C.T., Ma W., Garrison S.A., Huang M.T. (1996) Anti-tumor activity of the crude saponins obtained from asparagus. *Cancer Lett.*, V.104, p. 31-36.
- [13] Stajner N. (2013) Micropropagation of *Asparagus* by *in vitro* shoot culture. *Methods Mol. Biol.*, p. 341-351, <http://www.ncbi.nlm.nih.gov/pubmed/23179712>.
- [14] Wang H., Ng T.B. (2001) Isolation of a novel deoxyribonuclease with antifungal activity from *Asparagus officinalis* seeds. *Biochem. Biophys. Res. Commun.*, V. 289, p. 120-124.
- [15] Wang M., Tadmor Y., Wu Q.L., Chin C.K., Garrison S.A., Simon J.E. (2003) Quantification of protodioscin and rutin in asparagus shoots by LC/MS and HPLC methods. *J. of Agric. Food Chemistry*, V. 51, p. 6132-6136.
- [16] Yamamori A., Onodera S., Kikuchi M., Shiomi N. (2002) Two novel oligosaccharides formed by 1F-fructosyltransferase purified from roots of asparagus (*Asparagus officinalis* L.). *Bioscience Biotechnology Biochemistry*, V. 66, p. 1419-1422.