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IN VITRO PROPAGATION AND DETERMINATION OF THE NUTRIENT CONTENT OF NATURALLY GROWN *ASPARAGUS STIPULARIS* FORSSK

KAMILE ULUKAPI[1*], AYŞE GÜL NASIRCILAR[2], AHMET NACI ONUS[3] and İBRAHIM BAKTIR[3]

[1] *Akdeniz University, Vocational School of Technical Science, Organic Agriculture Program,* Antalya, Turkey
[2] *Akdeniz University, Faculty of Education, Department of Biology Education,* Antalya, Turkey
[3] *Akdeniz University, Faculty of Agriculture, Department of Horticulture,* Antalya, Turkey

*Corresponding author: kamileonal@akdeniz.edu.tr

Abstract - Wild *Asparagus* (*Asparagus stipularis* Forssk) is a species of the *Liliaceae* family. Although its fresh sprouts are collected from natural habitats in early spring for human diet, it has yet to be cultivated. The present study, aimed to establish an efficient *in vitro* propagation system and reveal the nutritive value of *A. stipularis*. To our knowledge, there are no reports on the nutritive value and *in vitro* propagation of *A. stipularis*. Bud scale, spear section and apical bud explants of *A. stipularis* were cultured on Murashige and Skoog (MS) medium containing various concentrations of 6-benzylaminopurine (BAP) and α-naphthaleneacetic acid (NAA). The best results were obtained from apical bud explants on MS medium containing 2.0 mg l^{-1} BAP and 0.5 mg l^{-1} NAA. In terms of nutritive value, it was found that the nitrogen content of *A. stipularis* was higher than the other macro elements. Although Zn- and Fe-containing compounds were higher in *A. stipularis*, Ca-containing compounds were lower than in other *Asparagus* species. It was determined that the portions of unprocessed *A. stipularis* had pH 7.6, 13.3% soluble solid content, 86.7% moisture, 0.09% titratable acidity and were about 31% ascorbic acid. We conclude that in terms of nutritional value, *A. stipularis* can be a good alternative to other *Asparagus* species for human nutrition, considering its high N, Fe and vitamin C contents.

Key words: Asparagus stipularis; *in vitro* culture; nutrition; nutrition analysis

INTRODUCTION

The genus *Asparagus* is a member of the *Liliaceae* family, consumed as a vegetable and native to Mediterranean countries. Although its farming is more difficult and costly than other vegetables, *Asparagus* is consumed worldwide because of its high nutritional value, especially due to vitamins A, C and its phosphorus content (Seçer et al., 2006; Ercan and Şensoy, 2007). Different parts of *Asparagus* species are used for various purposes due to their steroidal saponozid and sapogenol content (Güvenç and Koyuncu, 1999). Some *Asparagus* species such as *Asparagus adscendens* Roxb (Mehta and Subramanian, 2005) and *A. racemosus* Willd (Bopana and Saxena, 2008) have medicinal importance because of their biochemical properties. The rhizome extract of *A. adscendens* Roxb contains various important compounds such as steroids, essential oils, triterpenoids, glycosides and saponins, and it has been determined as a potential drug to control AIDS symptoms (Mehta and Subramanian, 2005). Additionally, another *Asparagus* species, *A. racemosus*, has antioxidant, phytoestrogenic and immunostimulant properties (Bopana and Saxena, 2008).

Although *Asparagus stipularis* Forssk is commonly known on the islands of Cyprus and Rhodes, it also has large distribution areas in Greece, Tunisia, Algeria and Egypt. Like other *Asparagus* species (Yılmaz et al., 2007), it is assumed that wild *A. stipularis* is rich in potassium, phosphorus, calcium and iron and its fresh spears are collected from natural habitats and sold in markets. Although *A. stipularis* has commercial value, it has not been cultivated yet. To our knowledge, there are no reports on the nutritive value and *in vitro* propagation of *A. stipularis* and this is the first report.

MATERIALS AND METHODS

In vitro propagation

A. stipularis plants were obtained from their natural habitat in Northern Cyprus. Bud scales, spear sections and apical buds were used as the explants for *in vitro* propagation. Whole plants were washed with detergent under running tap water and the explants were separated from the mother plant. The surfaces of all explant types were sterilized with three commercial bleach concentrations (10, 15 and 20%) for 10 min and then rinsed 3 times with sterile distilled water. After sterilization, spears were cut into about 5-mm pieces and bud scales, apical buds and spear sections were cultured on MS medium (Murashige and Skoog, 1962) containing 1.0, 2.0, 4.0 mg l^{-1} BAP and 0.25 or 0.5 mg l^{-1} NAA.

The pH of the medium that contained 30 g of sucrose and 7 g/L was adjusted to 5.7 before autoclaving at 121°C for 20 min. The cultures were kept at 25±1°C under a 16 h light photoperiod and were subcultured monthly during two months.

Shoots were transferred to hormone-free MS medium, half-strength MS medium, half-strength MS medium including 3 g l^{-1} activated charcoal, half-strength MS medium supplemented with 0.1, 0.5 mg l^{-1} NAA or half-strength MS medium supplemented 1.0, 2.0 mg l^{-1} Indole-3-butyric acid (IBA) and 3 gr L^{-1} activated charcoal for rooting.

Determination of nutrition values

Analytical determination of P, K, Fe, Mn, Zn, Ca and Mg was carried out by ICP-OES spectrophotometer and N value was determined by modified Kjeldahl method (Kaçar and İnal, 2008).

Statistical Analysis

The experiment was designed to be completely randomized, with three replications, and the results were analyzed for statistical significance by analysis of variance (SAS, 1985).

RESULTS AND DISCUSSIONS

Surface sterilization

Bud scales, apical buds and spear sections of *A. stipularis* were sterilized with 10, 15, 20% commercial bleach concentrations for 10 min. Twenty percent commercial bleach destroyed all of the explants so that 10% and 15% commercial bleach concentrations were used for further surface sterilization of explants. Although 15% commercial bleach application was found to be suitable for bud scale explant surface sterilization, 10% commercial bleach was convenient for apical buds and spear section sterilization (Table 1).

In vitro propagation

In nature, *Asparagus* species can be sexually propagated by seeds and clonally propagated by cutting. Because of the low germination percentage of *Asparagus* seeds and a limited number of plants obtained by clonal propagation, *in vitro* multiple propagation is preferred for propagation (Bopana and Saxena, 2008; Limanton-Grevet and Jullien, 2000). *In vitro* multiplication of *Asparagus* species has been reported but there are no reports on the *in vitro* propagation of *A. stipularis*. Pant and Joshi (2009) reported that while NAA promoted bud initiation, BAP promoted both shoot and bud initiation and their combination promoted shoot, bud, callus and root initiation in *in vitro* propagation of *A. racemosus*. Similarly, Afroz et

Table1. Multipropagation of *Asparagus stipularis* Forssk. shoots in *in vitro* conditions.

Explant	Medium	I. Subculture (shoot number per explant)		II. Subculture (shoot number per explant)	
		10% (NaOH)	15% (NaOH)	10% (NaOH)	
Bud scale	1 BAP (mg L^{-1}) + 0.25 NAA (mg L^{-1})	7.00c	0.00i	6.67b	0.00f
Bud scale	1 BAP (mg L^{-1}) + 0.5 NAA (mg L^{-1})	2.33gh	2.33gh	3.67dc	1.67e
Bud scale	2 BAP (mg L^{-1}) + 0.25 NAA (mg L^{-1})	0.00i	5.00d	0.00f	3.67dc
Bud scale	2 BAP (mg L^{-1}) + 0.5 NAA (mg L^{-1})	0.00i	3.67ef	0.00f	3.67dc
Bud scale	4 BAP (mg L^{-1}) + 0.25 NAA (mg L^{-1})	0.00i	5.00d	0.00f	5.00c
Bud scale	4 BAP (mg L^{-1}) + 0.5 NAA (mg L^{-1})	4.00ed	2.33gh	4.00c	2.33de
Spear section	1 BAP (mg L^{-1}) + 0.25 NAA (mg L^{-1})	0.00i	0.00ı	0.00f	0.00f
Spear section	1 BAP (mg L^{-1}) + 0.5 NAA (mg L^{-1})	0.00i	0.00ı	0.00f	0.00f
Spear section	2 BAP (mg L^{-1}) + 0.25 NAA (mg L^{-1})	0.00i	0.00ı	0.00f	0.00f
Spear section	2 BAP (mg L^{-1}) + 0.5 NAA (mg L^{-1})	1.33h	1.67gh	1.33fe	1.00fe
Spear section	4 BAP (mg L^{-1}) + 0.25 NAA (mg L^{-1})	0.00i	0.00ı	0.00f	0.00f
Spear section	4 BAP (mg L^{-1}) + 0.5 NAA (mg L^{-1})	0.00i	0.00ı	0.00f	0.00f
Apical bud	1 BAP (mg L^{-1}) + 0.25 NAA (mg L^{-1})	2.67gf	0.00ı	1.67e	0.00f
Apical bud	1 BAP (mg L^{-1}) + 0.5 NAA (mg L^{-1})	0.00i	0.00ı	0.00f	0.00f
Apical bud	2 BAP (mg L^{-1}) + 0.25 NAA (mg L^{-1})	0.00i	0.00ı	0.00f	0.00f
Apical bud	2 BAP (mg L^{-1}) + 0.5 NAA (mg L^{-1})	15.33a	0.00ı	10.67a	0.00f
Apical bud	4 BAP (mg L^{-1}) + 0.25 NAA (mg L^{-1})	0.00i	0.00ı	0.00f	0.00f
Apical bud	4 BAP (mg L^{-1}) + 0.5 NAA (mg L^{-1})	10.67b	0.00ı	6.67b	0.00f

LSD: 1,18, 1.39(respectively I. subculture, II. Subculture)

Table 2. The percentage of rooted explants on different nine media.

Media	**The percentage of rooting**
MS	9%
½ MS	0%
½ MS + 3 g L^{-1}activated charcoal	0%
½ MS + 0.5 mg L^{-1}NAA	9%
½ MS + 0.1 mg L^{-1}NAA	3%
½ MS + 1 mg L^{-1}NAA	0%
½ MS + 2 mg L^{-1}NAA	0%
½ MS + 3 mg L^{-1}activated charcoal+1 mg L^{-1}IBA	9%
½ MS + 3 g L^{-1}activated charcoal+2 mg L^{-1}IBA	0%

Table 3. Amount of nutritional value of *A. stipularis*

N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	pH	Moisture (%)	Soluble solids (%)	Ascorbic acid (vit. C)	Titratable acidity(%)
4.60	0.49	3.62	0.06	0.18	141	19	66	7.6	86.7	13.3	30.93	0.09

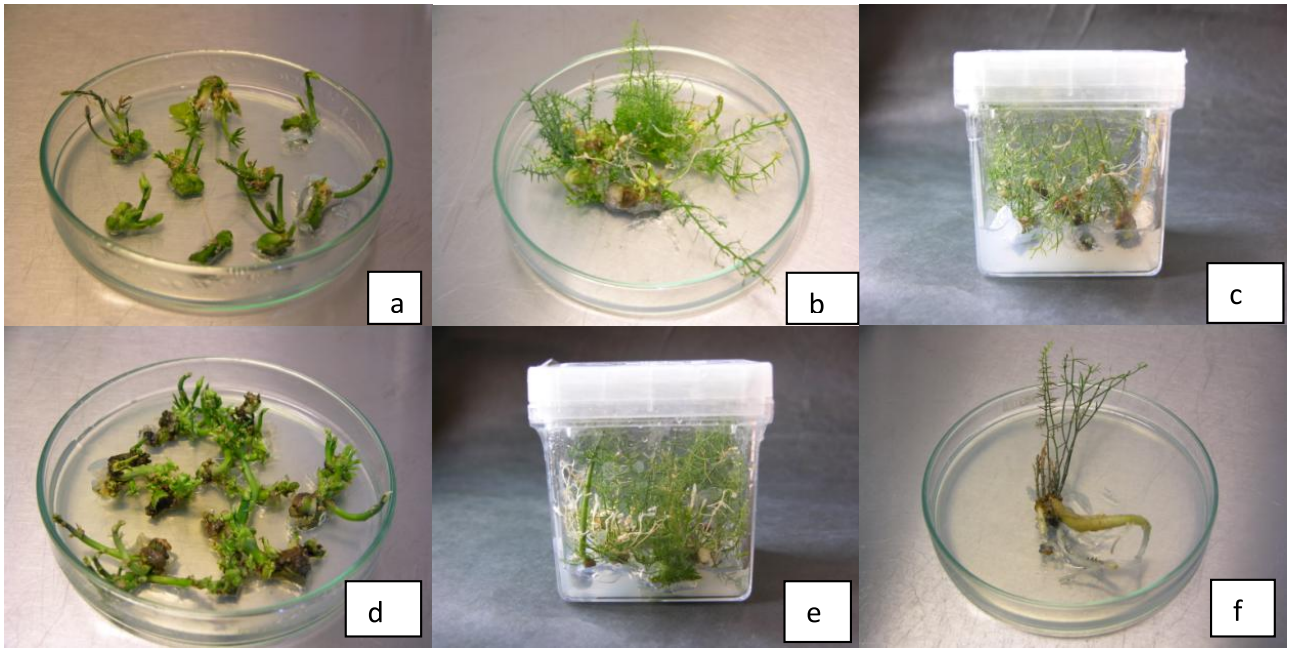

Fig. 1. Regeneration from bud scale explants (a,b,c); apical bud explants (d, e); root formation (f).

al. (2010) used BAP as a cytokine source and NAA and IBA as auxin source for the successful *in vitro* multiplication of *A. racemosus* as the best shoot multiplication was obtained from MS medium supplemented with BAP (0.1 mg l^{-1}) and NAA (0.05 mg l^{-1}). Half-strength MS medium containing 0.05 mg l^{-1} BAP and 0.1 mg l^{-1} IBA was suggested for root formation.

In order to establish an efficient propagation procedure, bud scales, spear sections and apical buds were used as explant sources (Fig. 1). The data obtained from the spear section explants indicated that this explant was not suitable for *in vitro* propagation of *A. stipularis* as 1.33 (10% NaOCl) and 1.67 (15% NaOCl) shoots per explant were obtained on MS medium supplemented with 2 mg l^{-1} BAP and 0.5 mg l^{-1} NAA. When the bud scales were used as the explants, better results (7.00 shoots per explant) were recorded in low concentration of cytokine (1 mg l^{-1} BAP and 0.25 mg l^{-1} NAA) (Table 1 and Fig. 1). Of the different explants, the apical bud was determined as the best explant type for *in vitro* multiplication of *A. stipularis* since the highest multiplication rate was obtained in MS medium supplemented with 2 mg l^{-1} BAP and 0.5 mg l^{-1} NAA (Table 1). Average numbers of shoots were 15.33 and 10.67, respectively, from the first and second subculture on the medium. Similarly, a high number of shoots per bud scale explant (10.67) was obtained on MS medium containing 4 mg l^{-1} BAP and 0.5 mg l^{-1} NAA. However in both media, the mean number of shoots decreased after the second subculture. Therefore, it is possible that the regeneration capacity of the shoots decreases in subsequent subcultures. In order to improve the shoot regeneration, different cytokine types should be considered.

Toma and Rasheed (2012) obtained the highest frequency of shoot multiplication on MS medium supplemented with BA (0.5 mg l^{-1}) and NAA (0.2 mg l^{-1}) in *A. densiflorus*. Stajner (2012) and Stajner et al. (2002) used different concentrations of BA, NAA and KN (kinetin) for *in vitro* regeneration of *A. maritimus* and *A. officinalis,* respectively. In both studies, the use of ancymidol was suggested, especially to promote the root induction. Ancymidol may also be used for shoot development and callus formation in different *Asparagus* species, including *A. stipularis*. In another research conducted on *in vitro* regenera-

tion of *A. officinalis,* the highest number of shoot per explants (15.50) was obtained from 0.015 mg l^{-1} NAA and 0.5 mg l^{-1} BAP (Sarabi and Almasi, 2010). According to Kumar (2009), nodal segments were suitable for *in vitro* shoot development and bud induction in *A. racemosus*. The best results were achieved on MS medium supplemented with 3.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA, and 40-45 shoot bud/explants were obtained on the medium. It is possible that a high BAP concentration positively affects shoot development.

Root formation

Root initiation of *Asparagus* species is difficult (Stajner, 2012). For this reason, different rooting media were used for root regeneration of the shoots. Among nine rooting media, root regeneration was obtained on MS, half-strength MS supplemented with 0.5 mg l^{-1} NAA, half-strength MS supplemented with 0.1 mg l^{-1} NAA and half-strength MS with 3 g activated charcoal added and supplemented with 1 mg l^{-1} IBA. The data were evaluated and presented as percentages (Table 2, Fig. 1). The rooting media can be modified by adding ancymidol as suggested by Stajner (2012) and Stajner et al. (2002).

Determination of nutritional values

Nutritional values were determined from *A. stipularis* plants grown in their natural habitat without fertilization and pesticide application in Northern Cyprus. All analyses determining nutritional value were carried out on 0.5 g dried ash samples. Green spears of *A. stipularis* were analyzed in terms of some trace elements and pH, moisture, soluble solid, ascorbic acid and titratable acid. Since there are no previous reports on the chemical components of *A. stipularis*, the results were compared with other *Asparagus* species.

Makus (1995), working on the nutritional value of green and white *A. officinalis* supplemented with and without nitrogen, reported that green *A. officinalis* contained higher total amounts of N, K, P, Ca, Mg, Fe, Zn and Mg than white ones. When these results (without nitrogen application) were compared with the results of *A. stipularis*, N (4.75%), P (0.692%), Ca (0.160%), Fe (191 μg g^{-1}) and Zn (69 μg g^{-1}) content was slightly higher than the results obtained for *A. Stipularis* in the present study (Table 3). On the other hand, Mg (0.184%) and Mn (19 μg g^{-1}) content was the same and K (2.98) content was lower than *A. stipularis*. The water and soil environment affect the nutritional content of plants (Qiao-Juan et al., 2010) and different species may contain different nutritional values. The Fe content of *A. stipularis* and *A. officinalis* (Qiao-Juan et al., 2010) was found to be rich. Seçer et al. (2006) determined the effects of mineral fertilization on plant nutrient contents of *A. officinalis*. When the results of the present study were compared with those obtained for *A. officinalis* by Seçer et al. (2006), the macronutrient elements of *A. stipularis* were higher than in the control group of *A. officinalis* that was not subjected to any treatment. Unlike other nutrients, the Ca content of *A. stipularis* was found to be less than *A. officinalis*. Contrary to the findings of Qiao-Juan et al. (2010), Seçer et al. (2006) found that the Fe and Mn content of *A. officinalis* was higher than the Fe and Mn content of *A. stipularis* determined in the present study. However, Amara Lopez et al. (1999) reported a lower Fe content than *A. stipularis* similar to Qiao-Juan et al. (2010), but the moisture, Mn and Zn content of *A. officinalis* was higher than *A. stipularis*.

As the first report on *in vitro* multiplication and the nutrient content of *A. stipularis*, it was revealed that while the apical buds were the best explants, MS medium containing 2.0 mg l^{-1} BAP and 0.5 mg l^{-1} NAA was the best propagation medium. However, it must be emphasized that further research is needed, especially on rooting. In terms of nutritional value, *A. stipularis* can be a good alternative to other *Asparagus* species for human nutrition, considering its high N, Fe and vitamin C content.

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