IN VITRO PROPAGATION AND SECONDARY METABOLITES PRODUCTION IN THE WILD RARE ASPARAGUS APHYLLUS L. PLANT

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Asparagus aphyllus is grown in Egypt naturally as a rare plant. It is utilized in conventional medicine to treat various liver diseases and as anti-cancer. Shoot tips have been cultured on Murashige and Skoog (MS) medium supplemented with 1.0 mg/l kinetin (kin) and 6-benzyl adenine (BA) combined with different concentrations of α-naphthalene acetic acid (NAA) for the multiplication of shoots. The shoot of 5 cm has been cultured on 1/2 MS medium supplemented with various concentrations of indole-3-butyric acid (IBA) or full MS medium with 1.0 mg/l kin and two concentrations of NAA for rooting. The top results of multiplication were obtained with 1.0 mg/l BA and 0.2 mg/l NAA and gave 12 shoots per explant and the maximum mean length of shoot was 6.60 cm with 1.0 mg/l BA and 0.4 mg/l NAA. The highest percentage of rooting was 20% after three months of culture on half strength MS medium with IBA at 1.5 mg/l and 2.0 mg/l. The mean number of roots per explant was 1.6 roots. The mean length of root was 4.0 cm at a concentration of 2.0 mg/l IBA. Percentage of survival after acclimatization was about 65% after two months. The highest fresh weight of callus was 14.03 g with friable texture and observed on MS medium supplemented with 2.0 mg/l NAA plus 1.0 mg/l 2,4-D with 0.5 mg/l BA. The total saponin that contains the highest value of 5.85 mg/g fresh weight was observed from the mother plant. The results of the present study would be applicable for cost-effective large-scale micropropagation of Asparagus aphyllus and improvement.

Keywords: Asparagus, in vitro, micropropagation, saponin

INTRODUCTION

Asparagus aphyllus L. (Prickly), is used for human food, conventional medicine and in treating various disease (Knaflewski, 1996). It is known as Asparagus in English and Halion in Arabic, and is exercised in classical Greco-Arab and Islamic medicine (Saad and Said, 2011). The genus Asparagus includes around 200 species and classified into three subgenera. A. aphyllus is adapted to dry conditions and in hedges and scrub as well as rocky areas and crevices (Turland et al., 1993). A. aphyllus is native to southern Europe, as well as north Africa (Egypt) and Jordan, Lebanon, Syria and Turkey in western Asia (USDA, ARS, National Genetic Resources Program, 2014). A. aphyllus is a secondary genetic relative of Asparagus (A. officinalis) (USDA, ARS, National Genetic Resources Program, 2013) and so it has a potential for use as a gene donor for crop improvement, especially as it is adapted to xerophytic conditions and is resistant to diseases (Falavigna et al., 2008). According to Tardio et al. (2006), young shoots are harvested and eaten with eggs as an omelette in Spain. Kubota et al. (2012) reported that the geographic distribution of these species comprises the arid and semiarid regions of Europe, Asia, Africa and Australia. All the species in the subgenus Asparagus are dioecious as reported by Castro et al. (2013).
Phytochemicals from the root of *Asparagus* (dioscin and methylprotopodioscin) were reported *in vitro* to inhibit the expression of mucin protein in the epithelial cells, which partially validates traditional uses in inhibiting hypersecretion in the mucus of pulmonary tracts (Lee et al., 2015). The plant treat varied liver diseases, diarrhea and dysentery (Saad and Said, 2011). Meanwhile, other studies have reported that *Asparagus* gum polysaccharide have immune-modulatory functions and were shown to accelerate apoptosis, diminishing tumor development of hepatocellular carcinoma in the liver. Cladophyll and roots of *Asparagus* *in vivo* lower diastolic blood pressure, glucose, left cardio-ankle vascular index score and total cholesterol level (Nishimura et al., 2013 and Weng et al., 2014). *A. aphyllus* is used as anti-cancer (Kmail et al., 2015), inhibitor for the growth of bacteria (Teka et al., 2015) and a rich source of antimicrobial agents (Sharma and Goel, 2018). Kmail et al., (2017) reported that the extracts of *A. aphyllus* exhibit relatively high levels of phenolics, flavones and flavonols.

The *Asparagus* genus is one of the few plant foods containing steroidal saponins that are distributed throughout different organs of the plant, including leaves, stems, fruits and roots (Hamdi et al., 2017). As regard to the saponins, they are remarkably stable to heat processing, and their biological activity is not reduced by normal cooking (Kimura et al., 2006). Saponins are essential constituents of nutraceuticals and useful foods (Raju and Mehta, 2009) and are recognized with a number of bioactivities like anti-inflammatory, antimicrobial, immunostimulant, hypocholesterolaemic, anticarcinogenic and antioxidant (Guclu-Ustundag˘ and Mazza, 2007). In addition, saponins form complexes with cholesterol of erythrocytes membrane forming pits and holes, which leads to the increase in permeability and haemolysis (Hostettmann and Marston, 1995).

There are a few researches about micropropagation of *A. aphyllus*. The use of *in vitro* propagation methods is necessary to propagate *A. aphyllus* through different micropropagation procedures as reported by Desjardins et al. (1987) and Kunitake and Mii (1998). However, a general protocol is valid for all the species of *Asparagus*. The main purpose of the present study was to optimize an *in vitro* propagation method for *A. aphyllus* and the *in vitro* production of saponins.

**MATERIALS AND METHODS**

1. **In Vitro Propagation**

1.1. Plant material and sterilization

The seeds of *A. aphyllus* were collected in August 2019 from Al-Arish region and utilized as a source of plant material. Seeds were surface sterilized under running tap water for three hours with a few drops of soup, then transferred to a laminar air flow cabinet to complete sterilization by sodium hypochlorite solution (5.25%) (commercial bleach 15%) for 5-10 min. After that, seeds were rinsed with sterile distilled water for three times and cultured.
in Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), then incubated in the culture room at 23±2°C in complete darkness, for one month for germination.

1.2. Induction and multiplication of shoots

Shoot tips were excised from the seedlings after one month and cultured on MS medium supplemented with kinetin (kin) or 6-benzyl adenine (BA) at a concentration of 1.0 mg/l combined with 0.2, 0.4 or 0.6 mg/l α-naphthalene acetic acid (NAA) for the multiplication stage. The mean number of the shoots and mean length (cm) were counted after 30 days of culture for each treatment.

1.3. Rooting stage

The multiplied clustered (5 cm long) were inoculated into ½ MS medium supplemented with 30 g/l sucrose, 2.5 g/l phytagel and 1.0, 1.5, or 2.0 mg/l of indole-3-butyric acid (IBA) or full MS medium with 1.0 mg/l kin with 0.4 or 0.6 mg/l NAA for rooting.

1.4. Acclimatization stage

Healthy rooted clusters were transferred into small pots (10 cm in diameter) containing 1:1:1 (v/v/v) sand: peat: perlite under greenhouse conditions. The pots were covered with polyethylene bags for 5 weeks to maintain humidity. Then plantlets were transferred into large pots for 3-4 months.

2. In Vitro Production of Saponins

2.1. Induction of callus

Stems cuttings were cultured on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) at different concentrations or NAA with BA at 0.5 mg/l and 2.0 mg/l 2,4-D or 2.0 mg/l NAA with 1.0 mg/l kin for the initiation of callus. The mean fresh weight, texture and color of callus were measured after 60 days of culture in complete darkness for each treatment.

2.2. Extraction and determination of saponins

The determination of total saponins was done by the standard method of Obadoni and Ochuko (2002) with minor modifications. Callus (1 g) was added to 100 ml of 20% aqueous ethanol and kept in a flask on a stirrer for half an hour, then heated for 4 h at 45°C with mixing. The mixture was filtered by using Whatman filter paper no. 1 and the residue again extracted with another 100 ml of 25% aqueous ethanol. The combined extracts were concentrated using a rotary evaporator in 40°C to get 40 ml approximately. The concentrate was transferred into a separation funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was kept and then re-extracted with 30 ml n-butanol. The n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated. After evaporation, the samples were dried in the oven at 40°C to a constant weight and the saponin content was calculated as a percentage:

Total yield of saponins (%) = (Weight of saponins / Weight of sample) x100.
3. Statistical Analysis

Each experiment was set up as a completely randomized design. Data were tested according to the analysis of variance (ANOVA) using Costat statistical package software. Means were separated according to the least significant difference (LSD) test at the 0.05 level probability

RESULTS AND DISCUSSION

In vitro propagation, rooting and callus induction of A. aphyllus on MS medium supplemented with different concentrations of growth regulators were evaluated.

1. In Vitro Propagation
1.1. Induction and multiplication of shoots

Shoot tips of seedlings were cultured on MS medium plus different concentrations of cytokinins and NAA. Data in table (1) show the effect of cytokinins and NAA on the number and length of A. aphyllus shoots. Data in table (1) show that the highest value for the mean number of axillary shoots per explant was observed in explants cultured on MS medium supplemented with 1.0 mg/l BA and 0.2 mg/l NAA and were 12 shoots. The explants cultured on MS medium plus 1.0 mg/l kin and 0.6 mg/l NAA significant had the lowest number compared with the other treatments. While, the highest mean length of axillary shoots (6.60 cm) was obtained with 1.0 mg/l BA and 0.4 mg/l NAA. It was observed that BA with high concentrations of NAA formed callus. It was noticed that BA was superior in multiplication in comparison with kin (Fig. 1A).

**Table (1). Effect of cytokinin and NAA on shoot multiplication of Asparagus aphyllus.**

| Concentrations (mg/l) | Mean number of axillary shoot / explant | Mean length of axillary shoot (cm) | Callus formation |
|-----------------------|----------------------------------------|----------------------------------|-----------------|
| 1.0 kin 0.2 NAA       | 8.25<sup>a</sup>                        | 5.56<sup>a</sup>                 | -               |
| 0.4 NAA              | 7.25<sup>a</sup>                        | 5.31<sup>a</sup>                 | -               |
| 0.6 NAA              | 3.25<sup>c</sup>                        | 4.45<sup>a</sup>                 | -               |
| 1.0 BA 0.2 NAA       | 12.00<sup>b</sup>                       | 5.31<sup>a</sup>                 | -               |
| 0.4 NAA              | 4.00<sup>bc</sup>                       | 6.60<sup>a</sup>                 | +++             |
| 0.6 NAA              | 9.00<sup>ab</sup>                       | 5.67<sup>a</sup>                 | ++              |

Data are expressed as mean columns by the LSD test and different lowercase letters indicate significant difference at a 5% level

Similar results were found with the best shooting on MS medium supplemented with kin and BA on Asparagus racemosus by Pandey et al. (2016). While, the highest shoot multiplication was achieved with MS medium enriched with BA at 0.1 mg/l plus NAA and 0.05 mg/l as concluded

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on *A. racemosus* by Patel and Patel (2015). While, Pant and Joshi (2018) noted that *A. racemosus* gave adventitious shoots on low concentration of IBA in combination with relatively higher concentrations of kin in MS medium. Among the treatments where hormones were tested singly, IAA at 0.5 mg/l and kin at 1.0 mg/l, Paudel et al. (2018) found that MS medium supplemented with 0.5 mg/l BA and MS medium plus NAA at 0.5 mg/l and kin at 1 mg/l gave the most significant shoot multiplication of *A. racemosus*. The interactions between endogenous plant growth regulators in varied plant organs at assorted developmental stages are very complex, and the manner in which a given explant will react to various concentrations of diverse exogenous growth regulators *in vitro* can vary greatly depending on the species, variety, age, and source of the explants, according to Pollard and Walker (1990).

### 1.2. Rooting stage

Data in table (2) indicate that there is no significant difference between the treatments and all the parameters recorded. The highest percentage of rooting per explant was 20% after three months of culture, when the explants cultured on ½ MS medium with IBA at 1.5 mg/l and 2.0 mg/l. The mean number of roots per explant was 1.6 roots. The mean length of the root was 4.0 cm at 2.0 mg/l IBA. It was noticed that all concentrations of IBA formed callus. These results were given by Patel and Patel (2015), who reported that *in vitro* shoots of *A. racemosus* were cultured on ½ MS basal medium and rooting was observed with IBA at 1.5 mg/l in nodal explants. While, Afroz et al. (2010) found that the shoots of *A. racemosus* were rooted best on ½ MS medium amended with 0.05 mg/l BA combined with 1.0 mg/l IBA.

| IBA concentration (mg/l) | Percentage of rooting (%) | Mean number of roots / explant | Mean length of root (cm) | Callus formation |
|--------------------------|---------------------------|-------------------------------|-------------------------|-----------------|
| 0.0                      | 0                         | 0.0                           | 0.0                     | -               |
| 1.0                      | 0                         | 0.0                           | 0.0                     | ++              |
| 1.5                      | 20                        | 1.6                           | 2.5<sup>a</sup>         | ++              |
| 2.0                      | 20                        | 1.6                           | 4.0<sup>a</sup>         | ++              |

Data are expressed as mean columns by the LSD test and different lowercase letters indicate significant difference at a 5% level

The important component was IBA, it was regulating root regeneration and is considered more potent than other auxins (Ludwig-Muller,
2000). IBA induces and enhances rooting quality (Hartmann et al., 2002). Root induction is dependent on the presence of IBA, whether endogenous or artificially applied (Haissig, 1972). In addition, cuttings treated with IBA stimulates a more uniform root to produce (Hartmann et al., 2002). However, the synthesis of rooting can significantly affected by IBA concentration (Raven et al., 1999). The effect of IBA on the rooting synthesis may be due to its effect on cell wall turgidity, which accelerates cell division (Hartmann et al., 2002).

Data of table (3) show clearly no significant differences among treatments. It was observed that the best treat was MS medium containing 1.0 mg/l kin in combination with 0.6 mg/l NAA, the mean number of root per explant was given 1.6 roots per cluster and the mean length of the root was 4.0 cm, and all treatment of kin plus NAA formed callus on explants (Fig. 1B). Regalado et al. (2015) reported that more than 70% of Asparagus developed shoots and the rooting rate on MS medium was 30–45%. The rooting rate increased to 60–85% when the unrooted shoots were subjected to an additional cycle of rooting, reaching 100% after two cycles of rooting.

**1.3. Acclimatization stage**

Healthy rooted clusters were successfully transferred to a sterilized mixture of 1:1:1 v/v/v sand: peat: perlite for three weeks in a growth chamber, then gradually acclimatized in the greenhouse with a percentage of survival of about 65% after two months (Fig. 1c).

**Table (3).** Effect of MS medium with kin and NAA on rooting of A. aphyllus, data were recorded after 6 months.

| Concentrations (mg/l) | Percentage of explant root (%) | Mean number of roots / explant | Mean length of root (cm) | Callus formation |
|-----------------------|-------------------------------|--------------------------------|--------------------------|-----------------|
| 0.0                   | 0                             | 0.0<sup>b</sup>               | 0.0<sup>b</sup>          |                 |
| 1 kin+0.4 NAA         | 25                            | 1.6<sup>a</sup>               | 3.17<sup>a</sup>         | +++             |
| 1 kin+0.6 NAA         | 25                            | 1.6<sup>a</sup>               | 4.00<sup>a</sup>         | ++              |

Data are expressed as mean columns by the LSD test and different lowercase letters indicate significant difference at a 5% level.

**Fig. (1).** *In vitro* propagation of Asparagus aphyllus; (a) multiplication of shoots (b) rooted plantlet, and (c) acclimatization of transplants in the greenhouse.

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2. In Vitro Production of Saponins

2.1. Induction of callus

It could be observed from data in table (4) that MS medium supplemented with different concentrations of NAA and/or 2,4-D and 0.5 mg/l BA initiated callus (Fig. 2a and b). Data indicate that the highest fresh weight of callus was observed on MS medium supplemented with 2.0 mg/l NAA plus 1 mg/l 2,4-D with 0.5 mg/l BA, it gave 14.03 g callus with friable texture and piege color, followed by 2.0 mg/l NAA plus 2.0 mg/l 2,4-D with 0.5 mg/l BA and 2.0 mg/l NAA only that gave 8.96 and 7.43 g, respectively, the texture of callus was compact and piege with green color. The lowest weight was observed at concentration of 2.0 mg/l 2,4-D only, it gave 0.33 g. Some treatments formed direct organogenesis, which were 1.0 mg/l 2,4-D, 1.0 mg/l 2,4-D + 0.5 mg/l BA, 1.0 mg/l NAA + 1.0 mg/l 2,4-D + 0.5 BA mg/l and 1.0 mg/l NAA + 2.0 mg/l 2,4-D + 0.5 mg/l BA). On the other hand, Pise et al. (2012) found that in A. racemosus the MS medium supplemented with 1.0 mg/l NAA plus 1.0 mg/l 2,4-D with 0.5 mg/l BA gave the highest weight of callus and percentage of callus initiation. Also, Patel and Patel (2015) found that the highest percentage of callus induction of A. racemosus was observed in MS medium supplemented with 0.1 mg/l NAA.

Table (4). The effect of MS medium supplemented with different concentrations of NAA and/or 2,4 D and/or 0.5 mg/l BA on callus induction of A. aphyllus

| Concentrations (mg/l) | Callus formation (%) | Callus fresh weight (g) | Texture | Colour |
|-----------------------|-----------------------|-------------------------|---------|--------|
| NAA 2,4-D BAP         |                       |                         |         |        |
| 0 0 0.0               | 0                     | --                      | Compact | Bage   |
| 1 0 0.0               | 100                   | 3.73<sup>b-d</sup>     | Compact | Piege+ |
| 2 0 0.0               | 100                   | 7.43<sup>a-c</sup>     | Compact | Piege+ |
| 1 0 0.5               | 30                    | 4.73<sup>b-d</sup>     | Compact | Green  |
| 2 0 0.5               | 60                    | 4.23<sup>b-d</sup>     | Compact | Piege+ |
| 0 1 0.0               | 0                     | -                       | Organogenesis | - |
| 0 2 0.0               | 30                    | 0.33<sup>cd</sup>      | Compact | Piege  |
| 0 1 0.5               | 0                     | -                       | Organogenesis | - |
| 0 2 0.5               | 20                    | 0.73<sup>cd</sup>      | Compact | Piege  |
| 1 1 0.5               | 0                     | -                       | Organogenesis | - |
| 2 1 0.5               | 100                   | 14.03                   | Friable | Piege  |
| 1 2 0.5               | 0                     | -                       | Organogenesis | - |
| 2 2 0.5               | 100                   | 8.96<sup>ab</sup>      | Compact | Piege+ |

LSD 0.5 8.43

Data are expressed as mean columns by the LSD test and different lowercase letters indicate significant difference at a 5% level.
Fig. (2). Induction of callus of *Asparagus aphyllus*; (a) MS medium plus 2,4-D (b) MS medium plus NAA.

2.2. Saponin content

Data in table (5) show the total saponin content in the mother plant, shootlet and callus of *Asparagus aphyllus*. The total saponin that had the highest value was observed in the mother plant and callus cultured on MS medium supplemented with 1 mg/l kin plus 2 mg/l 2,4-D, this medium followed by that supplemented with 1 mg/l kin plus 2 mg/l NAA was 5.85, 5.74 and 5.67 mg/g fresh weight, respectively. Pise et al. (2012) found that in *A. racemosus*, MS medium supplemented with 1.0 mg/l NAA plus 1.0 mg/l 2,4-D with 0.5 mg/l BA gave the maximum level of total saponin and biomass accumulation. Biomass and saponin accumulation patterns depended on the combinations of growth regulators and the pH of the medium.

**Table (5).** Total saponin content (TSC) in mother plant, shootlet and callus of *A. aphyllus*.

| Treatments                        | TSC mg/g |
|----------------------------------|----------|
| Mother plant                     | 5.85     |
| Shootlet                         | 5.55     |
| Callus (1 mg/l kin+ 2 mg/l 2,4-D) | 5.74     |
| Callus (1 mg/l kin+ 2 mg/l NAA)  | 5.67     |

CONCLUSION

The results of the present study highlight a fact that such shoots have excellent potential to be exploited as a starting material for the development of axenic cultures in this herb species. The use of forced *A. aphyllus* shoots resulted not only in the establishment of axenic shoot cultures but also in their further successful maintenance, rooting and establishment, thus providing new information for micropropagation of *A. aphyllus*. Long term maintenance of such axenic shoot cultures is possible. The interactive role of auxins proves to be quite promising for rooting of regenerated shoots.

The method described for the first time in the present study provides an efficient reproducible protocol for *in vitro* propagation of *A. aphyllus* as a potent natural antitumor source. This protocol can also be used for the rapid
production of *A. aphyllus* plants, thus contributing to the germplasm conservation of this endangered and valuable medicinal species in the wild. The application of the protocol will facilitate research into the improved production of antitumor components via different biotechnological strategies, such as cell suspension, tissue and organ cultures, and large-scale cultivation in bioreactors. This study recommends that the obtained model in this study can be applied for extraction of bioactive compounds from different types of plants.

These results clearly demonstrate the potential of *in vitro* cultivation of this plant as a source of pharmaceutically important metabolites such as cytotoxic triterpenoid saponins. This could be of special interest for future biotechnological approaches.

**REFERENCES**

Afroz, F., M.A.A. Jahan, S.A.K.M. Hassan and R. Khatun (2010). *In vitro* plant regeneration from axillary buds of *Asparagus racemosus* Wild, a medicinal plant. Bangladesh Journal of Scientific and Industrial Research, 45 (3): 255-260.

Castro, P., J. Gil, A. Cabrera and R. Moreno (2013). Assessment of genetic diversity and phylogenetic relationship in *Asparagus* species related to *Asparagus officinalis*. Genet. Resour. Crop Evol., 60: 1275–1288.

Desjardins, Y., H. Tiessen and P.M. Harney (1987). The effect of sucrose and ancymidol on the *in vitro* rooting of nodal sections of asparagus. HortScience, 22: 131–133.

Falavigna, A., P. Alberti, P. Casali, L. Toppino, W. Huaisong and G. Mennella (2008). Interspecific hybridization for Asparagus breeding in Italy. Acta Horticulturae, 776: 291–298.

Guclu-Ustundag˘, O. and G. Mazza (2007) Saponins: properties, applications and processing. Critical Reviews in Food Science and Nutrition, 47: 231-258.

Haissig, B.E. (1972). Meristematic activity during adventitious root primordium development: Influences of endogenous auxin and applied gibberellic acid. Plant Physiol., 49 (6): 886-892.

Hamdi, A., S. Jaramillo-carmon, R. Srairieji, R. Tej and Zaoui (2017). The phytochemical and bioactivity profiles of wild *Asparagus albus* L. plant. Food Res. Int., 99: 720-729.

Hartmann, H.T., D.E. Kester, F.T. Jr. Davies and R.L. Geneve (2002). In: “Plant Propagation: Principles and Practices”. Prentice Hall, Up. Saddle River, N.J., USA.

Hostettmann, K. and A. Marston (1995). In: “Saponins [Chemistry and Pharmacology of Natural Products]”. Cambridge University Press, UK.

Kimura, H., S. Ogawa, M. Jisaka, Y. Kimura, T. Katsube, and K. Yokota (2006). Identification of novel saponins from edible seeds

Egyptian J. Desert Res., 71, No. 2, 149-161 (2021)
of Japanese horse chestnut (Aesculus turbinata Blume) after treatment with wooden ashes and their nutraceutical activity. Journal of Pharmaceutical and Biomedical Analysis, 41: 1657-1665.

Kmail, A., B. Lyoussi, H. Zaid and B. Saad (2015). In vitro assessments of cytotoxic and cytostatic effects of Asparagus aphyllus, Crataegus aronia, and Ephedra alata in monocultures and co-cultures of HepG2 and THP-1-derived macrophages. Pharmacognosy Communications, 5 (3): 165-172.

Kmail, A., B. Lyoussi, H. Zaid, H. Imtara and B. Saad (2017). In vitro evaluation of anti-inflammatory and antioxidant effects of Asparagus aphyllus, Crataegus azarolus, and Ephedra alata in monocultures and co-cultures of HepG2 and THP-1-derived macrophages. Pharmacognosy Communications, 7 (1): 24-33.

Knaflewski, M. (1996). Genealogy of asparagus cultivars. Acta Horti., 415: 87–91.

Kubota, S., I. Konno and A. Kanno (2012). Molecular phylogeny of the genus Asparagus (Asparagaceae) explains interspecific crossability between the garden asparagus (A. officinalis) and other Asparagus species. Theor. Appl. Genet., 124: 345–354.

Kunitake, H. and M. Mii (1998). Somatic embryogenesis and its application for breeding and micropropagation in asparagus (Asparagus officinalis L.). Plant Biotechnol., 15: 51–61.

Lee, H.J, J.S. Park, Y.P. Yoon, Y.J. Shin, S.K. Lee and Y.S. Kim (2015). Dioscin and methylprotodioscin isolated from the root of Asparagus cochinchinensis suppressed the gene expression and production of airway MUC5AC mucin induced by phorbol ester and growth factor. Phytomedicine, 22 (5): 568-572.

Ludwig-Muller, J. (2000). Indole-3-butyric acid in plant growth and development, J. Plant Growth Regul., 32: 219–230.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology, 15: 473-497.

Nishimura, M., T. Ohkawara, K. Katsuyama, H. Sato and J. Nishihira (2013). Improvement of blood pressure, glucose metabolism, and lipid profile by the intake of powdered Asparagus (LüSun) bottom-stems and Cladophylls. J. Tradit. Complement Med., 3 (4): 250-5.

Obadoni, B.O and P.O. Ochuko (2002). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl. Sci., 8 (2): 203-208.

Pandey, P., P.K. Shukla, P. Misra and P.W. Ramteke (2016). In-vitro clonal propagation of Asparagus racemosus by nodal explants. Annals of Pharmacy and Pharmaceutical Sciences, 7 (1): 14-19.

Egyptian J. Desert Res., 71, No. 2, 149-161 (2021)
Pant, K.K. and S.D. Joshi (2018). Comparative study of in vitro root and shoot proliferation from the node explants of Asparagus racemosus Willd. J. Inst. Agric. Anim. Sci., 35: 121-125.

Patel, L.S. and R.S. Patel (2015). Rapid in vitro micropropagation of Asparagus racemosus Willd. from nodal explants. International Journal of Current Microbiology and Applied Sciences, 4 (5): 607-617.

Paudel, N., M.R. Aryal and R.H. Puri (2018). Effect of hormone for in vitro propagation of Asparagus racemosus Wild. Current Life Sciences, 4 (4): 53-61.

Pise, M., J. Rudra, S. Bundale, D. Begde, N. Nashikkar and A. Upadhyay (2012). Asparagus racemosus cell cultures: a source for enhanced production of shatavarins and sarsapogenin. In Vitro Cell. Dev. Biol. - Plant, 48: 85–91.

Pollard, J. and J. Walker (1990). In: “Plant Cell and Tissue Culture”. Humana Press; https://doi.org/10.1385/0896031616.

Raju, J. and R. Mehta (2009). Cancer chemopreventive and therapeutic effects of diosgenin, a food saponin. Nutrition and Cancer, 61: 27-35.

Raven, P., R. Evert and S. Eichhorn (1999). In: “Biology of Plants”. Freeman W.H. and Co., N.Y., USA.

Regalado, J.J., E. Carmona-Martín’, P. Castro, R. Moreno, J. Gil and C.L. Encina (2015). Micropropagation of wild species of the genus Asparagus L. and their interspecific hybrids with cultivated A. officinalis L., and verification of genetic stability using EST-SSRs. Plant Cell Tiss. Organ Cult., 121 (2): 501-510.

Saad, B. and O. Said (2011). In: “Greco-Arab and Islamic Herbal Medicine: Traditional System, Ethics, Safety, Efficacy and Regulatory Issues”. Wiley-Blackwell John Wiley and Sons Inc.

Sharma, P. and D. Goel (2018). Antimicrobial and minimum inhibitory concentration activity of leaf extract of Asparagus aphyllus. International Journal of Life Sciences Research, 6 (2): 144-148.

Tardio, J., M. Pardo-de-Santayana and R. Morales (2006). Ethnobotanical review of wild edible plants in Spain. Botanical Journal of the Linnean Society, 152 (1): 27–71.

Teka, A., J. Rondevaldova, Z. Asfaw, S. Demissew, P. Van Damme and L. Kokoska (2015). In vitro antimicrobial activity of plants used in traditional medicine in Guberge and Silti Zones, south central Ethiopia. BMC Complement Altern. Med. 18: 286.

Turland, N.J.; L.Chilton and J.R. Press (1993). Flora of the Cretan area: annotated checklist & atlas. The Natural History Museum, HMSO, London.

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USDA, ARS, National Genetic Resources Program (2013). Germplasm Resources Information Network - (GRIN) [Online Database]. Available at: www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl.

USDA, ARS, National Genetic Resources Program (2014). Germplasm Resources Information Network – (GRIN) [Online Database]. Beltsville, Maryland Available at: https://npgsweb.arsgrin.gov/gringlobal/search.aspx.

Weng, L.L., J.F. Xiang, J.B. Lin, S.H. Yi, L.T. Yang and Y.S. Li (2014). Asparagus polysaccharide and gum with hepatic artery embolization induces tumor growth and inhibits angiogenesis in an orthotopic hepatocellular carcinoma model. Asian Pac. J. Cancer Prev., 15 (24):10949-55.
IN VITRO PROPAGATION AND SECONDARY

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The effect of the peel of the Egyptian aloe vera

Proliferating and secondary shoots

Sayed Salam, M.

A study on the cultivation of Aloe barbadensis Miller in vitro.

Aloe barbadensis is a perennial plant which is used in the treatment of many diseases. It is known for its anti-inflammatory and anti-microbial properties. In this study, the in vitro propagation of Aloe barbadensis was investigated. The results showed that the plants propagated in vitro exhibited a higher percentage of shoot regeneration and a higher number of shoots per explant compared to the plants propagated in vivo. The optimal nutrient medium for in vitro propagation was found to be Murashige and Skoog medium supplemented with 0.4 mg/l of IAA and 2.0 mg/l of BAP. The plants propagated in vitro showed good growth and development, and were free from contamination. The study also indicated that the plants propagated in vitro had higher levels of total phenols and total flavonoids compared to the plants propagated in vivo. These results suggest that in vitro propagation is a promising method for the cultivation of Aloe barbadensis, which can be used for medicinal and cosmetic purposes.