Impact of indole-3-butyric acid (IBA) on the root induction of *Arbutus pavarii* Pamp (Lybian Strawberry tree) in *in vitro* culture

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ABSTRACT

The main objective of this study was to clarify the best concentration of the indole-3-butyric acid (IBA) in order to induce the formation of strong roots of *Arbutus pavarii* Pamp, an endangered plant in the El-Jabel El-Akhdar region in Libya. A study was carried out to find a protocol for its *in vitro* propagation. The present paper aimed to investigate the effects of different concentrations of IBA plant growth regulator on the rooting. Three weeks old seedlings obtained with *in vitro* germination were transferred to Murashige and Skoog (M&S) roots induction medium supplemented with different concentrations of IBA (0, 1, 1.5 and 2 mg L^{-1}). The highest response was obtained with the M&S medium half strength supplemented with IBA 1 mg L^{-1} concentration. In fact, all the measured growth indicators (rooting percentage, root length and dry weight) significantly enhanced when using this concentration.

Key words: micropropagation; germination; Indole-3-butyric acid; roots dry weight; sterilization.

INTRODUCTION

Lybian strawberry tree (*Arbutus pavarii* Pamp) spreads naturally in El-Jabel El-Akhdar region in the north-eastern of Libya (about between 20° and 23° East, 32° and 33° North) [1]. Its presence is concentrated in the northern and central parts of the region, in the valleys, slopes, mountain slopes and lands with shallow or rocky calcareous soils. Arbutus is a genus with 12 species with different local names in its spread areas [2]. Endemic species from around 4% from the total species of Libyan flora. *A. pavarii* Pamp. (Ericaceae) locally known as ‘‘Shmeri’’ is one of the endemic species in El-Jabel El-Akhdar. In this region this shrub grows mixed with many trees and shrubs such as *Pistacia lentiscus*, *Ceratonia siliqua* L, *Juniperus phoenicea* L, *Quercus coccifera*, *Rhus tripartita* (Ucria) Grande, *Phillyea media* L., *Ziziphus lotus*(L) Desf [3; 4], main species in the Maquis formation. Arbutus are shrubs with dense branches, growing as a small tree or a large evergreen shrub with a smooth reddish brown bark and a multi-patterned leaves with serrate or entire edge. Flowering occurs in late spring and fruits mature in late summer. The fruit is globose, a many-seeded berry, yellow to orange in colour, turning red when fully mature [5]. This plant suffers for increasing degradation due to negative human activities in many of the El-Jabel El-Akhdar areas, and also agricultural expansion, urbanization, overgrazing, charcoal making. Also due to the climatic factors, with high rainfall variation and temperature fluctuations accompanied by dry southern winds, which are the cause of physiological diseases due to drought, *A. pavarii* has never been widely cultivated by afforestation of other species.

The need for the continuous improvement of traits in crop species remains an ongoing effort for crop scientists and farmers. Different plant species have their own set of phenotypes that need to be improved in order to both add nutritional values and enhance economic gains for humankind. The increase in food demand worldwide, associated with unequal distribution, and the disequilibrium in the distribution of wealth have caused an increasingly important pressure on food producers who, in parallel, have increased their requirements for new technologies that allow greater yields and better quality of the products that they offer [5]. At the same time, there has been an increasing consumer led demand for lower environmental impact and greater sustainability in the food production chain. Strawberry tree is propagated by runners; therefore, the health of daughter plants depends on their mother plants. Moreover, *A. pavarii* is affected by numerous viruses that greatly reduce the yield [6].

Plant tissue culture (PTC) is a set of techniques for the aseptic culture of cells, tissues, organs and their components like genes and enzymes under defined physical and chemical conditions *in vitro* and controlled environment. PTC technology also explores conditions that promote cell division and genetic re-programming in *in vitro* conditions and it is considered an important tool in both basic and applied studies, as well as in commercial application [7]. PTC techniques have become of major industrial importance in the area of plant propagation, disease elimination, plant improvement, and production of secondary metabolites.

Growth regulators constitute one of the key and more expensive elements used for *in vitro* propagation. For this reason, they must be optimized or substituted for more efficient and cheaper bio regulators [8]. Indole-3-butyric acid (IBA) is a plant hormone in the auxin family and is an ingredient in many commercial horticultural plant rooting products.. In plant tissue culture IBA is used to initiate *in vitro* root formation in a procedure called micropropagation. In previous studies, the effect of three different auxins, IBA, Indole-3-acetic acid (IAA) and 1-Naphthaleneacetic acid (NAA) were examined to determine the relative effect of each auxin on root formation. According to the result for the species, IBA was shown to produce a higher yield of roots compared to the other auxins [9]. The effect of IBA is in concurrence with other studies where IBA is the most commonly used auxin for root formation [10].

Therefore, the aim of this study was find a protocol for the propagation of *A. pavarii* Pamp and verify seed germination using different concentrations of IBA to obtain seedlings to enable their re-planting in their natural environment.

MATERIAL AND METHODS

Fresh seeds of *A. pavarii* were collected in December in the outskirts of the city of Al Bayda (Libya). Taxonomists at the Department of Botany Herbarium, Faculty of Science, and at Omar Al-Mukhtar University identified the samples.

Preparation of culture media

Half of Murashige and Skoog 1962 (M&S) [11] basal salt nutrient medium with vitamins, glycine and supplemented with 30 g L^{-1} sucrose, 0.1 g L^{-1} myo-inositol (Table 1) was used for *in vitro* seedlings

germination. Full M&S nutrient salt medium was used for rooting media. After supplementation of full M&S media with different concentrations (0, 1, 1.5 and 2 mg L^{-1}) of indole-3-butyric acid (IBA) plant growth regulators for rooting, pH of all cultures was adjusted to 5.8 with 1N KOH or 1N HCl, then with 7 g L^{-1} agar prior to autoclaving at 121ºC and 1.2 kg cm^{-2} for 20 minutes. Culture medium was dispensed as 50 ml per jar (350 ml) for *in vitro* seedlings germination, and rooting. All types of culture media were kept for three days under completely darkness for test of contamination.

Tissue culture chemicals M&S medium and growth regulator indole-3-butyric acid were purchased from Sigma-Aldrich company.

Table 1: Composition of the M&S culture media used for *in vitro* seedlings germination.

Ingredients	Amount (mg/L)
Macronutrients	
NH_4NO_3	1650.00
KNO_3	1900.00
$CaCl_2.2H_2O$	440.00
$MgSO_4.\ 7H_2O$	370.00
KH_2PO_4	170.00
Micronutrients	
KI	0.83
H_3BO_3	6.20
$MnSO_4.4H_2O$	22.30
$ZnSO_4.7H_2O$	8.60
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2$	0.025
Iron stock	
$FeSO_4.7H_2O$	27.80
$Na_2.EDTA.2H_2O$	37.30
Vitamins	
Myo-inositol	100.00
Nicotinic acid	1.00
Pyridoxine HCl	1.00
Thiamine HCl	10.00
Glycine	2.00
Sucrose (g)	30.00
Agar (g)	8.00

Sterilization and germination

Seeds of the *A. pavarii* were washed with running tap water for 30 min. Then they were taken to the laminar air flow cabinet in which they were surface sterilized by dipping in 70% (v/v) ethanol for 2 min, rinsed with sterilized distilled water, then disinfected with 20% (v/v) of commercial Clorox (5.25% Cl_2) solution for 15 min (Rabha [12]) and rinsed three times with sterilized distilled water. In complete aseptic conditions, equal number from sterilized seeds represents were inoculated in culture medium aseptically as six seeds per each. Cultures were maintained under normal condition (16/8

hours light/dark) at 1500 lux using cool white fluorescent lamps and incubated in a controlled growth chamber at 26±1°C.

This experiment was carried out to study the effect of indole-3-butyric acid capacity to enhance rooting on seedling derived *in vitro*. *In vitro* germination three weeks old seedlings, reached about 5 - 6 cm in height were subjected as plant materials, which resulted from M&S free growth regulators were transferred to M&S roots induction medium (R) supplemented with different concentrations of IBA (0, 1, 1.5 and 2 mg L^{-1}) as follow:

R_0= control (M&S free growth regulators)
R_1 = M&S + 1 mg L^{-1} IBA
R_2= M&S + 1.5 mg L^{-1} IBA
R_3= M&S + 2 mg L^{-1} IBA

Each treatment consisted of 6 replicates (jar) and each replicate contained three seedlings. Cultures were incubated in a controlled growth chamber in complete darkness for 3 days at 26±1°C then transferred to normal condition. After 4 weeks from incubation, the number, length (cm) and dry weight (g/jar) for root were measured and recorded.

Statistical Analysis

The data were subjected to two-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at the 5% level using Microsoft Excel software.

RESULT AND DISCUSSION

Data reported in Table 2 clearly show that M&S medium supplemented with 1 mg L^{-1} IBA (R_1) gave the maximum value for rooting percentage (70%). In addition, there were non-significant differences among R_0, R_2, and R_3 recording the minimum value for rooting percentage (55%). About number of roots and concerning to seedling, clear differences among the treatments were noted. It was found that M&S medium fortified with 1.5 mg L^{-1} IBA (R_2) allowed to obtain in the highest number of roots (5) compared to 3 of the other treatments and 2 of the control. On the other hand, the data reported in the table 2 show that the longest root (7.4 cm) was obtain with R_1 treatment followed by R_2 treatment (5.4 cm of length). Furthermore, there were non-significant differences among R_0, and R_3 treatments with the lower length. The highest dry weight increment was obtained with R_1 and R_2 treatments (0.0449 and 0.0303, g/jar respectively) compared to others.

Many studies were dedicated to the cultivation of strawberry trees and the exact propagation has been reported about 30 years ago [13]. In recent years, many research groups have been involved in establishing reliable regeneration protocols for *A. pavarii*, because it would be a primary step to facilitate gene introduction and improvement of the crop. Our aim in the study was to investigate the effect of IBA hormone on root induction in vitro. Through our study of the effect of IBA on the root induction we found that it significantly enhanced the number and length and dry weight in strawberry seedling. In vitro plant regeneration of Strawberry from different parts, has been reported by seeds, leaves, petioles [14], stem [15], stipules [16], and roots [17]. The results in Table 2 showed that the

growth and formation of roots were very low in the treatment of control (MS free growth regulators) compared to all other treatments. These findings are somewhat similar to those previously reported by Ashraf [18]. Regarding the effect of IBA on the root response, the results indicate that the IBA with 1 and 1.5 mg L^{-1} showed the highest roots response compared to all other treatments. These results do not exactly match with those ones of Emarach [19]. Our results agreed with Gautam [20] indicating that the highest root induction frequency obtained was 95.23% on M&S medium with IBA at 1.0 mg L^{-1}. Mereti [21] found that the highest percentages of rooting were achieved in M&S medium contained 10 µM IBA (92%) and 10 µM IAA (82%). Additionally, by increasing the concentration of IBA the height of root was decreased. Haddadi et al [22] reported that the presence of NAA strength the rooting percentage and root number but the medium without any Auxin had the lower number of root. However, the highest root development was observed in the control treatment. Here it was concluded that the root phenotype (number and length) was diverse as influenced by different Auxin treatments. All different concentrations of IBA (0, 1, 1.5 and 2 mg L^{-1}) induced the root induction in strawberry and significantly differences were observed among treatments in number and length of regenerated seedling.

Table 2: Effect of culture media composition with different concentrations of IBA on rooting percentage number of roots, root length and root dry weight of A. *pavarii* after 4 weeks of culturing and incubation at normal condition.

Parameters / Treatments	rooting percentage (%)	No. of roots	Root length (cm)	Dry weight (g/jar)
R_0= control (M&S free growth regulators)	55^{b}	2^{c}	3.2^{c}	0.008^{d}
R_1 = M&S + 1 mg L^{-1} IBA	70^{a}	3^{b}	7.4^{a}	0.0449^{a}
R_2= M&S + 1.5 mg L^{-1} IBA	55^{b}	5^{a}	5.4^{b}	0.0303^{b}
R_3= M&S + 2 mg L^{-1} IBA	55^{b}	3^{b}	3.2^{c}	0.0183^{c}

Means having the same letters in a column were not significantly different at $p<0.05$

CONCLUSIONS

In vitro regeneration of *Arbutus pavarii* Pamp (Lybian Strawberry tree) is a requirement for genetic transformation, which involves induction and development to the whole plant. According to several studies, showing positive effects of IBA on root induction, also our study has demonstrated to promote the root growing and formation and the better results were obtained with the concentration of 1 mg L^{-1} in the M&S media.

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