

1 **Impact of indole-3-butyric acid (IBA) on the root induction of Strawberry tree**
2 **(*Arbutus pavarii*) culture in vitro**
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5 **ABSTRACT**

6 The main objective of this study was to clarify the best concentration of the indole-3-butyric acid (IBA)
7 in order to induce the formation of strong roots of the *Arbutus pavarii* plant, it is an endangered
8 plant in El-Jabel El-Akhdar- Libya, this study was created to find a protocol for the propagation of
9 *Arbutus pavarii* in vitro. We investigated the effect of different concentrations of IBA plant growth
10 regulator on the rooting. *In vitro* germination three weeks old seedlings were transferred to MS roots
11 induction medium which supplemented with different concentrations of IBA (0, 1, 1.5 and 2 mg L⁻¹).
12 the highest response was obtained when transfer the seedlings to the MS medium half strength and
13 supplemented with IBA (0.1 mg L⁻¹) . All the growth indicators [rooting percentage (77%) number,
14 length (7.4 cm) and dry weight (0.0449 g/jar)] that were measured significantly enhanced when using
15 concentration 0.1 mg L⁻¹ of IBA.

16 **Key words:** Sterilization and germination; *Arbutus pavarii*; root length ; roots dry weight and IBA.

17 **INTRODUCTION**

18 *Arbutus pavarii* spreads naturally in El-Jabel El-Akhdar region in the north-
19 eastern part of Libya, between latitudes 25° 20' east and latitude 33° 30' north Its
20 presence is concentrated in the northern and central regions, in the valleys, slopes,
21 mountain slopes and lands with shallow or rocky calcareous soils, *Arbutus* has about
22 20 species of trees and has different local names in its spread areas [1]. Endemic
23 species from around 4% from the total species of Libyan flora, *A. pavarii* Pamp.
24 (Ericaceae) locally known as “Shmeri” is one of the endemic species in El-Jabel El-
25 Akhdar it is shrubs grows in the El-Jabel El-Akhdar region, mixed with many trees
26 and shrubs such as *Pistacia lentiscus*, *Ceratonia siliqua* L, *Juniperus phoenicea* L,
27 *Quercus coccifera*, *Rhus tripartita* (Ucria)Grande, *Phillyea media* L., *Ziziphus*
28 *lotus*(L)Desf,[2 and 3]. These trees and shrubs are the main species in the Maquis
29 formation in -Jabel El-Akhdar region, in the form of shrubs dense of branches which
30 is a small tree or a large evergreen shrub with a smooth reddish brown bark and a
31 multi-patterned leaves with Serrate or Entire edge. Flowering occurs in late spring and
32 fruits mature in late summer. The fruit is globose, a many-seeded berry, yellow to
33 orange in colour, turning red when fully ripehence [4]. This plant suffers from
34 increasing degradation due to negative human activities in many of the El-Jabel El-
35 Akhdar areas and agricultural expansion, urbanization, overgrazing, charcoal making
36 In addition to the climatic factors of the fluctuation of rainfall and temperature

37 fluctuations which are accompanied by dry southern winds caused by physiological
38 diseases due to drought, and whereas *A. pavarii* has never been widely cultivated by
39 afforestation of other species.

40 The need for the continuous improvement of traits in crop species remains an
41 ongoing effort for crop scientists and farmers. Different plant species have their own
42 set of phenotypes that need to be improved in order to both add nutritional values and
43 enhance economic gains for humankind. The increase in food demand worldwide,
44 associated with unequal distribution, and the disequilibrium in the distribution of
45 wealth has caused an increasingly important pressure on food producers who, in
46 parallel, have increased their requirements for new technologies that allow greater
47 yields and better quality of the products that they offer [4]. While at the same time,
48 there has been an increasing consumer led demand for lower environmental damage
49 and greater sustainability in the food production chain. Strawberry is propagated by
50 runners; therefore the health of daughter plant depends on their mother plants.
51 Strawberry is affected by numerous viruses that greatly reduce the yield [5]. The
52 viruses caused the smaller leaves, decreasing the photosynthesis rate and eventually
53 reducing fresh and dry weight. In complex infections (more than one virus), the rate
54 of photosynthesis per unit area also was profoundly reduced [6].

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

63 Growth regulators constitute one of the key and more expensive elements used
64 for *in vitro* propagation. That is why; they must be optimized or substituted for more
65 efficient and cheaper bio regulators [8]. Indole-3-butyric acid (IBA) is a plant
66 hormone in the auxin family and is an ingredient in many commercial
67 horticultural plant rooting products.. In plant tissue culture IBA is used to initiate root
68 formation *in vitro* in a procedure called micropropagation. Micropropagation of
69 plants. the effect of three different auxins, IBA, IAA and NAA were examined to

70 determine the relative effect of each auxin on root formation. According to the result
71 for the species, IBA was shown to produce a higher yield of roots compared to the
72 other auxins.[9] The effect of IBA is in concurrence with other studies where IBA is
73 the most commonly used auxin for root formation.[10]

74 Therefore, the aim of this study was to try to **Propagation** the plant by seed
75 germination using different concentrations of IBA to obtain seedlings to enable them
76 to re-plant them in their natural environment.

77 **MATERIAL AND METHODS**

78 **MATERIAL**

79 Fresh **Seed** *A. pavarii* were collected in december month from the outskirts of the city
80 of Al Bayda – Libya. Taxonomist at the Department of Botany Herbarium, Faculty of
81 Science, and Omar Al-Mukhtar University further identified the samples.

82 **METHODS**

83 **Preparation of culture media**

84 Half basal of Murashige and Skoog 1962 (MS) [11] salts nutrient medium with
85 vitamins, glycine and supplemented with 30 g L⁻¹ sucrose, 0.1 g L⁻¹ myo- inositol
86 (Table 1) was used for *in vitro* seedlings germination. While full MS nutrient salt
87 medium was used for rooting media . After supplementation of full MS media with
88 different concentrations (0,1,1.5 and 2 mg L⁻¹) of **indole-3-butyric acid** (IBA) plant
89 growth regulators [for rooting], all cultures pH were adjusted to 5.8 with 1N KOH or
90 1N HCl, then with 7 g L⁻¹ agar prior to autoclaving at 121°C and 1.2 kg cm⁻² for 20
91 minutes. Culture medium was dispensed as 50 ml per jar (350 ml) for *in vitro*
92 seedlings germination, and rooting. All types of culture media were kept for three
93 days under completely darkness for test of contamination.

94 Tissue culture chemicals **MS** medium and growth regulator **indole-3-butyric acid**
95 were purchased from Sigma company.

96 **Sterilization and germination**

97 Seeds of the *A. pavarii* were washed with running tap water for 30 min. Then they
98 were taken to the laminar air flow cabinet in which they surface sterilized by dipping
99 in 70% (v/v) ethanol for 2 min and rinsed with sterilized distilled water. Further,
100 disinfected with 20 % (v/v) of commercial Clorox (5.25% Cl₂) solution for 15min

101 (Rabha [12])and rinsed three times with sterilized distilled water. In complete aseptic
102 conditions equal number from sterilized seeds represents were inoculated in culture
103 medium aseptically as six seeds per each. Cultures were maintained under normal
104 condition (16/8 hours light/dark) at 1500 lux using cool white fluorescent lamps and
105 incubated in a controlled growth chamber at $26\pm 1^{\circ}\text{C}$.

106 performed for studying effect of indole-3-butyric acid capacity to enhance rooting on
107 seedling derived *in vitro*. *In vitro* germination three weeks old seedlings (reached
108 about 5 - 6 cm in height were subjected as a plant materials) which resulted from MS
109 free growth regulators were transferred to MS roots induction medium (R) which
110 supplemented with different concentrations of IBA(0, 1, 1.5 and 2 mg L^{-1}) as follow:

R₀= control (MS free growth regulators)
R₁ = MS + 1 mg L^{-1} IBA
R₂= MS + 1.5 mg L^{-1} IBA
R₃= MS + 2 mg L^{-1} IBA

111 Each treatment was consisted of 6 replicates (jar) and each replicate contained three
112 seedling. Cultures were incubated in a controlled growth chamber in complete
113 darkness for 3 days at $26\pm 1^{\circ}\text{C}$ then transferred to normal condition. After 4 weeks
114 from incubation, the number, length (cm) and dry weight (g/jar) for root were
115 recorded.

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127 **Table 1: Culture media composition (Murashige and Skoog 1962).**

Ingredients	Amount (mg/L)
Macronutrients	
NH₄NO₃	1650.00
KNO₃	1900.00
CaCl₂.2H₂O	440.00
MgSO₄. 7H₂O	370.00
KH₂PO₄	170.00
Micronutrients	
KI	0.83
H₃BO₃	6.20
MnSO₄.4H₂O	22.30
ZnSO₄.7H₂O	8.60
Na₂MoO₄.2H₂O	0.25
CuSO₄.5H₂O	0.025
CoCl₂	0.025
Iron stock	
FeSO₄.7H₂O	27.80
Na₂.EDTA.2H₂O	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

128 **RESULT AND DISCUSSIONS**

129 This experiment was conducted to provide the *in vitro* growing seedlings needed for
 130 present study as explants source. Indole-3-butyric acid in different concentrations was
 131 used to study its effect on the roots. The effect of MS medium supplemented with
 132 different concentrations of IBA (1, 1.5 and 2 mg L⁻¹) on rooting frequency (%),
 133 number of roots, root length (cm) and roots dry weight (g/jar) of Arbutus seedlings
 134 was investigated. Data tabulated in Table 2 clearly showed that MS medium
 135 supplemented with 1 mg L⁻¹ IBA (R₁) gave the maximum value for rooting percentage
 136 (70%). In addition, there were non-significant differences among R₀, R₂, and R₃ and
 137 they recorded the minimum value for rooting percentage (55%).

138 About number of roots and concerning to Arbutus seedlings , There were clear
 139 differences between the treatments used in the study. It was found that MS medium
 140 fortified with 1.5 mg L⁻¹ IBA(R₂) in the best number of roots as the number of five is
 141 the highest compared to the control. On the other hand, the data are also shown in the
 142 table 2 the longest root (7.4 cm) recorded with R₁ followed by R₂ medium, which
 143 recorded 5.4 cm length. Furthermore, there were non-significant differences among

144 R₀, and R₃ media they were shorter in length. The highest dry (0.09 g/jar) weight
145 increment was scored on R₁ (0.0449) and R₂ media compared to other used media.

146 There have been many studies devoted to the cultivation of strawberry trees and the
147 exact propagation has been reported about 30 years ago [13]. In recent years, many
148 research groups have been involved in establishing reliable regeneration protocols for
149 agronomical important *A. pavarii*, because it would be a primary step to facilitate
150 gene introduction and improvement of the crop. Our aim in the study was to
151 investigate the effect of IBA hormone on root induction in vitro. Through our study of
152 the effect of IBA on the root induction we found that it significantly enhanced the
153 number and length and dry weight in strawberry seedling. In vitro plant regeneration
154 of Strawberry from different parts, has been reported by seeds, leaves, petioles [14],
155 stem [15], stipules [16], and roots [17]. The results in Table 2 showed that the growth
156 and formation of roots were very low in the treatment of control (MS free growth
157 regulators) compared to all other treatments. These findings are somewhat similar to
158 those previously reported by **Ashraf [18]** .Regarding the effect of IBA on the root
159 response, the results indicated that the IBA with (1 and 1.5 mg/L) showed the highest
160 roots response compared to all other treatments. These results do not exactly match
161 what he concluded [19]. Our results agreed with **Gautam [20]** indicated that the
162 highest root induction frequency obtained was 95.23% on MS medium with IBA at
163 1.0 mg/l . **Mereti [21]** found that the highest percentages of rooting were achieved in
164 MS medium contained 10 μM IBA (92%) and 10 μM IAA (82%). Additionally, by
165 increasing the concentration of IBA the height of root was decreased. **Haddadi et al**
166 **[22]** reported that the presence of NAA strength the rooting percentage and root
167 number but the medium without any Auxin had the lower number of root. However,
168 the highest root development was observed in the control treatment. Here it was
169 concluded that the root phenotype (number and length) was diverse as influenced by
170 different Auxin treatments. All different concentrations of IBA (0, 1, 1.5 and 2 mg L⁻¹)
171 induced the root induction in strawberry and significantly differences were
172 observed among treatments in number and length of regenerated seedling.

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176 **CONCLUSION**

177 In vitro regeneration of **Strawberry tree** (*Arbutus pavarii*) is
 178 a requirement for genetic transformation, which involves induction and development
 179 to the whole plant. Several studies have shown that IBA the effects of root induction
 180 in plants. This research demonstrated that IBA (0.1mg L⁻¹) was an effective
 181 concentration among different other concentrations on root induction.

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

Parameters Treatments	rooting percentage (%)	No. of roots	Root length (cm)	Dry weight (g/jar)
R ₀ = control (MS free growth regulators)	55 ^b	2 ^c	3.2 ^c	0.008 ^d
R ₁ = MS + 1 mg L ⁻¹ IBA	70 ^a	3 ^b	7.4 ^a	0.0449 ^a
R ₂ = MS + 1.5 mg L ⁻¹ IBA	55 ^b	5 ^a	5.4 ^b	0.0303 ^b
R ₃ = MS + 2 mg L ⁻¹ IBA	55 ^b	3 ^b	3.2 ^c	0.0183 ^c

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

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