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# Recent Unique Research for Enhancing Micropropagation Protocol of *Balanites aegyptiaca* (L.) *Via* Mammalian Sex Hormones

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## Abstract

In the present study, trials were done to investigate the influence of supplementing Murashige and Skoog's culture medium with mammalian sex hormones (progesterone, estrogen, testosterone, human chorionic gonadotropin (HCG) and anabolic steroids on enhancing regeneration of *Balanites aegyptiaca* L., *in vitro*. For this purpose, nodal segment explants which were excised from *in vitro* grown plantlets (5-6 weeks old) were cultured on MS medium supplemented with 0.5 mg/L BAP in addition to progesterone, estrogen, testosterone, HCG and anabolic steroids (1.0, 3.0 and 5.0 mg/L each). For rooting, the regenerated shoots were cultured on half strength, hormone-free MS medium (as a control) or supplemented with 1.0 mg/L anabolic steroids alone or combined with β-cyclodextrin (10 and 20mg/L) and/or IBA (1.0 and 2.0 mg/L). Results of the present study have revealed that impregnating the culture medium with mammalian sex hormones (MSHs) had, in the great majority, significant positive effects on number of shoots per explant, shoot length, number of leaves per single shoot, and leaf area of *Balanites aegyptiaca* after 5 weeks of incubation. For example, impregnating the culture medium with 5.0 mg/L testosterone had resulted in the maximum shoot length, number of leaves and leaf area (15.33cm, 29 and 2.78cm$^2$respectively). The highest significant shoots number per explant reached up 4.0using 1.0 mg/L testosterone. On other hand, the least significant number of shoots/nodal segment, shoot length, number of leaves and mean leaf area were(1.0, 1.93cm, 3.67, and 0.1cm$^2$respectively)in response to treatment with 5.0 mg/L HCG. The results obtained showed also that half strength MS medium supplemented with 1.0 mg/L anabolic steroid hormone with or without 1.0 mg/L IBA had the highest significant rooting percentage (75%) and number of rootlets per shoot (5.67). The highest significant root length (5.50cm) was obtained when regenerants were cultured on half strength MS medium supplemented with 1.0 mg/L anabolic steroids hormones combined with 20.0 mg/l *β*- cyclodextrin. The obtained regenerants were easily acclimatized within 4 weeks and transferred to greenhouse with a survival percentage of 95%. The results obtained in this study may indicate the promising future role that mammalian sex hormones (MSHs) can play in enhancement of shoot and root morphogenesis *in vitro*

**Keywords:** *Balanites aegyptiaca* L., Mammalian sex hormones (MSHs), and Regeneration, Anabolic steroids, Micropropagation.

## 1. Introduction

*Balanites aegyptiaca* L. (Del., Balanitaceae), commonly known as Heglig or Desert Date, is a spiny evergreen xerophytic tree found in tropical and northern Africa, Syria, west Asia, Sudan, Egypt, neighboring parts of east and west Africa particularly Senegal, Nigeria, Arabia and Burma. It is distributed throughout drier parts of India like Rajasthan **(Bhandari, 1995).**The oil content of *Balanites aegyptiaca* has been tested for biodiesel production as it was found meeting with the International Biodiesel Standards (**Chapagain *et al*., 2009).**Medicinally, it has been stated that the active constituents of this plant has anti-leishmanial activity **(El-Tahir *et al*., 1998)**, anti-molluscidal, anti-fertility, anti-bacterial, antifungal, and antitumor properties **(Corbiere *et al*., 2003).** Fruits are used as gastric pain reliever and as an oral antidiabetic, seed extract is used in treatment of bilharziasis **(Mohamed *et al*., 2002),** roots are used in the treatment of abdominal pains and asthma as a purgative and antiasthmatic **(Neuwinger, 1996)**. Due to its significant multipurpose properties, the tree has been over exploited and included in the list of endangered plant species (**El-Nour *et al*., 1991).**

*Balanites* is propagated through seeds, root suckers and vegetative cuttings but these methods are not efficient in producing sufficient number of elite planting stocks due to poor seed germination frequency, age dependent root suckers production and very low survival rate of vegetative cuttings during transport and plantation and so alternative propagation methods would be beneficial in large scale multiplication, improvement and conservation of its elite clones **(Anis *et al*., 2010).** The use of biotechnology has opened up new possibilities for rapid mass multiplication of existing stocks of germplasm, as well as conservation of medicinally important plants/plant parts **(Bajaj 1986; Haissig *et al*., 1987; Gupta and Agrawal, 1992; Anis *et al*., 2005; Husain *et al*., 2007, 2008).** Some success has been achieved by **(Ndoye *et al*., 2003; Anis *et al*., 2010; Siddique and Anis,2009)** on efficient *in vitro* regeneration through high frequency axillary shoot proliferation from nodal explants *of Balanites aegyptiaca*.

MSHs which are lipophilic and low molecular weight compounds are a group of steroids. These hormones play a key role in controlling the process of development and reproduction, and are also involved in the control of mineral and protein metabolism in mammals **(Kliewer *et al*., 1998; Janeczko and Skoczowski, 2005).** MSH previously known to occur in only animals are now isolated from various plant parts like roots, leaves and flowers **(Dogra and Kaur, 1994**; **Erdal and Dumlupinar, 2010 a,b)**.

To the best of our knowledge, the effect of exogenous application of MSHs on micropropagation of *B. aegyptiacain vitro* has not been studied. The main objective of the present investigation is to study the effect of exogenous application of MSHs (Progesterone, Estrogen, Testosterone, HCG and Anabolic Steroids Hormones) on micropropagation of *Balanites aegyptiaca in vitro.*

## 2. Materials and Methods

This study was conducted in Tissue Culture Res. Lab., Bot. & Microbiol. Dept., Fac. Sci., Al-Azhar University, Cairo, Egypt in collaboration with Tissue Culture & Germplasm Conservation Laboratory, Horiculture Research Institute, Agricultural Research Center, Giza, Egypt, during the years from 2015 to 2017.

### Plant materials

Nodal segment explants of *Balanites aegyptiaca* were excised from sterile *in vitro* plantlets (5-6 weeks old) growing in Tissue Culture & Germplasm Conservation Laboratory, Horiculture Research Institute, Agricultural Research Center, Giza, Egypt.

### Culture medium and incubation conditions

The basal salts mixture of MS medium **(Murashige and Skoog, 1962)** full and half strength supplemented with 25 g/L sucrose and pH adjusted to 5.7±1 was solidified with 7 g/L agar (Sigma). All the culture treatments were incubated in growth room under controlled conditions, where temperature was maintained at 25 ± 1°C day/night schedules and illumination intensity of 1500 lux using white cool fluorescent lamp (120 cm long 40 watts), the light/dark photoperiod was adjusted to 16/8 by electronic timer.

### Microcutting and growth of shootlets under the effect of MSHs

This experiment aimed to study the effect of MSHs on the number of shootlets, their lengths, the number of leaves per shoot and mean leaf area. Nodal explants were transferred to semisolid MS medium supplemented with combination of 0.5 mg/L BAP plus different concentrations of MSHs (progesterone, estrogen, testosterone, HCG and anabolic steroidal hormones at concentrations of 1.0, 3.0 and 5.0 mg/L for each one). The obtained regenerants from each one of the above tested MSHs treatments were aseptically removed, divided to (1-2 cm length) and re-cultured into fresh culture media of the corresponding components. These procedures were successively repeated for three subculture cycles. Each treatment consisted of ten replicates.

### Rooting stage of *Balanites aegyptiaca* L

In this stage, the obtained shoots were subjected to the following treatments:

Half strength MS (free hormonal)

1- Half strength MS + 1.0 mg/L anabolic steroid
2- Half strength MS + 1.0 mg/L anabolic steroid + 10 mg/L Beta Cyclodextrin
3- Half strength MS + 1.0 mg/L anabolic steroid + 20 mg/L Beta Cyclodextrin
4- Half strength MS + 1.0 mg/L anabolic steroid + 1.0 mg/L IBA
5- Half strength MS + 1.0 mg/L anabolic steroid + 2.0 mg/L IBA
6- Full strength MS 1.0 mg/L anabolic steroid
7- Full strength MS 2.0 mg/L anabolic steroid

All cultures were examined after 6 weeks of incubation at 25±1ºC under 16 hrs of light and 8 hrs of darkness provided by cool florescent light intensity of 1500 lux to record rooting percentage, average numbers of shoot and Shoot length (cm) of the initiated roots per shoot. Each treatment consisted of ten replicates.

### Acclimatization Stage:

Rooted plantlets were transferred to greenhouse in (peat moss and sand 1:1 v/v) and covered with polyethylene bags for 2 weeks, then, one pore was made in every bag for two another week. After that the bags were removed.

## 3. Results and Discussion

### Effect of MSHs on Shoot number/explant, shoot length, number of leaves/shoot and mean leaf area of *B. aegyptiaca*

As regards to the effect of MSHs on number of shoots resulted from the first microcutting, the data presented in table (1) show that the highest significant shoots numbers (4.0) was obtained when explant treated with 1.0 mg/L testosterone then (3.33) in response to 5.0 mg/L HCG and (1.67) as a result of the treatment with 3.0 mg/L progesterone. On the second round of microcutting as presented in table (2) show that the highest significant shoots number (3.0) was obtained when explants were treated with 1.0 mg/L estrogen and also 3 shoots per explant were obtained in response to treatment with 5.0 mg/L progesterone while the lowest significant shoots number (1.0) was detected when explants were cultured on media supplemented with 1.0, 3.0 and 5.0 mg/L HCG. During the second subculture (third round of microcutting as presented in table (3) show that the highest significant shoots number (3.33) was obtained when explant was obtained on medium supplemented with 3.0 mg/L estrogen then (3.0 shoots per explant) were obtained due to treatment with 1.0 mg/L estosterone. The lowest significant shoots number (1.0) was detected in culture media which contained 3.0 and 5.0 mg/L HCG.

With respect to shoot length, it seems that some of the MSHs hormones could significantly increase the length of regenerated shootlets but this rate of increase declined on the second and third rounds of microcutting. The data presented in table (1) showed that the highest significant shoot length reached up (15.13cm) when explants were cultured on medium supplemented with 5.0 mg/L testosterone followed by (12.43cm) in response to the treatment with 1.0 mg/L testosterone while the lowest significant shoot length (5.33cm) was detected when the shoots emerged from explants cultured on medium supplemented with 5.0 mg/L Estrogen. During the first subculture, data presented in table (2) show that the highest significant shoot length (7.27cm) was obtained when explants were cultured on medium supplemented with 1.0 mg/L estrogen then (6.5 cm) with 1.0 mg/L testosterone while the lowest significant shoot length (1.93 cm) was detected when explants were treated with 5.0 mg/L HCG. Results of the second round of microcutting, as presented in table (3), show that the highest significant shoot length (6.93cm) was obtained

when explant in response to the treatment with 1.0 mg/L estrogen then (6.067cm) in response to the treatment with 1.0 mg/L testosterone while the lowest significant shoot length (2.17cm) resulted from the treatment with 3.0 mg/L HCG.

As regards to the effect of MSHs on leaf number, the data presented in table (1) show that the highest significant number of leaves (29.0) was obtained 5.0 mg/L testosterone followed by (25.67) with 1.0 mg/L testosterone and (10.67) in response to the treatment with 3.0 mg/L progesterone. Regenerants of the second round of microcutting (table 2) have revealed that the highest significant leaves number (23.33) had resulted from the treatment with 1.0 mg/L testosterone then (21.33 leaves per shoot) was obtained in response to treatment with 3.0 mg/L testosterone. The lowest significant leaves number (3.67) was detected when explants were cultured on medium supplemented with 5.0 mg/L HCG. Results of the third round of microcutting (data presented in table 3) show that the highest significant leaf numbers (22.67, 21.67 and 4.0) were obtained in response to the treatment with 1.0 mg/L testosterone,3.0 mg/L testosterone and 5.0 mg/L progesterone respectively.

The leaf area of the primarily obtained regenerants, data presented in table (1) showed that the leaf areas reached up (2.78cm$^2$, 1.37cm$^2$ and 0.21cm$^2$) in response to the treatment with obtained 5.0 mg/L testosterone, 3.0 mg/L HCG and 3.0 mg/L Estrogen. Regenerants of the second round of microcutting (table 2) resulted in leaf areas of (2.69cm$^2$, 1.5cm$^2$ and 0.11cm$^2$) as a result of the treatment with 5.0 mg/L testosterone 3.0 mg/L anabolic steroids and 5.0 mg/L estrogen respectively. Plantlets resulted from the third round of microcutting (table 3) had leaves with mean leaf areas of 2.17cm$^2$, 1.4cm$^2$ and 0.1cm$^2$ due to the treatment with 5.0 mg/L testosterone,3.0 mg/L anabolic steroids and 3.0 or 5.0 mg/L HCG respectively.

**Table (1):** Effect of addition of MSHs to 0.5 mg/L BAP on shoot morphogenesis from *B. aegyptiaca* nodal segments

| Hormone | Conc. mg/L | Number of shootlets | Shoot Length | Number of leaves | Leaf area |
|---|---|---|---|---|---|
| | | **Means ± SE** | **Means ± SE** | **Means ± SE** | **Means ± SE** |
| Control | 0 | 2±0.58$^{cd}$ | 9.58±1.06$^{b-f}$ | 14.67±2.40$^{def}$ | 1.05±0.23$^{bcd}$ |
| Progesterone | 1 | 2.33±0.33$^{bcd}$ | 11.73±1.75$^{bc}$ | 18.67±1.45$^{bcd}$ | 0.53±0.066$^{cde}$ |
| | 3 | 1.67±0.33$^{d}$ | 6.7±1.37$^{fgh}$ | 10.67±3.76$^{f}$ | 0.68±0.067$^{cde}$ |
| | 5 | 3±0.58$^{abc}$ | 11.33±0.84$^{bc}$ | 19.67±3.18$^{bcd}$ | 0.75±0.27$^{b-e}$ |
| Estrogen | 1 | 2.67±0.33$^{bcd}$ | 7.17±0.73$^{e-h}$ | 18±1.53cd$^{e}$ | 0.48±0.29$^{de}$ |
| | 3 | 2.67±0.33$^{bcd}$ | 8.13±0.46$^{e-h}$ | 10.67±0.88$^{f}$ | 1.11±0.15$^{bc}$ |
| | 5 | 2±0.58$^{cd}$ | 5.33±0.49$^{h}$ | 11±1.528$^{ef}$ | 0.21±0.055$^{e}$ |
| Testosterone | 1 | 4±0.58$^{a}$ | 12.43±0.61$^{ab}$ | 25.67±2.96$^{ab}$ | 0.68±0.28$^{cde}$ |
| | 3 | 2.33±0.33$^{bcd}$ | 11.73±0.46$^{bc}$ | 21.67±2.40$^{bcd}$ | 0.65±0.12$^{cde}$ |
| | 5 | 3±0.58$^{abc}$ | 15.13±0.67$^{a}$ | 29±3.51$^{a}$ | 2.78±0.5$^{a}$ |
| Anabolic steroids | 1 | 2.667±0.33$^{bcd}$ | 9.97±0.291$^{b-e}$ | 21±0.57$^{bcd}$ | 0.57±0.11$^{cde}$ |
| | 3 | 2±0$^{cd}$ | 8.83±1.186$^{c-f}$ | 23±1.53$^{abc}$ | 0.24±0.15$^{e}$ |
| | 5 | 2.667±0.33$^{bcd}$ | 7.5±0.87$^{e-h}$ | 16±3.46$^{c-f}$ | 0.29±0.037$^{e}$ |
| HCG | 1 | 2±0$^{cd}$ | 11.3±2.35$^{bcd}$ | 11±3.055$^{ef}$ | 0.74±0.17$^{cde}$ |
| | 3 | 1.667±0.33$^{d}$ | 8.33±0.63$^{e-h}$ | 14.667±2.33$^{def}$ | 1.08±0.12$^{bcd}$ |
| | 5 | 3.33±0.33$^{ab}$ | 5.43±0.47$^{gh}$ | 18.333±1.86$^{cd}$ | 1.37±0.2$^{b}$ |
| **F ratio** | | 2.4 | 6.95 | 5.05 | 8.65 |
| **P value** | | * | *** | *** | *** |

Each value is a mean of three determinations± standard error. Columns with similar letters are not significantly different according to Fisher LSD. NS=non-significant, * = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0. 001, (Note: each Colum of means compared according to different treatments showed in first Colum).

**Table (2):** Effect of MSHs on shoot multiplications of *Balanites aegyptiaca*, explants cultured on MS medium supplemented with 0.5 mg/L BAP . (second round microcutting).

| Hormone | Conc. mg/L | Number of shootlets | Shoot length | Number of leaves | Leaf area |
|---|---|---|---|---|---|
| | | **Means ± SE** | **Means ± SE** | **Means ± SE** | **Means ± SE** |
| Control | 0 | 1.33±0.33$^{bc}$ | 4.7±0.9$^{b-e}$ | 10.67±1.20$^{d-h}$ | 0.66±0.12$^{cde}$ |
| Progesterone | 1 | 2.33±0.88$^{abc}$ | 4.53±0.49$^{b-e}$ | 13.33±0.88$^{c-f}$ | 0.53±0.035$^{c-g}$ |
| | 3 | 1±0$^{c}$ | 4.1±0.31$^{cde}$ | 7.67±0.88$^{f-i}$ | 0.52±0.11$^{c-g}$ |
| | 5 | 3±0.578$^{a}$ | 5.47±1.58$^{a-d}$ | 18.67±4.1$^{abc}$ | 0.7±0.23$^{cd}$ |
| Estrogen | 1 | 3±1.53$^{a}$ | 7.27±0.98$^{a}$ | 12.67±2.91$^{c-g}$ | 0.4±0.15$^{d-g}$ |
| | 3 | 1±0$^{c}$ | 5.67±0.32$^{abc}$ | 15±1.53$^{cde}$ | 0.91±0.061$^{c}$ |
| | 5 | 1±0$^{c}$ | 4.07±0.3$^{cde}$ | 8±1.16$^{f-i}$ | 0.11±0.0077$^{g}$ |
| Testosterone | 1 | 2±0.58$^{abc}$ | 6.5±0.87$^{ab}$ | 23.33±2.40$^{a}$ | 0.54±0.17$^{c-g}$ |
| | 3 | 1.67±0.33$^{abc}$ | 6.27±1.18$^{ab}$ | 21.33±4.06$^{ab}$ | 0.57±0.15$^{c-f}$ |
| | 5 | 2.67±0.33$^{ab}$ | 5.13±0.12$^{b-e}$ | 15.33±2.03$^{bcd}$ | 2.69±0.41$^{a}$ |
| Anabolic steroids | 1 | 1.67±0.33$^{abc}$ | 5.6±0.65$^{a-d}$ | 14.33±1.20$^{cde}$ | 0.52±0.12$^{c-g}$ |
| | 3 | 2.67±0.33$^{ab}$ | 6.07±0.58$^{abc}$ | 15.33±1.76$^{bcd}$ | 1.5±0.10$^{b}$ |
| | 5 | 1.33±0.33$^{bc}$ | 3.6±0.27d$^{ef}$ | 9±1.53$^{e-i}$ | 0.29±0.037$^{d-g}$ |
| HCG | 1 | 1±0$^{c}$ | 3.333±0.12$^{ef}$ | 5.67±0.67$^{hi}$ | 0.23±0.033$^{efg}$ |
| | 3 | 1±0$^{c}$ | 3.2±0.058$^{ef}$ | 7±2.08$^{ghi}$ | 0.17±0.067$^{fg}$ |
| | 5 | 1±0$^{c}$ | 1.93±0.27$^{f}$ | 3.67±0.88$^{i}$ | 0.17±0.033$^{fg}$ |
| **F ratio** | | 2.15 | 4.07 | 7.15 | 18.09 |
| **P value** | | * | *** | *** | *** |

Each value is a mean of three determinations± **SE standard error.** Columns with similar letters are not significantly different according to Fisher LSD. NS=non-significant, * = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0. 001, (Note: each Colum of means compared according to different treatments showed in first Colum).

**Table (3):** Effect of MSHs on shoot multiplications of *Balanites aegyptiaca*, explants cultured on MS medium supplemented with 0.5 mg/L BAP .(third round microcutting).

| Hormone | Conc. mg/L | Number of shootlets | Shoot length | Number of leaves | Leaf area |
|---|---|---|---|---|---|
| | | Means ± SE | **Means ± SE** | **Means ± SE** | **Means ± SE** |
| Control | 0 | 1.67±0.33$^{bcd}$ | 4.13±0.5$^{def}$ | 11.67±0.33$^{d}$ | 0.27±0.03$^{e-h}$ |
| Progesterone | 1 | 2±0.58$^{a-d}$ | 5.8±0.99$^{abc}$ | 18.33±3.28$^{bc}$ | 0.52±0.06$^{d}$ |
| | 3 | 2±0.58$^{a-d}$ | 2.83±0.067$^{fg}$ | 6.33±0.88$^{f}$ | 0.27±0.03$^{e-h}$ |
| | 5 | 2±1$^{a-d}$ | 2.93±0.088$^{fg}$ | 4±1.528$^{f}$ | 0.2±0.1$^{fgh}$ |
| Estrogen | 1 | 2.67±0.33$^{abc}$ | 6.93±0.088$^{a}$ | 6±1.53$^{f}$ | 0.37±0.03$^{def}$ |
| | 3 | 3.33±0.33$^{a}$ | 6±0.49$^{abc}$ | 16±1$^{c}$ | 0.73±0.03$^{c}$ |
| | 5 | 1.33±0.33$^{cd}$ | 2.8±0.23$^{fg}$ | 7.67±0.88$^{ef}$ | 0.3±0$^{efg}$ |
| Testosterone | 1 | 3±0.58$^{ab}$ | 6.07±0.29$^{ab}$ | 22.67±1.20$^{a}$ | 0.53±0.03$^{d}$ |
| | 3 | 2.67±0.67$^{abc}$ | 5.2±0.35$^{bcd}$ | 21.67±0.88$^{ab}$ | 0.4±0.058$^{de}$ |
| | 5 | 3±0.58$^{ab}$ | 4.63±0.77$^{cde}$ | 15.67±0.33$^{c}$ | 2.17±0.13$^{a}$ |
| Anabolic steroids | 1 | 1.33±0.33$^{cd}$ | 3.8±0.9$^{def}$ | 7±1.16$^{ef}$ | 0.53±0.09$^{d}$ |
| | 3 | 1.67±0.33$^{bcd}$ | 3.43±0.24$^{efg}$ | 10.33±1.20$^{de}$ | 1.4±0.1$^{b}$ |
| | 5 | 1±0$^{d}$ | 2.9±0.65$^{fg}$ | 5.67±1.20$^{f}$ | 0.13±0.03$^{gh}$ |
| HCG | 1 | 1.333±0.33$^{cd}$ | 3.7±0.31$^{ef}$ | 7.67±2.33$^{ef}$ | 0.2±0$^{fgh}$ |
| | 3 | 1±0$^{d}$ | 2.17±0.067$^{g}$ | 5±0.58$^{f}$ | 0.1±0$^{h}$ |
| | 5 | 1±0$^{d}$ | 2.97±0.44$^{fg}$ | 5±0.58$^{f}$ | 0.1±±0$^{h}$ |
| **F ratio** | | 2.8 | 8.72 | 20.24 | 80.28 |
| **P value** | | ** | *** | *** | *** |

Each value is a mean of three determinations± standard error**.** Columns with similar letters are not significantly different according to Fisher LSD. NS=non-significant, * = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0. 001, (Note: each Colum of means compared according to different treatments showed in first Colum).

1

2

3

4

5

6

**Figure (1):** Microshoot of *Balanites aegyptiaca* explants culturing on MS medium supplemented with 0.5 mg/L BAP as affected by MSHs at 1500 Lux and 25±1 ºC. (1 control = no MSHs; 2= culture produced from Progesterone hormone; 3= culture produced from Estrogen hormone; 4= culture produced from testosterone hormone; 5= culture produced from Anabolic steroids hormone; 6 = culture produced from HCG hormone)

With respect to the use of MSHs in *in vitro* culture of plants, it can be concluded that the interest in such mammalian hormones has started after the stimulation of the growth of an isolated pea embryo *in vitro* by estrone treatment **(Bonner & Axtman, 1937**; **Kogl & Haagen-Smit, 1936).** Androsterone and androstenedione (1 µM) promoted the germination and growth of immature embryos of winter wheat (*Triticum aestivum* L.) as reported by **(Janeczko, 2000; Janeczko *et al.*, 2002)**. Direct organogenesis (caulogenesis, rhizogenesis) and callus proliferation were observed during *in vitro* culture of hypocotyls and cotyledons of *Arabidospis thaliana* 3-day-old seedlings cultured on medium supplemented with estrone for 28 days (**Zabicki *et al.*, 2014)**. More recently, *in vivo* study by **Upadhyay and Maier (2016)** found out that 17β-estradiol enhanced root growth and shoot biomass on *Arabidospis thaliana*. Some supporting evidences of the results obtained in this study may be understood on the light of the poor previous observations made by others like**Martınez-Honduvilla *et al.*(1976)**who declared that these compounds augmented seed germination and seedling growth in *Pinus pinea* seeds. Similarly, MSH increased growth and sugar content in pea and pine seedlings **(Kopcewicz; 1969, Kopcewicz; 1970 and Kopcewicz and Rogozinska; 1972),** and the growth of winter wheat seedlings **(Janeczko; 2000).**

## Rooting Stage:

It can be noticed from data in table (4), that the examined MSHs exhibited a significant effect on the rhizogensis behaviors of plantlet *in vitro* (i. e. rooting percentage, number of rootlets per regenerant and root length of *Balanites aegyptiaca*). Data presented in table (4) showed that the highest significant rooting percentage (75%) and rootlets number (5.67) were obtained when plantlets were cultured on half strength MS medium supplemented with to up to1.0 mg/L anabolic steroids hormones with or without 1.0 mg/L IBA.

**Table (4):** Effect of medium strength, MSHs, beta cyclodextrin and IBA on rooting of heglig shoots *in vitro*.

| Treatment | Rooting % | Number of rootlets | Root length (cm) |
|---|---|---|---|
| Free half strength MS media | 25 % | $2\pm0.58^{c}$ | $1.73\pm0.64^{c}$ |
| 1/2 MS + 1.0 mg/L AS | 75 % | $5.67\pm1.45^{a}$ | $3.27\pm0.55^{bc}$ |
| 1/2 MS + 1.0 mg/L anabolic steroid + 10 mg/L Beta Cyclodextrin | 75 % | $3.67\pm1.20^{a-c}$ | $1.67\pm0.59^{c}$ |
| 1/2 MS + 1.0 mg/L anabolic steroid + 20 mg/LBeta Cyclodextrin | 50 % | $5\pm0^{ab}$ | $5.5\pm1.16^{a}$ |
| 1/2 MS + 1.0 mg/L anabolic steroid + 1.0 mg/l IBA | 75 % | $5.67\pm0.67^{a}$ | $4\pm0.87^{ab}$ |
| 1/2 MS + 1.0 mg/L anabolic steroid + 2.0 mg/L IBA | 25 % | $2\pm0.58^{c}$ | $2.07\pm0.55^{bc}$ |
| Full strength MS + 1.0 mg/L anabolic steroid | 75 % | $2\pm0.58^{c}$ | $2.13\pm0.73^{bc}$ |
| Full strength MS + 2.0 mg/L anabolic steroid | 75 % | $2.67\pm0.33^{bc}$ | $1.33\pm0.44^{c}$ |
| F Ratio | | 4.26 | 3.96 |
| P Value | | ** | *** |

Each value is a mean of three determinations ± standard error Columns with similar letters are not significantly different according to Fisher LSD. NS=non-significant, * = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0. 001, (Note: each Colum of means compared according to different treatments showed in first Colum).

The highest significant root length (5.5 cm)was obtained when plantlets were transferred to half strength MS medium supplemented with to 1.0 mg/L anabolic steroids hormones in addition to 20 mg/l *Beta* Cyclodextrin (**figure 2)** while the lowest significant rooting percentage (25%)and rootlet numbers (2.0) were obtained when plantlets were allowed to grow on half strength, hormone-free MS medium. The lowest significant root length (1.33 cm) was detected when plantlets were cultured on full strength MS medium supplemented with up to 2.0 mg/L anabolic steroids hormones.

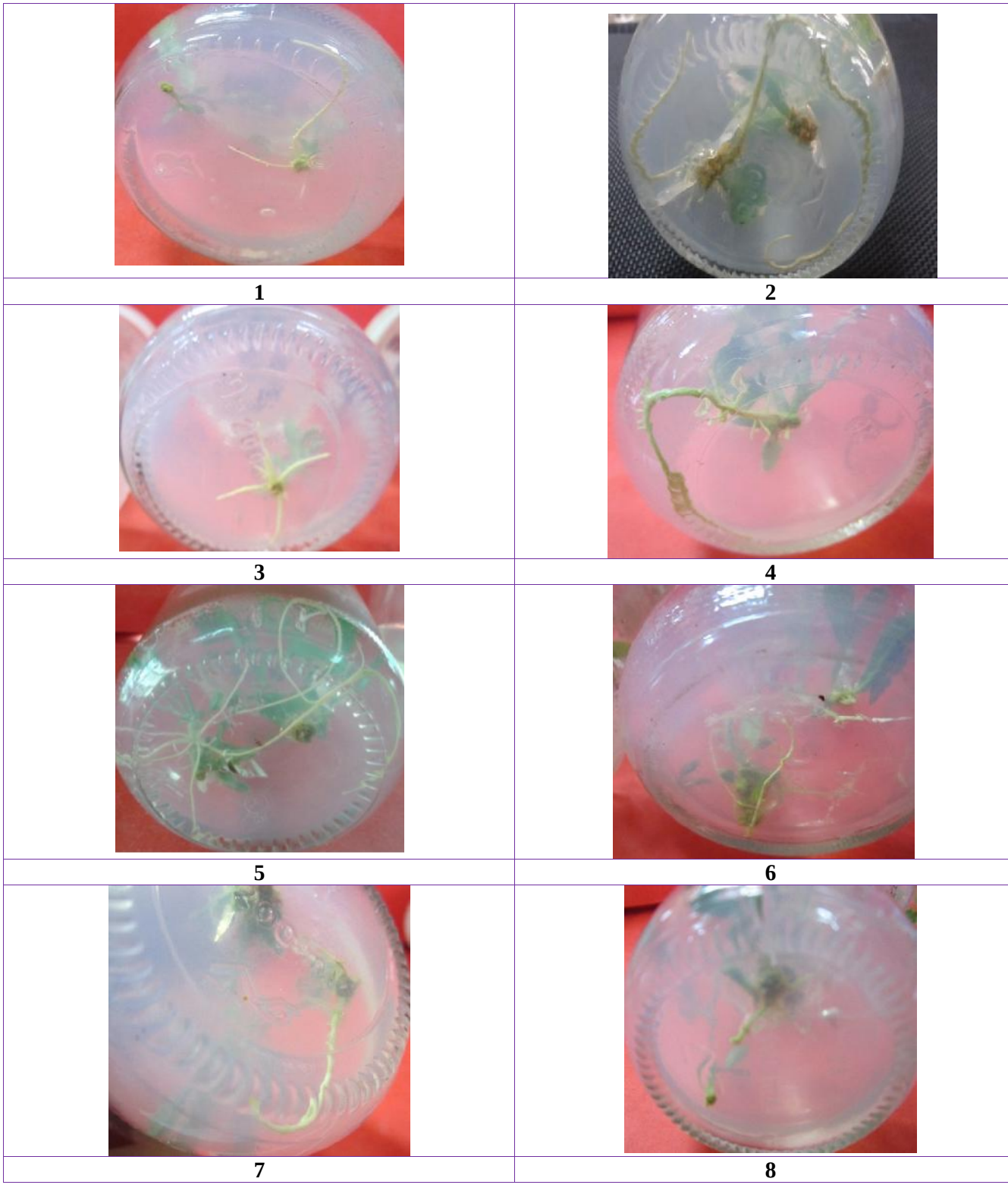


**Figure (2):***in vitro* rooting stage of *Balanites aegyptiaca*, (1) explants culturing on ½ MS free hormonal (2) explants culturing on ½ MS + 1.0mg/L anabolic steroid hormones (3) explants culturing on ½ MS1.0 mg/L anabolic steroid + 10 mg/L Beta Cyclodextrin (4) explants culturing on ½ MS1.0 mg/L anabolic steroid + 20 mg/L Beta Cyclodextrin (5) explants culturing on ½ MS + 1.0 mg/L anabolic steroid + 1.0 mg/l IBA (6) explants culturing on ½ MS+ 1.0 mg/L anabolic steroid + 2.0 mg/l IBA (7) explants culturing on MS +1.0 mg/L anabolic steroid (8) explants culturing on MS + 2.0 mg/L anabolic steroid at 1500 Lux and 25±1 ºC.

With respect to rooting, **Abd El-Naby, (2006)** reported that the highest rooting percentage (38.89%) of *B.aegyptiaca* was obtained using half strength MS medium. The results obtained in this study may be considered as an additional evidence of the possibility to induce rooting *in vitro* without the use of plant growth regulators but MSHs, a results or observation that might reflect the importance of this study knowing that rooting in woody trees *in vitro* is considered as a general and huge problem in many cases (**Hartmann *et al*., 1990** and **DE klerk, 2002).**It might be worthy to mention that the percentage of rooting using anabolic steroids exceeded the rooting percentage using growth regulators free or enriched culture medium. The results obtained in this study may encourage us to reevaluate earlier studies like **Love and Love (1945) who** reported that meristem activity in the roots of *Rubrum* (Weigel) Garcke and *Rumex acetosa* L. was accelerated after treatment with testosterone and **Helmkamp and Bonner (1952)** who rooted *in vivo Melandrium dioecium, Rumex acetosa and Anthoxanthum aristatumplants in vivo*using estrone and testosterone.

### Acclimatization Stage:

An average of 95% of the acclimatized plantlets survived after four weeks of transferring into the peat moss: sand mixture (1:1 v/v) **figure (3).**

| 1 | 2 | 3 |
|---|---|---|

**Figure (3): *ex vitro* acclimatization of** *Balanites aegyptiaca*, **on peatmoss: sand mixture (1:1 v/v).**
1 (rooted plantlet), 2 (pots covered with plastic bags for acclimatization), 3 (acclimatized plantlet)

## 4. Conclusion

In conclusion, the present study has revealed enhancing micropropagation protocol of *balanites aegyptiaca* (l.) through effect of mammalian sex hormones (progesterone, estrogen, testosterone, human chorionic gonadotropin (HCG) and anabolic steroids) on nodal segment explants. The results obtained in this study may indicate the promising future role that mammalian sex hormones (MSHs) can play in enhancement of shoot and root morphogenesis *in vitro*.

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