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## Research Article

# CULTURE OF LEAVES AND IMMATURE FRUITS OF *MAERUA CRASSIFOLIA* IN VITRO

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**ABSTRACT**

Leaves and immature fruits of *M. crassifolia* were cultured on Murashige and Skoog medium (MS) supplemented with different concentrations of auxins and cytokinins. When the leaves were inoculated on MS +Indolebutyric acid (IBA) 1 mg/l, a small green branches were directly formed from basal part as well as radicles covered with root hairs after two weeks, the branches were increased in growth and reached 0.3, 1and 1.3 cm in length after 14, 30,45 days respectively. Those branches were sub cultured on MS+ Benzyl adenine (BA) (2mg|l), no any further responses were observed, but their color was changed to brown after two weeks. In the case of using immature fruits as explants on MS+2,4-Dichlorophenoxy acetic acid 2,4-D (2mg/l)+kinetin (0.5mg/l), friable white calluses were obtained after 7 days, their growth were increased after 12 weeks. When the callus subculture on MS+2,4-D (2mg/l)+kin (1.5mg/l) and on MS+ Naphthalene acetic acid NAA(1.5 mg/l) + kin (3.5mg/l), no any responses were noticed after 4 weeks.



## INTRODUCTION

The *Maerua crassifolia* plants belongs to the family of Capparaceae as perment green trees, their length reached ten meters high, the leaves were oval shaped and flowers were white (e flora 2010, Jstor 2010). Those trees considered multipurpose uses as food for Cows, Goats and wood for house furniture (Diatta *et al.,* 2007). Leaves were edible for human and animals food and many medical beneficial as for fever, stomach troubles and skin diseases (Bur kill *et al.,* 1985). The *Maerua crassifolia* contain high amounts of fats and minerals such as Calcium, Magnesium, Potassium and Sodium (Rhim 2003). The Leaves contain low amount of fibers (Calabro *et al.,* 2007) and rich in protein content especially as phenyl aniline, tyrosine (Cook *et al.,* 2008; Freiberger 1998). Due to heavy grazing, the numbers of *Maerua crassifolia* were decreased (Le Houerou 1980). The *Maerua crassifolia* distributed through the world such as in Singal, Mauritania, Maroco, Algeria, Libya, Egypt, Sudan, Somalia, Ethiopia, Kenya, Uganda, Tanzania and Palestine (Arbonnier 2004), Justor 2010, U SDA 2010, Jafri 1978). The aim of present work is to culture Leaves and immature fruits of *Maerua crassifolia* in vitro in order to micropropagate of those plants.

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## MATERIALS AND METHODS

### The explants

The leaves and immature fruits were used as explants, they collected from Teminhent village about 30 km from Sebha University south of Libya.

### Sterilization

The Leaves and immature fruits were washed under running tap water to remove the dusts, later they rinsed in 70% ethyl alcohol for 1 min and then surface sterilized with 0.5 % Sodium hypochlorite for 5 min and then rinsed in sterile double distilled water for 5 min of each respectively. All the work were done under laminar flow hood.

### Media preparation

The Murashige and Skoog (1962) with 30 g sucrose and %8 agar, PH was adjusted between 5.7-5.8, different concentrations of different of plants hormones used : Naphthalene acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D), Indole 3-Acetic Acid (IAA), Indole 3-butyric Acid (IBA), Kinetin (Kin), benzyl adnine (BA), Zeatin (Z) and Activated charcoal (AC). The media were sterilized by autoclave 121 $^{0}$C, pressure 1 bar for 20 min.

**Culture conditions**

The cultures were kept for 7 days in dark at $25^0$C±2 and later transferred to 16/8 (light-dark) photoperiod with light supply by white fluorescent tubes at 3500 lux. Samples were photographed by digital camera and fixed on binocular microscope.

## RESULTS

**The effect of 2,4-D, IAA, NAA, IBA, ZEA, BA, KIN and AC on Leaves**

Different parts of leaves were inoculated on MS medium (Fig.1)


**Fig. 1. Different parts of leave used as explants**

- MS free hormones: no any observation were recorded after 2 weeks.
- MS+ 2, 4-D (4.5mg/l) +BA(0.4mg/l) no any sign of response were recorded and changed in color only after 20 days.
- MS+ IAA (2mg/l), no any response were observed and the color of leaves were changed into dark after 30 days.
- MS +IBA(1 mg/l), a green shoot with roots covered with root hairs were formed directly and showed rapid growth then reached 1.3 cm in long after 45 days (Fig.2, Table 1).


**Fig.2. green shoot with roots covered by root hairs**

In case of sub culturing of those explants on MS + (2mg/l), the plantlets were changed in brown color and stopped growing after 4 days (Fig.3, Table 1)

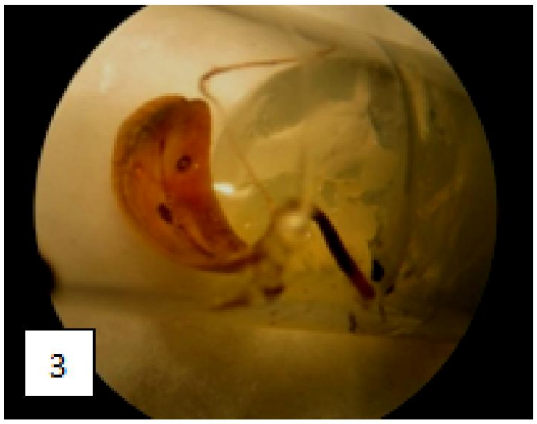


**Fig.3. plantlets were change in brown color**

- MS + NAA (1.5 mg/l) + Kin (3.5mg/l, no any responses were noticed and the color changed after 22 days.
- MS +Kin (0.5mg/l), no any observation only changed in color after 30 days.
- MS + Zeatin (0.5mg/l)+Activated charcoal (0.5mg/l), only changing in color after 30 days.
- MS + BA (2mg/l) small spots were observed after 20 days and also change in color of explants were observed (Fig. 4, Table 1).

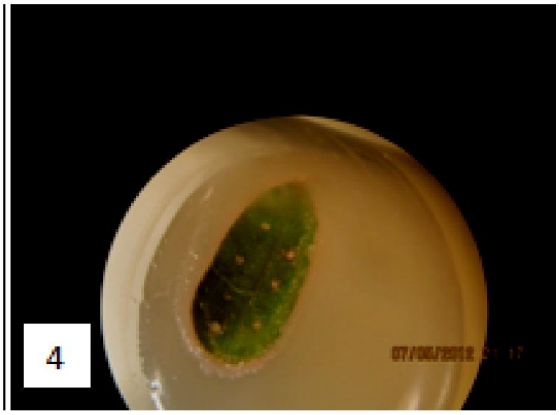


**Fig.4. a small pink spots on leaves**

**Table 1. Effect concentrations of growth regulators on leaves(mg/l)**

| Explants | 2.4- | D | IAA | NAA | IBA | ZEA | BA | KIN | AC | Results |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | - |
| 2 | 4.5 | | | | | | 0.4 | | | |
| 3 | | | 2 | | | | | | | - |
| 4 | | | | | 1 | | | | | + |
| 5 | | | | 1 | | | | 0.5 | | - |
| 6 | | | | | | | | 0.5 | | |
| 7 | | | | | | 0.5 | | | | - |
| 8 | | | | | | 0.5 | | | 0.5 | - |

+ green shoot
- no response

**Table 2. Effect of concentrations growth regulators on Immature fruits (mg/l)**

| Explants | 2.4-D | NAA | KIN | Results |
|---|---|---|---|---|
| 1 | | | | - |
| 2 | 2 | | 0,5 | + |
| 3 | | 1.5 | 3.5 | - |

+ callus
- no callus

**The effect of NAA, 2,4-D, KIN on Immature fruits**

- MS free hormones
- No any responses were observed after 2 weeks
- MS + NAA (1.5mg/l) +Kin (3.5mg/l) no any results were noticed after two weeks.
- MS+2,4-D (2mg/l)+ Kin (0.5mg/l) small amounts of calluses were induced after 7 days (Fig. 5,Table 2) the calluses were increased in growth after 12days. Those calluses were fragmented and subculture on MS+2,4-D (2mg/l) + kin (1.5 mg/l), they increased in growth after 1 week. But on MS+ NAA (1.5mg) + kin (3.5mg/l), no any responses were noticed after 4 weeks (Fig. 6, Table 2).

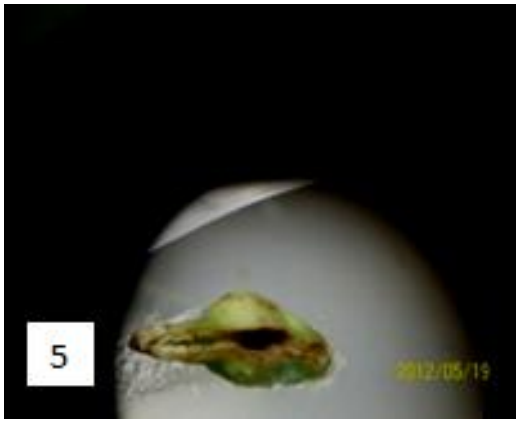


**Fig.5. small amounts of calluses from immature fruits**

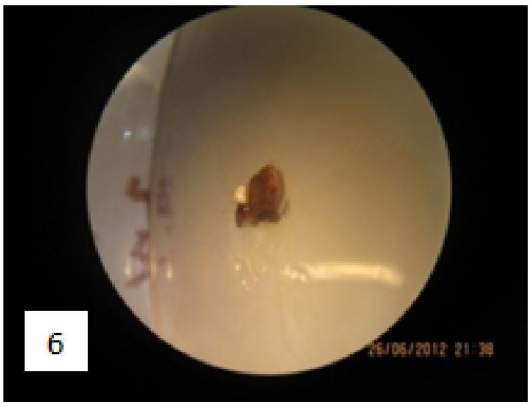


**Fig.6. calluses sub cultured on MS+2.4-D (2mlg/l) after 1 week from immature fruits**

## DISCUSSION

According to literature data that responses of desert plants to in vitro technique by using different parts of explants as in *Ceratonia sliqua* El-Shafey *et al.* (1998), *Albizzia lebeck* (Gharyal *et al.,* 1983), *Calligonum comosum* (Mohamed and Alkayali 2005). In present study high concentration of IBA showed high concentration of IBA formed directly shoots and roots as compared with other hormones Kin, IAA, BA, 2,4-D, Zeatin. Among hormones applied to medium Kin, IAA, BA, 2,4-D, Zeatin, IBA, the IBA(1mg/l) proved the most suitable for shoots and roots. When young leaves of *Callogonium comosum* (Mohamed and Alkayali 2005) were used, the induction of calluses depend on the type used and concentration of plant hormones supplement to medium as friable calluses were obtained, Those results disagree with our finding.

When the immature fruits were used as explants in the present study, calluses were obtained in case of using 2,4-D, Kin on the other hand (Mohamed and Alkayai 2005) were reported that no any responses were observed on *Callogonium comosum*.

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