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REGULAR ARTICLE

ENHANCEMENT OF ADVENTITIOUS SHOOT REGENERATION IN SESAME (*SESAMUM INDICUM* L.) CULTIVAR PROMO KY USING ETHYLENE INHIBITORS

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SUMMARY

Ethylene produced by tissue, callus and plantlets in closed vessels may lead to abnormal plantlet growth and branching *in vitro*. Silver nitrate ($AgNO_3$) and Cobalt chloride ($CoCl_2$) are known as ethylene inhibitors. Therefore, the objective of this study was to evaluate the *in vitro* regeneration capacity of sesame cultivar Promo KY when exposed to culture media containing ethylene inhibitors. A protocol is presented for direct adventitious shoot organogenesis and complete plant regeneration. MS medium enriched with 1.0 mg/L Benzyl adenine (BA) induced adventitious shoot in axenic seedling-derived shoot tips. Addition of ethylene inhibitors $AgNO_3$ (0.5- 5.0 mg/L) enhanced number of shoots from 2.7 to 3.7 shoot/explant as well as shoot length from 1.3 to 2.9 cm . In the case of a medium comprising of MS salts with Naphthyl-Acetic Acid (NAA) 0.05 mg/L, 63 root/explant was observed and root emergence occur after 25 days of culture. Addition of 3.0 mg/L $AgNO_3$ improved root initiation to 79 root/explants and root emerged after 14 days. Addition of 5.0 mg/L $CoCl_2$ increase the root length from 3.5 cm to 17 cm. These promotive effects may result from a reduction in ethylene concentration or inhibition of ethylene action. The results found in this study may be used to improve the multiple shoot and rooting efficiency of Sesame cultivars and possibly of other plant species.

Keywords: Sesame, *Sesamum indicum,* ethylene inhibitor, multiple shoot, rooting.
Abdellatef et al.. Enhancement of Adventitious Shoot Regeneration in Sesame (Sesamum indicum l.) cultivar Promo ky Using Ethylene InhibitorsJ Phytol 2 (2010) 61-67
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1. Introduction

Sesame (*Sesamum indicum* L.) a member of the family Pedaliaceae, is widely grown in Sudan under rain fed conditions [1]. In spite of the economical importance of sesame for the Sudan economy big fluctuations in production and yield occurred. [1]. The average seed yield in Sudan is about 350 kg/ha [2]. This low yields are mainly due to absence of non-shattering cultivars suited for mechanical harvest, indeterminate growth, uneven ripening of capsules and biotic and abiotic stresses such as diseases, pests, drought etc. Moreover, Hamid *et al.,* [3] pointed out that low productivity was attributed partially to the traditional variety used.The potential benefits of using advanced agricultural biotechnology in sesame genetic improvement have not yet been realized in Sudan mainly, because the successful utilization of plant biotechnology for plant improvement requires the development of an efficient shoots regeneration system from cultured cells or tissues.

The development of an efficient micropropagation protocol can highly

support breeding of this potential and adaptive oil crop. Moreover, the establishment of cell culture has considerable potential to facilitate successful wide crosses using embryo culture techniques. Therefore there is an urgent need for developing an efficient *in vitro* regeneration protocol involving Sudanese sesame cultivars with regard to multiple shoot induction.

Sesame in general, has proved to be very recalcitrant to regenerate *in vitro* [4], nodal [5] and leaf [6] cultures. Hypocotyl and/or cotyledon explants [7, 8, 9], has been reported but at low frequencies.

Ethylene (C_2H_4), a gaseous plant hormone, which is produced by almost all plants, mediates a range of different plant responses and developmental steps [10]. It plays an important role in seed germination, tissue differentiation, the formation of shoot and root primordial [11], and also seems to be involved with the poor regeneration potential or recalcitrant behavior of cultured materials [12]. In members of Brassicaceae, ethylene produced by explants in *in vitro* culture conditions was responsible for the recalcitrancy in regeneration, reported in cauliflower [12] and Chinese cabbage [13]. The use of ethylene inhibitors in the shoot regeneration medium has been shown to be effective in overcoming the recalcitrance problem [13], or significantly enhancing the regeneration response in chili [14], Brassica [15,16], cassava [17], radish [18], and somatic embryo formation from coffee [19], and anther culture of cabbage [20]. It has also been used successfully in several monocotyledonous species (rice, maize) to enhance embryogenic callus initiation and plant regeneration [21, 22].

In this paper, we first report the simultaneous addition of ethylene inhibitors, to both shoot and root induction to obtain high frequency plant regeneration from shoot tip explants of poorly regenerative elite Sudanese sesame, cultivar Promo KY.

2. Materials and methods

Plant material

Seeds of sesame cultivar promo KY used in this study were obtained from the Agricultural Research and Technology Corporation (ARTC) Algdarief, Sudan.

Surface sterilization and seed germination:

Seeds were washed by continuously running tap water for 15 minutes then washed by sterile distilled water under laminar flow cabinet seeds were disinfected with clorex 25% (0.5% free chlorine) v\v for 15 minutes with continuous shaking then rinsed five times with sterile distilled water. After surface sterilization, twenty seed were directly transferred to culture bottle contain half strength B5 basal media solidified 0.6% agar and incubated for 10 days at 25 °C ± 2 with a 16 hr photoperiod [23]. (Fig.1.A).

Explants preparation:

In vitro produced Seedling 10 days- old were used as a source of explants Cotyledon and hypocotyls was removed and dischared, shoot tip 0.5 cm were used as explant for induction of multiple shoots (Fig.1.B).

Effect of ethylene inhibitors on multiple shoot induction:

Shoot tip derived from 10 days old seedlings were cultured on *in vitro* multiple shoot induction media (MS + 0.05 mg/L BA) [23], supplemented with different concentrations of silver nitrate and cobalt chloride (0.1,0.5,1.0,2.0,3.0,5.0 mg/L).

Effect of Ethylene inhibitor on *in vitro* root induction:

In vitro derived shoots were cultured on root induction media (MS + NAA 0.1 mg/L) [23], supplemented with silver nitrate and cobalt chloride in concentrations (0.1, 0.5, 1.0, 2.0, 3.0, 5.0 mg/L).

Statistical analysis:

All parameters were collected after 6 weeks of incubation then standard error was calculated by Excel computer program. Means were separated by Duncan's multiple range test (DMRT) [24].

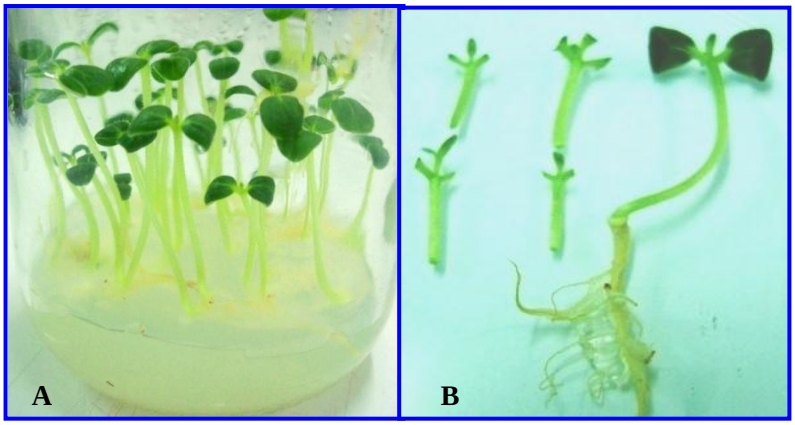

Figure 1. *In vitro* seed germination of sesame cultivar Promo KY and shoot tip explants. **A**: *In vitro* germinated seedling on half-strength B5 medium after 10 days of culture **B**: 10 days-old *in vitro* germinated seedling and shoot tip explants.

3. Result and Discussion

Effect of Ethylene inhibitors on shoot induction

Cytokines are ethylene inducing plant hormones and known to increase ethylene production several folds in many plants [25].

Based on the above-mentioned evidences the effects of ethylene inhibitors on sesame multiple shoot induction was addressed by adding different levels of silver nitrate and cobalt chloride to the culture media containing 1.0 mg/L BA.

Our result showed that in control experiment, without ethylene inhibitors, the shoot tip explant showed low frequency of shoot regeneration. the presence of ethylene inhibitors namely $AgNO_3$ (3.0 mg/L) in the shoot regeneration medium (1.0 mg/L BA,) was found to be beneficial as they significantly enhanced the percentage of shoot regeneration and number of regenerated shoots per explants. On the other hand, a slight improvement in shoot regeneration was observed from shoot tip explants when $CoCl_2$ added to the control media. (Table 1). Another important finding in this study was the induction of high length shoots when 1.0 mg/ L $AgNO_3$ added to control media (Fig. 2. B)

These observations suggest that the poor regeneration response found in control experiment (without ethylene inhibitors) of sesame may be associated with ethylene production by the *in vitro* cultured cells or tissues. *In vitro* tissue cultures produce ethylene in sealed containers [26], which inhibits shoot regeneration [27] and impairs plant growth and development [28].

Table.1: Effect of Ethylene inhibitors (silver nitrate and cobalt chloride) on multiple shoot induction from shoot tip explants cultured on Ms media supplemented with 1.0 mg/ L BA.

Ethylene inhibitor (mg/L)		Regeneration (%)	No of Shoot /explants	Shoot Length (cm)	No of Leaves/ Explants
$AgNO_3$	$CoCl_2$				
0.0	0.0	100	2.7±0.1^{e}	1.3±0.0^{f}	3.2±0.2^{f}
0.1	0.0	100	2.7±0.1^{e}	1.6±0.0^{d}	3.2±0.2^{f}
0.5	0.0	100	2.8±0.4^{d}	2.3±0.1^{c}	3.7±0.5^{d}
1.0	0.0	100	3.1±0.3^{c}	2.9±0.1^{a}	5.8±0.4^{a}
2.0	0.0	100	3.4±0.1^{b}	2.7±0.1^{a}	5.1±0.2^{b}
3.0	0.0	100	3.7±0.2^{a}	2.7±0.1^{a}	5.0±0.2^{b}
5.0	0.0	100	3.1±0.1^{c}	2.6±0.1^{b}	4.9±0.2^{b}
0.0	0.1	100	2.8±0.2^{d}	2.0±0.0^{c}	4.3±0.2^{c}
0.0	0.5	100	2.9±0.1^{d}	2.2±0.1^{c}	4.3±0.2^{c}
0.0	1.0	100	2.9±0.3^{d}	1.7±0.0^{d}	3.4±0.3^{e}
0.0	2.0	100	3.0±0.3^{c}	1.5±0.0^{e}	3.6±0.2^{d}
0.0	3.0	100	2.8±0.1^{d}	1.5±0.0^{e}	3.6±0.3^{d}
0.0	5.0	100	2.8±0.1^{d}	1.4±0.0^{e}	2.8±0.3^{g}

Means with same letters are not significantly different at 5% using Duncan's multiple range tests

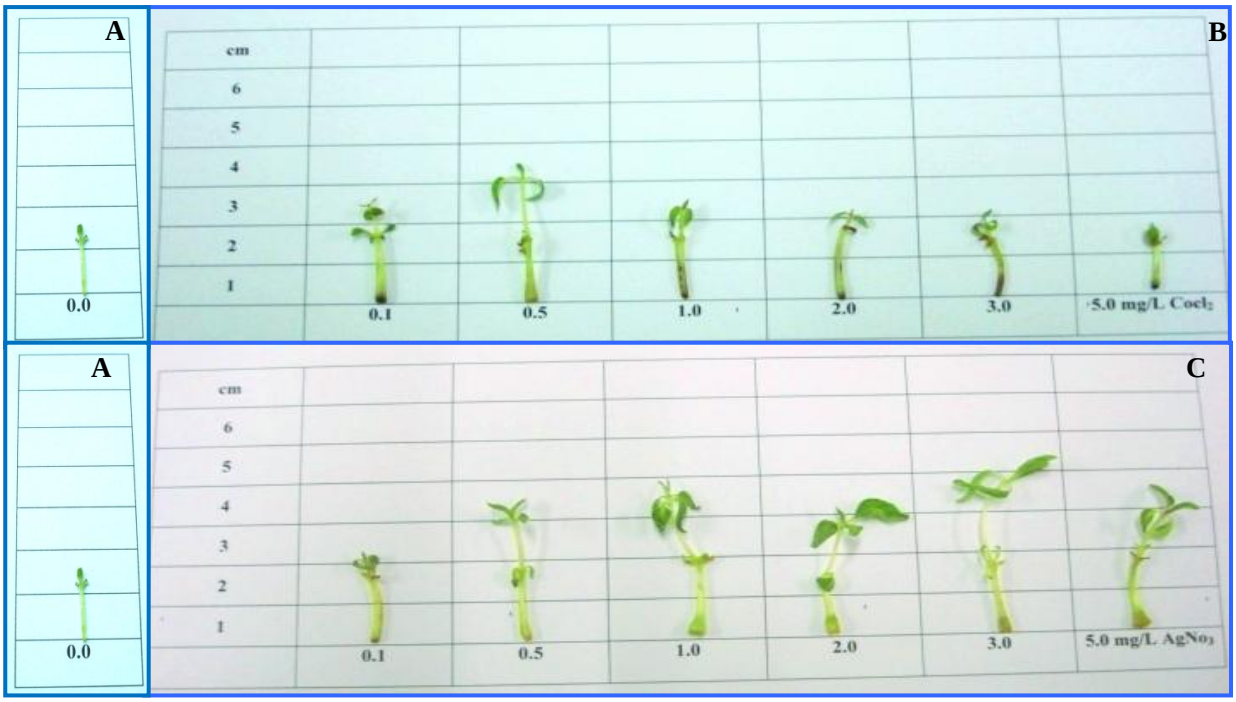

Figure 2. Effect of ethylene inhibitors on shoot length of elite Sudanese sesame cultivar Promo KY after three weeks of culture. **A.** Control. **B.** Effect of Cobalt chloride **C.** Effect of Silver nitrate

Effect of Ethylene inhibitors on root formation

Roots emerged after 30 days on the control rooting medium (NAA 0.05 mg/L). Rooting media containing $AgNO_3$ between 1 to 5 mg/L advanced the root emergence by 14 days (Figure 3.A). On average 63 roots per shoot were produced on the control rooting medium. $AgNO_3$ and $CoCl_2$ at Low concentrations did not significantly change the root number per shoot. While AgNO3 at 1–3 mg/L significantly increased the root number per shoot. Also $CoCl_2$ significantly induced number of roots on concentrations 0.5-5.0 mg/L (Figure 3.B, Table 3). $AgNO_3$ at the same concentrations significantly increased root length. But $CoCl_2$ on concentrations 0.1-5 mg/L induced the maximum root length (Figure 3. C, Table 3). This result clearly demonstrate that $AgNO_3$ and $CoCl_2$ can enhance root emergence, root growth rate, root number per shoot, root length and improve rooting efficiency.

. In accord with our results, ethylene was found to inhibit adventitious root formation from pea cutting [29], tomato leaf discs [30], and *Prunus avium* shoot cultures [31].

Table 2. Effect of Ethylene inhibitors (silver nitrate and cobalt chloride) on root induction from *in vitro* derived shoots cultured on Ms media supplemented with 0.05 mg/L NAA

Ethylene inhibitor mg/L		Rooting (%)	No of root /shoot	Root Length (cm)
AgNO3	$CoCl_2$			
0.0	0.0	100	63±0.3 ^{d}	3.5±0.0^{i}
0.1	0.0	100	63±0.1^{d}	5.2±0.8^{h}
0.5	0.0	100	64±0.0^{cd}	7.5±0.5^{f}
1.0	0.0	100	69±0.2^{b}	9.1±0.2^{e}
2.0	0.0	100	71±0.3^{b}	8.6±0.5^{e}
3.0	0.0	100	79±0.4^{a}	7.9±0.3e^{f}
5.0	0.0	100	65±0.1^{c}	6.3±0.1^{g}
0.0	0.1	100	63±0.6^{d}	9.0±0.0^{e}
0.0	0.5	100	65±0.2^{c}	10±0.3^{d}
0.0	1.0	100	65±0.3^{c}	11±0.3^{c}
0.0	2.0	100	66±0.1^{c}	14±0.1^{b}
0.0	3.0	100	67±0.2^{c}	14±0.2^{b}
0.0	5.0	100	65±0.5^{c}	17±0.1^{a}

Means with same letters are not significantly different at 5% using Duncan's multiple range tests

Figure 3. Effect of ethylene inhibitors on *in vitro* root induction of elite Sudanese sesame cultivar Promo KY after three weeks of culture.
A. Silver nitrate in all concentrations induced root emergence after 14 days
B. Silver nitrate in 1-5 mg/L induced maximum No of root (79±0.4) from *in vitro* derived shoots.
C. $CoCl_2$ induced maximum root length (17±0.1) from *in vitro* derived shoot

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