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Full Length Research Paper

Propagation of Medicinal Plant *Ferula assa foetida* L. Through Indirect Somatic Embryogenesis

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***Ferula assa foetida* L. (Asafetida) is a highly valuable medicinal plant, which is categorized as an endanger plant species. Thus, the experiments were carried out to examine the effect of explants, medium and plant growth regulators, on the *in vitro* indirect somatic embryogenesis of *F. assa foetida*. Explants of root, hypocotyl and cotyledon (leaf) were tested on MS and B5 media combined with 2, 4-D (0, 0.5, 1, 2, 3 or 4 mg/L), BAP (0, 0.5, 1, 2, 3 or 4 mg/L) and NAA (0, 0.5, 1, 2, 3 or 4 mg/L), each of them was combined with Kin (0, 0.2 or 0.5 mg/L). Callogenesis was observed on different media and after 3-4weeks some of them regenerated somatic embryos. The best indirect somatic embryogenesis was obtained from explants of hypocotyl and the highest value for indirect somatic embryogenesis was found in B5 medium enriched with 2,4-D (0.5 mg/L) + Kin (0.2 mg/L).**

Key words: Asafetida, callogenesis, callus, hypocotyls, plant growth regulators.

INTRODUCTION

Ferula assa foetida (Apiaceae), commonly known as Asafetida is a monocarpic, herbaceous, perennial medicinal plant indigenous to Iran and Afghanistan. About 140 species of genus *Ferula* have been distributed from Mediterranean region to central Asia. This plant is one of the most important among thirty species of *Ferula* distributed in Iran. This species is growing wild or recently cultivated in several areas of Iran (Sadraei et al., 2003; Ivan 2007).

Asafetida or Anghouzeh (in Persian) is an oleo gum resin obtained by incision from the living rhizome and roots. Reports about the chemical composition of the oil of oleo gum resin of Asafetida has also been shown that the major constituents were sulphur containing compounds with disulphidesas most important components and various monoterpins (Sadraei et al., 2003). Resin gum that obtain from roots is antispasmodic, aromatic, carminative, digestive, expectorant, laxative, sedative, nervine, analgesic, anthelmintic, aphrodisiac and antiseptic (Khajeh et al., 2005; Sadraei et al., 2003).

Cultivation of this valuable medicinal plant is limited due to insufficient quality of materials for planting. The commercial and pharmacological need of *F. assa foetida* is achieved generally through exploiting the wild population. Recently, overexploiting and lack of organized cultivation has posed a serious threat to its existence in the wild population, particularly when the plants are harvested before seed set. Conventional propagation of *F. assa foetida* is restricted by edaphic and climatic factors as well as low percentage of seed set and seasonal dormancy. Consequently, *F. assa foetida* is recorded as an endangered species in the Red Data Book of Iran (Jalili and Jamzad, 1999). On the other hand, Improvement of Apiacea family through the conventional breeding method is commonly slow, laborious and time consuming (Hunault et al., 1989). Therefore, there is a strong need for practical method of rapid micropropagation of this species.

Micropropagation through somatic embryogenesis can be extremely applied to shorten the long sexual cycle and other difficulties like limited seed availability (Chandrasekhar et al., 2006). *In vitro* embryogenesis may provide an alternative to plant micropropagation (Amirato 1987). The first, callus culture was studied on *Daucus carota* L. (carrot). Consequently, somatic embryogenesis was reported in *Foeniculum vulgare* Mill (fennel) and *Apium graveolens* L. (celery) (Hunault et al., 1989; Tawfik and Noga, 2002). Sharifi, (1995) used hypocotyls and

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cotyledon of *Buninm persicum* for indirect somatic embryogenesis in B5 medium containing NAA and Kin and MS medium containing 2,4-D. In addition, in *Buninm persicum* the maximum somatic embryogenesis occurred in MS medium combined with NAA and Kin (Valizadeh et al., 2006).

Zare et al. (2010) reported that MS medium with 1 mg/L NAA and 2 mg/L BAP was most effective for the proliferation of callus for root explants. Hassaniet al., (2008), used hypocotyls of *F. assa foetida* for indirect somatic embryogenesis in MS medium supplemented with Kin and NAA and reported that the highest embryogenesis frequency observed in medium containing 1.5 mg/L Kin and 1.0 mg/L NAA.

Despite the high medicinal value, health benefits and economic importance in some countries, only few data is available on tissue culture of *F. assa foetida*. Furthermore, this plants is categorized as an endanger herbal plants. Therefore, the present study was conducted to find out a new *in vitro* culture system for induction of indirect somatic embryogenesis in *F. assa foetida* L. leading to plantlet regeneration.

MATRIALS AND METHODS

The experiments were carried out at the Tissue Culture Lab of Agricultural Biotechnology Research Institute of Iran in 2010. The mature seeds of *F. assa foetida* were obtained from medicinal plant collection of Shahid Fozveh Research Center, Isfahan, Iran.

Seed Sterilization and Dormancy Breaking

In order to remove the surface contaminations, seed were washed with sterile distilled water 10with tap water; and then were soaked in a 2.5 g/L benomyl for 4 hours; and then they were washed with sterile distilled water. The seeds were soaked in 70% (v/v) ethanol for 90 second, then the seeds were put in 100 ml of sodium hypochlorite 30% commercial bleach with one drop of tween 20 for 20 minutes and finally rinsed 3 times with sterile distilled water in laminar flow cabinet, and were cultured in Petri dish containing breaking dormancy medium (0.25 mg/L BAP and 7 g/L agar) as reported by Otroshy et al., (2009). The cultures were incubated at 5°C and full darkness for a period of 1 month. The germinated seeds were transferred to the Petri dishes containing 1/2 MS medium. Plantlets were produced after 2 weeks incubation at 25°C, the light intensity of 3000 lux and a photoperiod of 16 h light.

Induction of somatic embryogenesis

Items that were testing were included of explants: roots, hypocotyls and cotyledon (leaf); two kind of media: MS (Murashig and Skoog) and B5 (Gamborg et al., 1968); growth regulators: 2,4-D (0, 0.5, 1, 2, 3 or 4 mg/L), BAP (0, 0.5, 1, 2, 3 or 4 mg/L) and NAA (0, 0.5, 1, 2, 3 or 4 mg/L) each of them was combined with Kin (0, 0.2 or 0.5 mg/L). After preparing media and adjustment of pH to 5.8 for MS and 5.5 for B5 the media were autoclaved. Immediately after autoclaving required doses of growth regulators were added to the nutrient medium before it was allowed to solidify.

To take explants, the plantlets were separated into 3 parts: root, hypocotyl and cotyledon (leaf), each of them was separately cut into 0.5-1 cm segments. The explants were cultured in sterilized Petri dishes containing 30 ml of above mentioned media combined with required doses of growth regulators and without growth regulators (as controls).

The Petri dishes were closed with cap and sealed with household plastic foil and were randomly placed in a growth chamber set at 25◦C and 16 h photoperiod; and the light intensity of 1500 lux light intensity for a period of 1 month. After one month the above mentioned explants were transferred to the Petri dishes containing media (MS and B5) without any growth regulators for embryogenesis and development.

Data Collection

The percentage of explants producing embryogenic callus in the different media was recorded 4 weeks after culture. At this stage the percentage of callogenesis, the percentage of indirect somatic embryogenesis and number of produced embryos in different stages (globular, torpedo and cotyledonary) were measured.

Experimental design and Statistical Analysis

Each experiment was arranged as completely randomized factorial design with three replicates. Data were analyzed by using the SAS version 8 statistical computer programs. When the ANOVA indicated significant treatment effects (5 or 1%) based on the F-test, the Duncan's Multiple Range Test ($P \leq 0.5$) was used as a method to determine which treatments were statistically different from other treatments.

RESULTS

Hormonal treatments and explants showed significant effects on percentage of callogenesis, percentage of embryogenic callus and on the number of embryos in different embryonic stages (globular, torpedo, cotyledonary) and their interaction effect was significant as well (Table 1).

Table 1: Analysis of variance of the effect of some plant growth regulators on indirect somatic embryogenesis, the number of total, globular, torpedo, cotyledonary and conversion embryos, percentage of callogenesis (EC) and embryogenic callus and relative callus weight (RCW).

Source	DF	Callus (%)	RCW	EC (%)	Total	globular	torpedo	cotyledonary	Conversion
Growth regulators	53	0.71**	14.67**	0.02**	1.88**	0.34**	0.07**	0.88**	0.31**
Medium	1	0.03 ns	5.17*	0.00 ns	0.01 ns	0.01 ns	0.00 ns	0.00 ns	0.02 ns
Explants	2	35.17**	9.78**	0.02**	2.39**	0.42**	0.09**	1.17**	0.45**
medium × growth regulator	53	0.23**	9.23**	0.02**	2.03**	0.36**	0.07**	0.95**	0.33**
Growth regulators × explant	106	0.16**	4.67**	0.01**	1.49**	0.30**	0.06**	0.67**	0.25**
medium × explants	2	0.11*	1.06 ns	0.00 ns	0.12 ns	0.18**	0.01 ns	0.02 ns	0.01 ns
medium × growth regulator × explant	106	0.09**	3.30**	0.01**	1.53**	0.31**	0.06**	0.69**	0.26**
Mean Square of Error	648	0.03**	1.12**	0.00**	0.26**	0.02**	0.01**	0.14**	0.05**

*$P \leq 0.05$,** $P \leq 0.01$, ns = non-significant

Table 2. The effect of some hormonal treatments on the percentage of callus, relative callus weight (RCW), percentage of embryogenic callus (EC), the number of total, globular, torpedo, cotyledonary and converted embryos.

Growth regulators	Callus (%)	RCW	EC (%)	Total	Globular	Torpedo	Cotyledonary	Conversion
2,4-D(0)+Kin(0)	0.00 z	0.00 t	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(0)+Kin(0.2)	14.44 y	0.88 st	0.00d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(0)+Kin(0.5)	21.11 xy	2.84 q-t	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(0.5)+Kin(0)	34.44 t-x	2.47 q-t	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(0.5)+Kin(0.2)	51.67 g-l	8.17 h-p	13.48 a	24.39 a	6.89a	1.61a	10.94a	4.94a
2,4-D(0.5)+Kin(0.5)	62.22 ab	12.48 b-j	4.92 b	13.92 b	1.97b	0.58b	8.78b	2.58b
2,4-D(1)+Kin(0)	36.11 o-u	5.35 l-r	3.00 c	1.42de	0.00d	0.06c	0.69e	0.72de
2,4-D(1)+Kin(0.2)	34.44 n-t	9.81 f-n	1.11 c	1.22de	0.00d	0.11c	1.06de	0.06e
2,4-D(1)+Kin(0.5)	31.67 s-x	31.70 ab	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e

Table 2. cont.

2,4-D(2)+Kin(0)	33.33 n-t	5.82 j-q	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(2)+Kin(0.2)	31.11 wx	1.90 q-t	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(2)+Kin(0.5)	28.33 r-x	5.01 m-r	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(3)+Kin(0)	39.44 m-r	3.24 o-s	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(3)+Kin(0.2)	38.33 m-r	5.81 k-q	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(3)+Kin(0.5)	30.56 r-x	2.93 p-s	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(4)+Kin(0)	26.11 u-x	3.79 o-s	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
2,4-D(4)+Kin(0.2)	22.22 v-x	1.98 q-t	0.69 d	0.89e	0.33cd	0.11c	0.44e	0.00e
2,4-D(4)+Kin(0.5)	31.11 q-w	3.11 o-s	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(0)+Kin(0)	0.00 z	0.00 t	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(0)+Kin(0.2)	14.44 y	0.88 st	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(0)+Kin(0.5)	21.11 xy	2.84 q-t	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(0.5)+Kin(0)	40.00 n-t	6.19 k-r	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(0.5)+Kin(0.2)	40.56 h-n	8.86 g-n	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(0.5)+Kin(0.5)	40.00 m-r	5.31 l-r	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(1)+Kin(0)	58.89 a-c	24.65 a	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(1)+Kin(0.2)	52.22 e-j	10.19 e-l	2.78c	1.61de	0.00d	0.00c	0.61e	1.00cd
BAP(1)+Kin(0.5)	49.44 h-m	12.31 c-k	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e

Table 2. cont.

BAP(2)+Kin(0)	62.78 a	13.96 a-f	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(2)+Kin(0.2)	50.00 f-k	7.83 g-n	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(2)+Kin(0.5)	50.56 f-l	15.02 a-i	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(3)+Kin(0)	58.33 a-e	17.44 ab	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(3)+Kin(0.2)	51.67 g-l	12.00 d-k	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(3)+Kin(0.5)	47.22 d-i	17.04 a-c	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(4)+Kin(0)	39.44 i-n	7.74 i-p	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(4)+Kin(0.2)	31.11 s-x	5.45 o-s	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
BAP(4)+Kin(0.5)	62.78 ab	20.48 a-d	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(0)+Kin(0)	0.00 z	0.00 t	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(0)+Kin(0.2)	14.44 y	0.88 st	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(0)+Kin(0.5)	21.11 xy	2.84 q-t	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(0.5)+Kin(0)	41.11 k-p	12.51 c-k	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(0.5)+Kin(0.2)	40.56 m-q	8.59 h-o	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(0.5)+Kin(0.5)	51.11 c-g	8.89 e-m	4.21b	5.53c	0.50c	0.72b	2.75c	1.56c
NAA(1)+Kin(0)	35.56 r-x	3.34 o-s	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(1)+Kin(0.2)	51.67 c-h	14.53 a-g	1.39c d	0.00e	0.00d	0.00c	0.06e	0.00e
NAA(1)+Kin(0.5)	44.44 g-l	9.64 c-k	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e

Table 2. cont.

NAA(2)+Kin(0)	33.33 u-x	6.79 k-r	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(2)+Kin(0.2)	54.44 e-j	19.02 a-e	2.78c	0.42e	0.11d	0.00c	0.22e	0.08e
NAA(2)+Kin(0.5)	40.56 k-p	12.01 a-i	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(3)+Kin(0)	32.22 p-v	21.84 ab	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(3)+Kin(0.2)	38.33 j-o	15.73 b-i	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(3)+Kin(0.5)	57.78 a-d	7.65 g-n	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(4)+Kin(0)	35.56 m-s	5.17 l-r	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(4)+Kin(0.2)	54.44 a-f	13.53 a--h	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e
NAA(4)+Kin(0.5)	31.11 x	1.80 r-t	0.00 d	0.00e	0.00d	0.00c	0.00e	0.00e

Values followed by the same letter(s) within a column are not significantly different $p \leq 0.05$

Table 3. The effects of Kind of basal medium on the percentage of callogenesis, relative callus weight (RCW), Percentage of embryogenic callus (EC), the number of total, globular, torpedo, cotyledonary and converted.

Medium	Callus (%)	RCW	EC (%)	Total	Globular	Torpedo	Cotyledonary	Conversion
MS	36.77a	7.63b	0.47a	0.77 a	0.11 a	0.05 a	0.45a	0.16a
B5	37.86a	9.42a	0.81 a	1.06a	0.26 a	0.07 a	1.49 a	0.25a

Values followed by the same letter(s) within a column are not significantly different $p \leq 0.05$

The highest percentage of callogenesishas been obtained by using BAP with concentrations of (2, 1 and 3 mg/L); BAP (4 mg/L) + Kin (0.5 mg/L) and also other treatments containing 2,4-D (0.5m g/L) + Kin (0.5 mg/L) respectively and NAA (3 mg/L) + Kin(0.5 mg/L) and finally NAA (4 mg/L) +Kin (0.2 mg/L) showed highest percentage of callogenesis (Table 2).

The highest percentage of callus was obtained from 2,4-D (0.5 mg/L) +Kin (2 mg/L), also the highest number of globular, torpedo, cotyledon and converted embryos were observed in this media as well. The highest percentage of embryogenesis were obtained with treatment of 2,4-D (0.5 mg/L) + Kin (0.5 mg/L) (Table 2).

The medium had not a significant effect on percentage of callogenesis, embryogenic callus, number of globular, torpedo, cotyledon and conversion percent. The kind of medium showed significant difference just in the relative weight of callus (Data not shown). The highest relative weight of callus was observed in B5 medium (Table 3).

The kind of explants showed a significant effect on callogenesis (Data not shown). Production of callus in root explants is higher than the others. While production of embryogenic callus in root explants was weaker than other explants. Explants of cotyledon and hypocotyl had a high percentage of embryogenic callus, whereas the highest total number of globular, torpedo, cotyledonary

Table 4. The effect of Kind of explant on the percentage of callogenesis, relative callus weight (RCW), percentage of embryogenic callus (EC), the number of total, globular, torpedo, cotyledonary and converted embryos.

Explant	Callus (%)	RCW	EC (%)	Total	globular	torpedo	cotyledonary	Conversion
Root	75.62a	7.67b	0.04 b	0.05 b	0.02b	0.01b	0.02b	0.00b
Hypocotyl	17.93b	8.55a	1.11 a	2.17a	0.47 a	0.14 a	1.11a	0.45a
Leaf	18.40b	9.35a	0.76a	0.52b	0.05b	0.04b	0.28b	0.15b

Values followed by the same letter(s) within a column are not significantly different $p \leq 0.05$

and converted embryosare related to hypocotyl explants (Table 4).

The interaction effects of medium and hormonal treatments on the percentage of embryogenesis and callogenesis was very significant. Highest percentage of embryogenesis and callogenesis were observed in B5 medium combined with NAA (2 mg/L) + Kin (0.2 mg/L) and MS medium combined with BAP (4 mg/L) + (0.5 mg/L) and 2,4-D (0.5 mg/L) + Kin (0.5 mg/L). The B5 basal medium with 2,4-D (0.5 mg/L) + Kin (0.2 mg/L) showed the highest percentage of embryogenic callus. The highest number of total embryo, globular, torpedo, cotyledon and converted embryos was found in B5 medium containing 2,4-D (0.5 mg/L) + Kin (0.2 mg/L) (Data not shown).

DISCUSSION

The results of this investigation showed that the treatment of BAP with or without low concentrations of Kin leads to increase of callogenesis. This finding is in accordance with the results of Martin (2005) that showed production of callus is the features of medium containing BAP. In the treatments containing NAA also observed that the highest concentration of NAA (3 mg/L) + Kin (0.2 mg/L) and NAA (4 mg/L) + Kin (0.5 mg/L) lead to increase of callogenesis. Pant and Manandhar, (2007) also reported production of callus in high concentrations of BAP and NAA. Tiwari et al. (2000) obtained that, increase in the NAA amount leads to enormous callus production. In *C. asiatica*, using higher concentrations of BAP and NAA resulted to increase in amount of callus production (Banerjee et al., 1999). Sharifi, (1995) also reported that, in B5 medium containing NAA and Kin production of callus is faster. Karimi et al. (2009) indicated various effects of using BAP and NAA on callogenesisas of *Ducrosia flabellifolia* well. In the same study, Zare et al. (2010) reported that percentage of callus induction in *F. assa foetida* was lower on media with 2,4-D plus Kin when compared to NAA plus BAP. These results clearly indicate that presence of both BAP and NAA in the medium in essential for optimum callus induction in *F. assa foetida.* Although both hypocotyl and leaf explants produced callus, the highest amount of callus was produced from root explants, which indicates that callogenesis in *F. assa foetida* is not resulting only from placing explants tissues in specific culture conditions, but it is intensively depends on the kind of explants. The performed studies by Vuorela et al. (2004) confirm this result as well. In the previous study in *F. assa foetida* Zare et al., (2010) reported that hypocotyls and cotyledon explants cultured on MS basal medium did not form callus.

The results of this experiment showed that 2,4-D (0.5 mg/L) + Kin (0.2 mg/L) followed by 2.4-D (0.5 mg/L) + Kin (0.5 mg/L) caused the highest somatic embryogenesis. Mizukami et al. (2008) reported that 2,4-D induced somatic embryogenesis. Dudits et al. (1991) also reported the similar results. As already mentioned, low concentrations of 2,4-D +Kin caused embryogenic callus induction in *F. assa foetida*. This finding is in accordance with results obtained from studies on *C.aasiatica* L. by Paramageethan et al. (2004) as well. It was observed that in treatments with low concentration of 2,4-D without Kin, no embryo was observed. Therefore, we can conclude that Kin existence is very important for embryogenesis in Asafetida. Tawfik and Noga, (2002) believed that presence of Kin beside 2,4-D is the factor of increasing reproduction in callus medium and differentiation in embryogenesis medium and said that Kin is necessary for embryogenesis. In *Tylohoraindica* species, the embryogenic callus was obtained from the medium containing 2,4-D and embryogenesis was induced by Kin (Tawfik and Noga, 2002). The explants of *Foeniculum vulgare* in MS medium containing 2,4-D was succeeded to production of callus, and transition of this calli to the free hormone medium caused to somatic embryogenesis (Anzidei et al., 2000). The results of the embryogenesis experiments showed that, after aforementioned treatments the medium containing NAA (0.5 mg/L) +Kin (0.5 mg/L) has a high ability for embryo-

genesis, as well.

In this research, embryogenesis was not observed until the explants stayed in the induction medium, and embryogenesis occurred after transition of explants to the hormone free medium. Cho et al. (2003) reported that, in many plants such as carrot, after inducing in medium containing 2,4-D the somatic embryogenesis performed in MS medium without hormone. The results of this research showed that, the maturity of normal embryos was successful on MS medium too. Martin, (2007) reported that, in *C. asiatica* the grown callus on medium containing 2,4-D or NAA combined with Kin is very suitable for inducing somatic embryogenesis. Bernard et al. (2007)reported the same results.

Our results showed that, the highest percentage of embryogenesis is related to hypocotyl and cotyledon (leaf) explants. According to the results of Taefik and Noga, (2002) for embryogenesis in green cumin, calluses obtained from hypocotyl and the initial leaf which as grown in medium containing 2,4-D and Kin must be used. Using leaf explants in Coriander leads to creation of very embrogenic callus in the medium containing 2,4-D and NAA (Stephan and Jayabalan, 2001). Obtaining *Solanum melongena* L. embryo of leaf explants is more successful than the other organs (Tarre et al., 2004). In *Eryngium foetidum* reported that, in presence of 2,4-D and Kin, embryogenesis was done in leaf explants (Martin, 2005). Ignaciumuthu et al., (1999) verified similar results in this case as well. The best direct embryogenesis obtained from hypocotyl explants (Bernard et al., 2007).

CONCLUSION

1. The high concentrations of BAP and NAA with low concentrations of Kin have a great effect on callus production in F*. assa foetida*.
2. In F*. assa foetida* low concentrations of 2,4-D with Kin (0.5 or 0.2 mg/L) results in somatic embryogenesis.
3. In experiments related to embryogenesis of F*. assa foetida*, 2,4-D with Kin give the best result and NAA with Kin is in the second degree of importance.
4. In F*. assa foetida* hypocotyl is the best explants for embryogenesis, and root is the best explants for callagenesis.

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