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Somatic Embryogenesis and Histological Aspect of Galbanum (*Ferula gummosa* Boiss.) Endangered Medicinal Plant

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ABSTRACT

Objective: The aim of this study was to develop tissue culture and somatic embryogenesis techniques to overcome seed dormancy and propagation challenges in *Ferula gummosa* Boiss., a valuable but endangered plant endemic to Iran.

Method: To minimize seed dormancy period and micropropagation, callus induction and embryogenesis were evaluated. The plantlets of the seeds were separated and cultured in the 1/2 MS medium. After 14 days, root, hypocotyl, cotyledon, and leaf explants were separated. Then they were transferred to the basal MS medium containing different concentrations of growth regulators. Different developmental stages of somatic embryos were evaluated.

Results: The results show that, after placing seedlings (2-3 old-days) in 1/2 MS medium germinated and whole plantlets were obtained after 12 days. In the callus induction phase growth regulator composition 1.5 mgl-1 of NAA and 0.5 mgl^{-1} of BA with root explants had proper results (100% callus formation). In the somatic embryogenesis phase MS medium containing 0.5 mgl^{-1} of 2,4-D accompanied by 1 mgl^{-1} of BA led to desirable results. Via taken sections from the embryos, different developmental stages of somatic embryos including pre-embryo, globular, heart-shaped, torpedo, and cotyledonary embryos were observed.

Conclusions: *In vitro* culture of the embryo to accelerate germination and elimination of long dormancy period and using this optimized method are strongly suggested for micropropagation of this plant so this valuable endemic plant may survive from extinction, too.

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Introduction

Somatic embryogenesis defines as embryo formation from asexual cells under in vitro conditions which has similar development capabilities as the seed embryos to develop into a complete plant (Fujimura 2014; Bayarmaa et al., 2018). A somatic embryo is similar to a seed embryo the embryogenesis process. Somatic embryogenesis has also been consumed as a model for studying seed embryogenesis. Somatic embryogenesis is also a possible tool to produce virus-free plants (Olah et al., 2022). Studies on somatic embryogenesis have several scientific and practical aspects and due to providing promising production and continuous embryonic mass, it is considered as an appropriate method for propagation (Kärkönen 2000; Ahmadpour et al., 2018).

The somatic embryogenesis (SE) is a common method in the large-scale production of plants. This will be very useful in protecting endangered plants in nature and fast and simple propagation of economical plants in industry and medicine (Avilés-Viñas et al., 2013).

Different species of Ferula belonging to the Apiaceae (Umbeliferae) family were reported from Iran. All of them, especially F. gummosa, F. assafoetida, and F. persica are important for industry, economy, medicine, and biodiversity (Nasri et al., 2018). Hence some research has been carried out on the micro propagation or phytochemical evaluation of them in recent years (Zare et al., 2010; Sarvi et al., 2018).

Ferula gummosa Boiss. is known as galbanum too, is an endangered valuable pharmaceutical and industrial plant that grows naturally in the North and West highlands of Iran. This species is a perennial plant with monocarpic flowering behavior as in the initial years of germination (5 to 7 years) it produces rosette leaves and in the final year it blooms, then roots decay and the plant perishes (Mozafarian 1983). *F. persica* extract has antioxidant and anti-inflammatory activities and can heal damaged tissue in wounds (Huang et al., 2022). Extracted resin from the roots of *F. gummosa* has made it an important meadow plant of Iran which is exported to the European countries in large amounts. *Ferula*'s oleo-gum resin consists of 5 to 30% essential oils (EOs), 60 to 70% resin, 20 to 40% resin materials, and 1 to 10% moister and mineral materials (Mortazaienezhad & Sadeghian, 2006). Evaluating the components of galbanum implicated that this plant's resin can be used as invisible glues to combine gemstones such as diamonds or jewelry, and also the latex can be utilized in the printing, textile, and perfume industries (Mortazaienezhad & Sadeghian, 2006).

According to its high medicinal and industrial values, *F. gummosa* is one of the most valuable plants among genetic reserves. On the other hand, due to the monocarpic flowering behavior and the prolonged seed dormancy period, multiple propagation of galbanum has faced obstacles. Also, this valuable plant is in dangered of extinction due to improper and indiscriminate harvesting. Ferula species have promising bioactive compounds such as auraptene, umbelliprenin, galbanic acid, daucane esters, ferutinin, ferulenol, ferprenin, sinkiangenorin C and E, farnesiferols A and B, which can be used as valuable resources in the production of drugs, especially chemo preventive agents (Salehi et al., 2019). Therefore, effective approaches are needed to minimize seed dormancy and germination time as well as to maximize propagation (Zardari et al., 2019). Based on this, the application of tissue culture techniques and somatic embryogenesis methods to achieve multiple proliferation and appropriate and practical solutions to prevent the extinction of this valuable plant (Otroshy & Roozbeh 2015). Therefore, in this study, the effective methods of callus generation and somatic embryogenesis were optimized by selecting appropriate explants and using different

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concentrations of growth regulators. Also, structural studies were carried out on embryogenic calli to investigate the development stages of somatic embryos.

Method

Sterilization and Seed Dormancy Breaking

Ferula gummosa seeds are very recalcitrant and their germination is difficult. Cold pretreatments at 4°C were performed for different periods without or with various concentrations of gibberellic acid hormone (GA3) for 72 hours were used to get the germinated seed. Despite this, a suitable explant was used for callus formation and somatic embryogenesis. We had to separate the seed coat and transfer plantlets to in vitro culture. So, seeds were primarily put in water at room temperature for 72 hours to soften endosperm tissue surrounding the plantlets. Seeds were sterilized by ethanol 70% for two minutes and sodium hypochlorite 3% for twenty minutes, consecutively. Then they were eluted by sterilized distilled water three times. Embryogenic axes were expelled from the coats by forceps and scalpel and grown plantlet were transferred into petri dishes including prepared 1/2 MS culture medium (Murashige & Skoog, 1962). Cultures were incubated in the following conditions: 23±2°C temperature and photoperiod of 16 / 8 hours for 14 days.

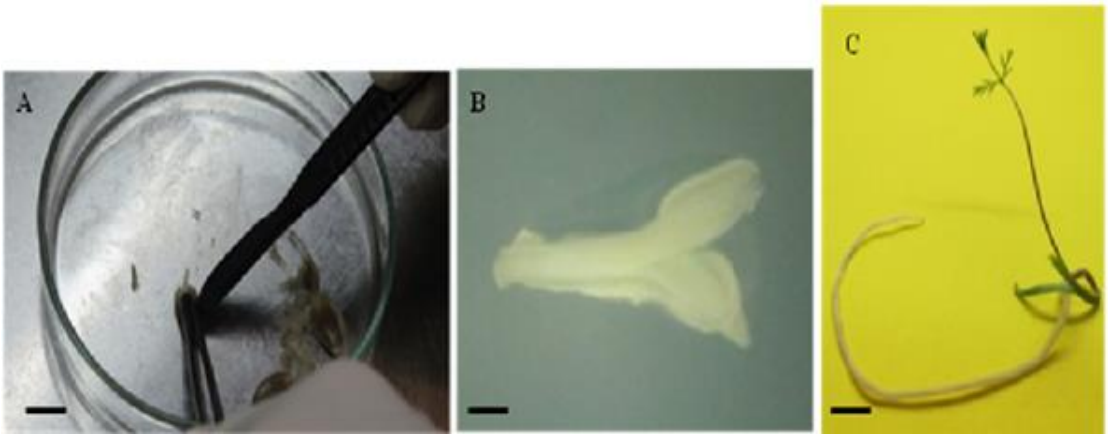

Figure. 1. (a) Taking out the plantlet from the seed coat. (Scale Bar =0.5 cm); (b) Complete plantlet (Scale Bar = 0.25 cm); (c) Seedling consisting of root, cotyledons, and leaves after two weeks in 1/2 MS medium (Scale Bar = 0.5 cm).

Callus Induction Medium and Statistical Analysis

Explants, with 1cm length, were separated from cotyledon, hypocotyl, roots and leave (Fig.1) of *F. gummosa* and set in the MS medium for callus induction. It was evaluated in a factorial experiment as the base of a randomized complete design with three replications. Treatments were 2,4-D (0, 1.5, 3 and 4.5 mgL^{-1}) or NAA (0, 1.5, 3, and 4.5 mgL^{-1}) and BA (0, 0.5, 1, and 2 mgL^{-1}). Two subcultures were performed for three months and callus formation percentage were measured in the petri dishes. Statistical analysis was performed by SAS software (version 2.0) and means comparison was done by Duncan's test at the probability level of 5%.

Somatic Embryogenesis

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Root explants were used for somatic embryogenesis. The explants were set on MS, 1/2 MS ،1/4 MS, and 1/8 MS salts media including 0.5, 1, 1.5, 2, 3, 4, and 5 mgl^{-1} of 2,4-D. In order to increase embryos, the next subcultures were performed once a month in the same condition. Somatic embryos with appropriate growth (more than 3 mm) were isolated from calli and transferred to 1/2 MS culture medium without a growth regulator for growth and root induction.

Embryonic Callus Histology

To evaluate and identify the somatic embryogenesis process, histological studies were done. The embryonic calli were located in the FAA fixation solvent (formaldehyde, acetic acid, and ethanol 100% in the ratio of 1: 2: 17) for four hours. After elution, they were dehydrated by additive degrees of ethanol, and finally, saturation was achieved by a toluene-paraffin mixture followed by pure paraffin and sectioned at 4-6 µm with microtome (Sanderson, 1994). Staining was carried out with the hematoxylin and eosin techniques**.** Several sections were viewed with a Zeiss light microscopy during the embryological stage.

RESULTS

Seed Germination

Because the seed germination rate of *Ferula gummosa* was very low and its germination was difficult, we preferred to use plantlets grown *in vitro*. Isolated plantlets from uncoated seeds grew after 2-3 days on 1/2 MS medium. Root, hypocotyl, and cotyledon explants were separated from the fourteen-day-old seedlings (Fig. 1).

Callus Induction

The first signs of inflation and cellular proliferation for root explants were observed 10-15 days after locating in the MS medium containing 4.5 mg l^{-1} of NAA accompanied by 2 mg l^{-1} of BA (Fig. 2A). The first considerable mass was observed in the third to fourth weeks. The appropriate calli in terms of quality and size were transferred to the embryogenesis medium. Among the explants used, root and hypocotyl succeeded in callus formation in all growth regulator treatments (Figs. 2B and 2C), while the leaf explant did not induce callus at all (Fig. 2E). Cotyledon explants produced the most callus in medium containing 1.5 mg l^{-1} of NAA and 0.5 mg l^{-1} of BA (Fig. 2D). The results indicated that the made cutting in the terminal parts of root and hypocotyl explants can stimulate callus induction and it seemed that providing direct contact between the cutting section of the explant and the culture medium containing growth regulator was essential for callus induction.

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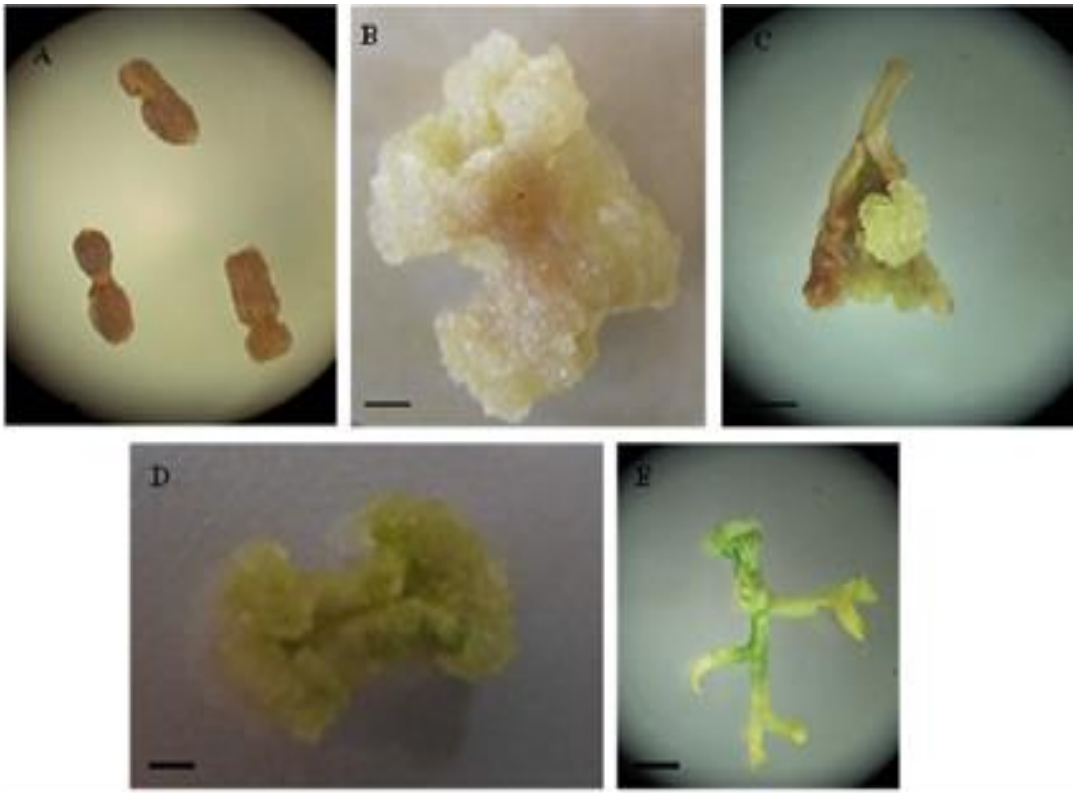

Figure. 2 Callus formation in *Ferula gummosa* : **a** Swelling of root explants on MS medium containing 4.5 mg l^{-1} of NAA and 2 mg l^{-1} of BA; **b** Callus formation from root explant 45 days after transferring to callus induction medium containing 3 mg l^{-1} of NAA and 2 mg l^{-1} of BA; **c** Callus formation from hypocotyl explant 45 days after transferring to callus induction medium containing 2 mg l^{-1} of BA; **d** Callus formation from cotyledon explant 45 days after transferring to callus induction medium containing 1.5 mg l^{-1} of NAA and 0.5 mg l^{-1} of BA; **e** No callus induction from leaf explant (Scale Bar = 0.25 cm)

Analysis variance of callus formation percentage for root, hypocotyl, and cotyledon explants demonstrated that the simple and interaction effects of each growth regulator factor were significant. Comparing the means obtained from Duncan's test elucidated that the root explant had the highest callus formation percentage among all of the explants (Tables1 and 2). This claim is made because despite the greater weight of the hypocotyl-originated callus compared to the root-originated callus, more quality and durability were observed for root-originated callus (Figs. 2B and 2C).

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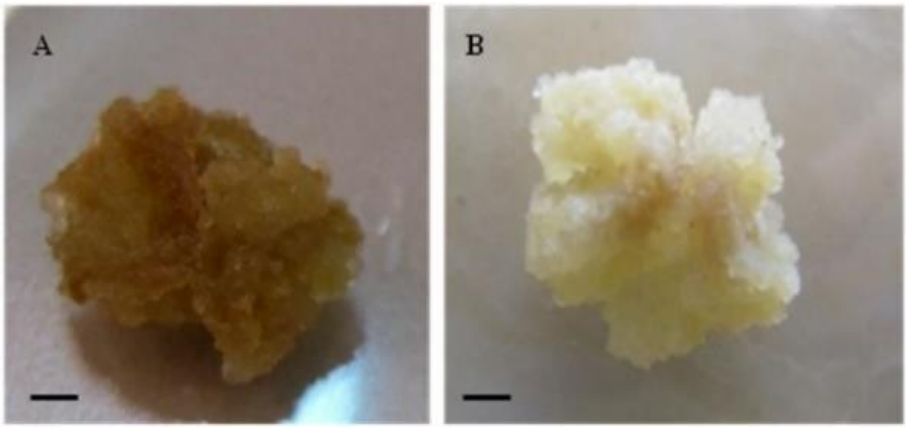

Figure. 3 The effect of different growth regulator treatments on root explants callus formation; **a** Callus browning in the MS medium containing 3 mg l^{-1} 2,4-D; **b** Formation of white, fragile, and smooth callus in the 1.5 mg l^{-1} of NAA and 0.5 mg l^{-1} of BA treatment (Scale Bar = 0.5 cm).

The best callus (based on quality, fragility, and survival rate after successive subcultures) was obtained from media containing NAA and BA from the root explant. The use of NAA 1.5 mgl^{-1} with 0.5 mgl^{-1} BA was the best treatment compared to another for callus weight and callus induction percentage (Fig. 3B). The results indicated that consuming high concentrations of 2,4-D decreased qualities and quantities of the callus (Fig. 3A). Low concentration of 2,4-D was more suitable for callus formation in root culture (Table 2).

Table1. Effect of NAA and BA levels on percentage of callus induction from root, hypocotyl and cotyledon explants.

NAA (mg l^{-1})	BA (mg l^{-1})	Root (%)		Hypocotyl (%)		Cotyledon (%)	
0		80.8	b	83.3		25.0	b
1.5		88.9	b	72.2		78.2	a
3		100.0	a	65.0		72.7	a
4.5		100.0	a	83.3		63.2	a
	0.5	100.0	a	70.8		62.5	
	1	88.5	b	73.8		55.2	
	2	88.8	b	83.3		61.6	
0	0.5	100.0	a	75.0	abc	37.5	d
1.5	0.5	100.0	a	83.2	ab	87.5	a
3	0.5	100.0	a	75.0	abc	50.0	cd
4.5	0.5	100.0	a	50.0	bc	75.0	abc
0	1	87.5	b	75.0	abc	0.0	e
1.5	1	66.6	b	75.0	abc	75.0	abc
3	1	100.0	a	45.0	c	87.5	a
4.5	1	100.0	a	100.0	a	58.2	a-d
0	2	55.0	b	100.0	a	37.5	d
1.5	2	100.0	a	58.3	bc	72.2	a-c
3	2	100.0	a	75.0	abc	80.5	ab
4.5	2	100.0	a	100.0	a	56.3	bcd

There is not any significant difference among treatments with the same letters in each column based on Duncan's test at 5%

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Table2. Effect of 2,4-D and BA levels on percentage of callus induction from root, hypocotyl and cotyledon explants.

2,4-D (mg l^{-1})	BA (mg l^{-1})	Root (%)		Hypocotyl (%)		Cotyledon (%)	
0		82.5	a[1]	62.3		23.3	a
1.5		91.3	a	59.0		10.0	c
3		81.0	a	57.3		15.2	b
4.5		64.6	b	61.3		7.5	c
	0	85.8		78.3	a	30.0	a
	0.5	76.3		56.3	b	3.1	c
	1	74.2		37.1	c	15.4	b
	2	83.1		68.1	a	7.5	c
0	0	70.0	abc	45.8	c-f	26.7	a
1.5	0	93.4	a	100.0	a	40.0	a
3	0	86.7	ab	87.5	a	23.3	a
4.5	0	93.3	a	80.0	ab	30.0	a
0	0.5	80.0	ab	90.0	a	0.0	c
1.5	0.5	85.0	ab	30.0	def	0.0	c
3	0.5	90.0	a	60.0	bc	12.5	c
4.5	0.5	50.0	c	45.0	cde	0.0	c
0	1	100.0	a	53.3	cd	36.7	a
1.5	1	93.3	a	50.0	cde	0.0	c
3	1	93.3	a	24.9	ef	25.0	a
4.5	1	60.0	bc	20.0	f	0.0	c
0	2	43.3	c	60.0	bc	30.0	a
1.5	2	93.3	a	55.8	bcd	0.0	c
3	2	87.5	ab	56.7	bcd	0.0	c
4.5	2	71.7	abc	100.0	a	0.0	c

[1]There are not any significant difference among treatments with the same letters in each column based on Duncan's test at 5%

Somatic embryogenesis

After 4 months, root-originated calli in the MS medium containing 2,4-D and BA were evaluated by a stereomicroscope. The results showed that using an MS medium consisting of different concentrations of macro and microelements (1/2, 1/4, and 1/8) led to callus browning after 1-2 weeks and a sign of cellular proliferation and consequently somatic embryogenesis was not observed (Fig. 4A). The puff -shaped calli in MS medium containing 2,4-D were transformed into bubble-shaped calli after one month (Fig. 4B). Observations also demonstrated that increasing 2,4-D concentration until 5 mg l^{-1} bubble-shaped callus was enhanced but this callus could not be able to embryogenesis.

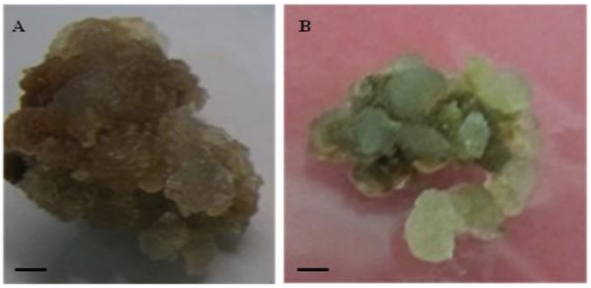

Figure. 4 Callus formation in different embryogenesis media; **a** Production of browning callus in 1/4 MS medium; **b** Bubble- shaped callus formation in MS medium containing 0.5 mg l^{-1} of 2,4-D and 1 mg l^{-1} of BA which is suitable for embryogenesis (Scale Bar = 0.5 cm).

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Calli in MS medium containing 0.5 mg l^{-1} of 2,4-D and 1 mg l^{-1} of BA proliferated intensively and formed smooth and puff- shaped (Fig. 5A). The first sign of embryogenesis in this treatment was observed via consecutive subcultures after 4 months (Fig. 5B). Embryos were white and exhibited different developmental stages. However, most of them had formed globular and heart shapes (Figs. 5A-5J). The embryos did not reach maturity in all of the applied growth regulator treatments, but their number increased after each subculture. The cotyledon embryos were able to form leaves and roots, then they grew into plantlets by being located in an appropriate medium with 1/2 MS salts (Fig. 5J). Evaluating embryo maturation on MS and 1/2 MS media showed that MS medium containing 1/2 concentration of macro and micro elements was more effective on embryos maturation.

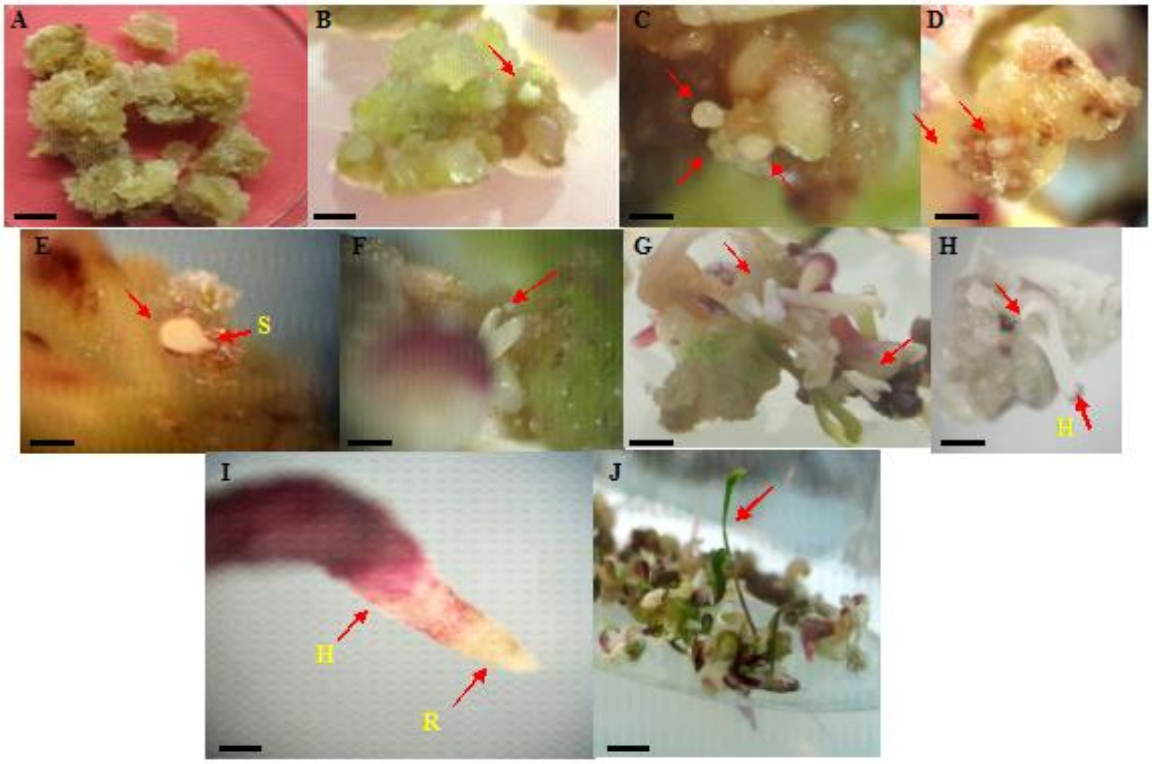

Figure. 5 Macroscopic and stereomicroscopic developmental stages of somatic embryos in the *Ferula gummosa*; **a** Developed embryogenic callus in the MS medium containing 0.5 mgl-1 of BA and 1 mgl-1 of 2,4-D; **b** The first sign of somatic embryogenesis in this medium after 4 months; **c** and **d** Globular stages; **e** Globular embryo with suspensor; **f** Heart shape embryo; **g** Torpedo shape embryo; **h** Cotyledon embryo and hypocotyl formation; **i** Root formation; **j** Developed plantlet from somatic embryo. H: Hypocotyl, R: Root, S: Suspensor, (Scale Bars **a, b, j** = 0.5 cm and Scale Bars **c-i** = 0.05 cm).

Histology of Somatic Embryogenesis

Different embryogenesis stages were observable in the sections of embryogenic mass that included the pre-embryonic stage, globular stage, heart- shaped, torpedo and cotyledon stages (Fig. 6). Non-embryogenic cells in comparison with embryogenic cells were bigger with a smaller nucleus and less condensed cytoplasm (Figs 6A and 6B). Embryonic cells were smaller than their adjacent cells, their cytoplasm was strongly stained, and they had a high nuclear volume compared to their cytoplasm (Fig. 6B). In the periphery of embryogenic callus, meristematic areas were observable in which continuous divisions lead to somatic embryogenesis (Fig. 6C). These small cells were capable of developing new somatic embryos via continuing cellular divisions. Globular embryos were derived from embryogenic cells that were attached to the lower cells by a conservator structure (the suspensor- like organ) (Fig. 6D). Through cell divisions in two poles of the embryos, globular embryos initiated the elongation

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gradually and moved toward bipolarization, therefore cotyledon primary structure was formed and embryos developed a heart- shaped appearance. In this stage, the suspensor was observed too (Fig. 6E). After this stage, a torpedo embryo was observed in which the cotyledons were more developed and the suspensor could still be observed (Fig. 6F). Consecutive divisions in two poles of the embryo lead to induce somatic development and finally, they achieved to form of mature embryos with complete cotyledon development (Fig. 6G). Gradually, vascular bundles were formed in the cotyledon embryos, which were visible at the base of the root (Figs. 6I and 6H). A mature embryo with cotyledon leaves, apical meristem, hypocotyl, and procambium strand can be seen in Fig. 6J.

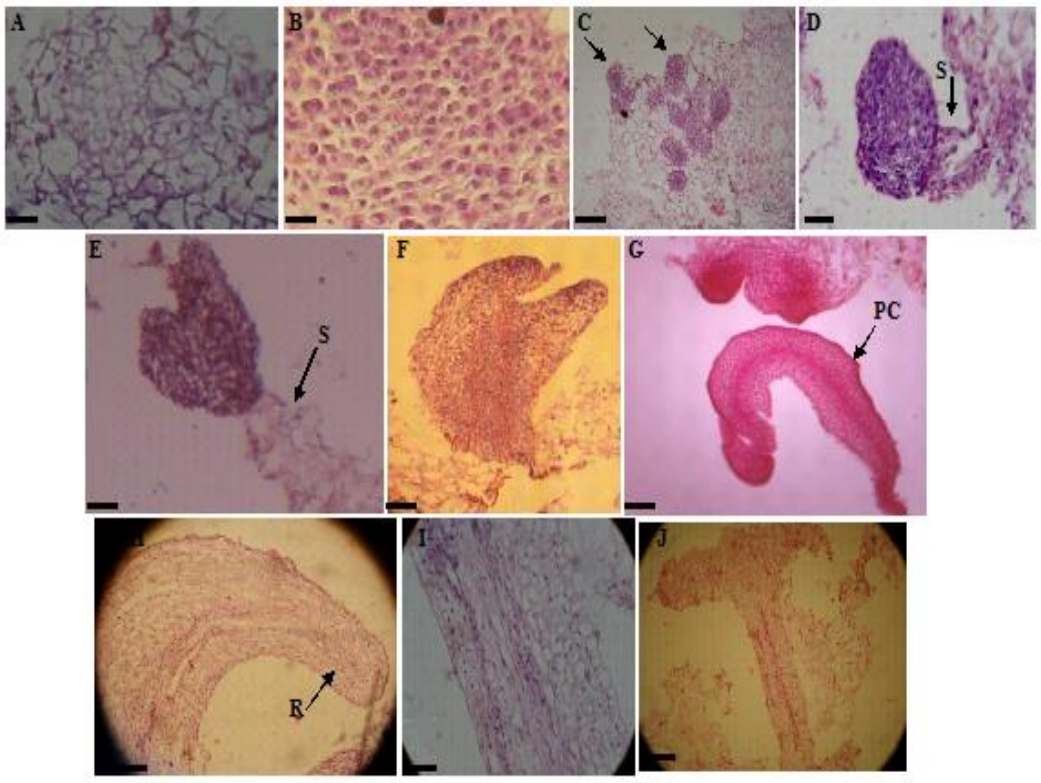

Figure. 6 Sections of embryogenic and non-embryogenic calli and different stages of somatic embryo development; **a** Non-embryogenic cells; **b** Embryogenic cells; **c** Active meristematic area that induces somatic embryo (arrow); **d** Globular embryo with conservator structure; **e** Heart shape embryo; **f** Torpedo embryo; **g** A section of cotyledon embryo in with vascular bundles are determined by arrows; **h** Root formation; **I** Vascular bundles in the root; **j** Complete plantlet including leaf and root. PC: Procambium, R: Root, S: Suspensor (Scale Bars **a** and **b** = 50 µm; **c** = 100 µm; **d, e, f, h, i** = 400 µm; **g** = 500 µm).

DISCUSSION

MS medium was the most effective on root explants' callus induction in comparison with hypocotyls and cotyledon leaf explants. Calli in the growth regulator-free MS medium turned brown and would perish if not transferred to a medium containing growth regulators. Therefore, the activation of somatic cells and the initiation of the cell division cycle to induce callus requires external growth regulators, especially auxin and cytokinin (Pasternak et al., 2002). In this research, comparing the percentage of callus induction for different explants (cotyledon leaf, hypocotyl, and root) in the MS medium containing variable growth regulator components of NAA, 2,4-D, and BA demonstrated that callus formation percentage for root explants was higher than other explants. This result was in compliance with the research of Sarabadani et la., (2008) who reported that root explants of *F. gummosa* respond mostly to callus induction (Sarabadani et al., 2008).

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According to *in vitro* embryo culture results, germination occurred 2-3 days after subculture. It showed a significant reduction in comparison with seed culture treated with cold to reduce dormancy and accelerate germination which was as same as some previous research (Zardari et al., 2019). Our study showed removing the seed coat induced germination and growth. Removing the seed coat caused to inhibit growth inhibitory factors activity, then germination occurred faster (Nadjafi et al., 2006).

In the callus formation process, callus induction occurred mostly at the cutting edge of explants that were in direct contact with the culture medium and gradually spread to the entire explant after a few days. This result was consistent with the results obtained from *Cuminum cyminum* L. (Tawfik and Noga 2002). Also, the application of NAA as auxin along with BA as cytokinin despite the concentrations led to the highest percentage of callus induction, which was consistent with *Lavandula vera* results (Dronne et al., 1999).

The somatic embryogenesis (SE) serves as a valuable model for studying plant cell totipotency, though the molecular mechanisms behind the reprogramming of somatic cells into totipotent cells remain poorly understood. The process requires endogenous auxin biosynthesis, polar auxin transport, and auxin responses, particularly when induced by exogenous auxin (Tang et al., 2020). A key insight from the analyses of the SE-related transcriptomes in numerous plants, is that besides auxin biosynthesis other aspects of auxin action including auxin perception, polar transport and response/signalling are also involved in the mechanism of SE induction. Importantly for the role of auxin in establishing the SE-transcriptome in the explant cells, the specific expression pattern of the auxin-responsive genes is likely due to the specific cellular concentrations of several of the Aux/IAA proteins that seem to result from the auxin content in a cell (Wójcik et al., 2020**)**.

Species belonging to Apiaceae are considered pharmaceutical plants and several studies have been conducted on their somatic embryogenesis so far (Tawfik & Noga, 2002; Ignacimuthu et al., 1999). However, there are scarce studies on *F. gummosa* somatic embryogenesis. The results of a previous study on the embryogenesis of this plant applied by Bernard et al., (2007) indicated that none of the explants responded to growth regulator treatments in the case of somatic embryogenesis induction. Recently, Sharma and Khajuria (2020) succeeded in obtaining somatic embryos from petiole explants in the MS medium supplemented with 2.0 mgl^{-1} 2,4-D and 1.0 mgl^{-1} Kn in *Ferula jaeschkeana*. In this research, callus culture on MS medium containing 0.5 mgl^{-1} of 2,4-D and 1 mgl^{-1} of BA leads to somatic embryogenesis after 4 months. This result was in accordance with Jiménez (2005). They explained that 84% of dicotyledons need a proper ratio of auxin and cytokinin growth regulator components for somatic embryogenesis induction. It has also been reported that the most suitable culture medium for the growth of callus and somatic embryogenesis of *F. gummosa* was the MS culture medium without growth regulator and callus could not grow in other treatments (Bernard et al., 2007). The present results did not confirm the report of Bernard et al., (2007), because the transfer of calli on MS medium without a growth regulator led to their browning and death.

Both the expression of embryonic identity genes and the accumulation of endogenous auxin are essential for this reprogramming. Embryonic identity genes enable somatic cells to gain embryonic competence, while high auxin levels drive the transition to totipotent cells and the initiation of SEs (Zeng et al. 2007). Auxin signaling activates embryogenic programs, while embryonic gene expression enhances auxin biosynthesis, suggesting a feedback loop between these pathways that warrants further investigation (Tang et al., 2020). The polar transport of auxin is crucial for establishing bilateral symmetry during embryogenesis in dicotyledonous somatic and zygotic embryos (Jiménez, 2001). Studies show that auxin polar transport inhibitors can disrupt this process, leading to developmental abnormalities such as fused cotyledons, highlighting its essential role in early embryo patterning (Liu et al, 1993).

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A model proposed by Guzzo et al. (1994) links auxin response, asymmetric division, and totipotency. It suggests that environmental stimuli can make cells competent to respond to auxin morphogenetically. Proper receptor presence leads to complete embryogenesis, while improper receptors result in organogenesis or unorganized proliferation. AUXIN RESPONSE FACTORs (ARFs) and other regulatory proteins are involved in auxin signaling and play crucial roles in somatic embryogenesis (Guzzo et al., 1994).

Somatic embryogenesis is regulated by transcription factors (e.g., LEC1, LEC2, BBM, AGL15, and WUS) that promote totipotency and embryogenic transitions in response to auxin (Yuan et al., 2023). Auxin biosynthesis occurs via tryptophan-dependent (TAA1-YUC) and -independent pathways, with YUC genes being key for SE induction (Li et al., 2022). Auxin also regulates perception, transport (e.g., PIN proteins), and signaling through ARFs like ARF5 (MONOPTEROS), which interacts with WOX2 to balance hormonal pathways. Chromatin accessibility changes induced by auxin activate totipotency-related genes like WOX2 and WOX3 (Wang et al., 2020). Cytokinins play a significant role during the initial cell division phase of somatic embryogenesis but are less critical for later stages like embryo differentiation and maturation. This suggests that cytokinins primarily facilitate cell division rather than embryo differentiation (Wongtiem et al. 2011). Moreover, fewer and rootless SEs are formed when the cytokinin response is suppressed by overexpression of type-A Arabidopsis response regulator (ARR) genes, indicating that cytokinin acts as an important factor for auxin-induced SE formation (Su et al. 2015).

Cytokinin and auxin response patterns play an important role in WUS and WOX5 regional expression and the subsequent embryonic shoot–root axis establishment during somatic embryogenesis (Su et al. 2015). The interplay between auxins and cytokinins is vital for embryogenic responses, where high free indole-3-acetic acid (IAA) levels are often associated with embryogenic potential (Tretyakova et al, 2021).

There are numerous factors influencing somatic embryo maturation such as nutrition poverty, dryness, osmotic alterations, type and concentrations of the sugars in the medium culture, and growth regulators composition (Kärkönen, 2000; Batista et al., 2018). In order to investigate the effect of mineral decrement on embryo maturation, MS and 1/2 MS media were utilized. It was demonstrated that 1/2 MS medium had the most effect on embryo maturation.

Two types of cells were observed in embryonic calli which were in compliance with Onay (2000). Among embryonic calli cells, activated cellular masses in cell division were observed that confirmed. Nucleus compression of embryonic cells is due to asymmetric division of a primary cell forming subsequent small cells that originate embryonic cells (Onay, 2000). Similar characteristics were observed for embryonic cells such as small size, enriched and condensed cytoplasm content, big nucleus, small vacuoles, and few starch granules. These findings agreed with Kärkönen (2000) and Batista et al., (2018).

Apical meristem differentiation of somatic embryos begins at the late globular stage, but the formation of the root apical meristem appears later in the pyramid stage, because it needs auxin polar transport from the shoot apical meristem. Each stage of embryonic development is normally directly or indirectly controlled through the activity of protein kinase, under the regulation of plant hormones by cell signaling (Feraru and Friml, 2008). In the first stage of embryogenesis, a low cytokinin concentration activated the protein kinase, thereby promoting the transcription, stimulating the synthesis of specific proteins and enzymes in cells (signaling pathway) (Osborne and McManus, 2005).

In the anatomical section, a cellular attachment between globular embryos and callus tissue was observed which was reported previously too (Quiroz-Figueroa et al., 2006). Primary procambium

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generated in the final stage of heart-shaped embryos also was observed by Santos et al., 2006. Observation of the procambium strand inside the cotyledon embryo that had no vascular connection between the embryo and maternal tissue, was in accordance with Canhoto et al., 2006 as well. Investigating the process of somatic embryogenesis and studying the anatomical structures of embryogenic calli provides the possibility of more detailed studies on the mechanisms involved in the induction of somatic embryos and the stages of cell differentiation.

Conclusion

According to the obtained results, *in vitro* culture of the embryo to accelerate germination can be consumed for the elimination of long dormancy obstacles. Utilizing variable culture mediums, different growth regulator levels, and explants in the callus induction phase for producing strong and high quality calli, considered as a basic study, is suggested to be used in the suspension culture and enhancing secondary metabolites through it. Micropropagation and somatic embryogenesis of *Ferula gummosa* can be used as an effective technique in the multiple propagation of this valuable endemic plant to save this plant from extinction.

Author Contributions

The following statements should be used "Conceptualization, PJ and MGN; methodology, PJ, MGN and AA; validation, PJ; formal analysis, PJ and MGN.; investigation, PJ, MGN, HH and AA; resources, MGN; data curation, PJ and MGN.; writing—original draft preparation, PJ, MGN, HH and AA; writing—review and editing, PJ; supervision, PJ and MGN; project administration, PJ; funding acquisition, PJ and MGN. All authors have read and agreed to the published version of the manuscript." All authors contributed equally to the conceptualization of the article and writing of the original and subsequent drafts.

Data Availability Statement

Not applicable

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Ethical Considerations

The authors avoided data fabrication, falsification, plagiarism, and misconduct.

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Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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