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Improvement Germination and Conversion of Somatic Embryos and Production of Normal Plantlets in *Ferula Assa-foetida* L. (A Rare Medicinal Plant)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Secondary somatic embryogenesis leads to the formation of abnormal somatic embryos and produces abnormal seedlings. Normal plants are difficult to obtain from these embryos, due to the asynchronous maturation of the embryogenic tissues and low germination and conversion rates. The effects of some media additives and different strengths of MS medium on germination and plantlet formation of in vitro derived somatic embryos of Ferula *Assa-foetida* were studied. The highest number of normal embryos was observed in MS medium containing 30g/l sucrose with 0.5% or 1% AC and in MS medium supplemented with PEG and 0.5% or 1% AC. The treatments of MS medium with 30g/l sucrose and 0.5% AC × MS medium containing sorbitol and MS medium containing PEG and 1% AC × ½ MS had maximum number of normal germinated embryos without secondary somatic embryogenesis (SSE). In some of the treatments the embryos were converted better than the others, such as; the interaction effect of MS medium with 30g/l sucrose and 0.5% AC× MS, MS medium with 30g/l sucrose × MS medium with glutamine. Using different strength of MS medium and presence of some media additives is effective on germination and conversion of somatic embryos into normal plantlet. Presence of Activated Charcoal in the culture medium can reduce secondary somatic embryogenesis.

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Keywords: Somatic embryo germination; conversion; manitol; polyethylene glycol; Activated Charcoal (AC).

1. INTRODUCTION

Ferula Assa-foetida from *Apiaceae* family, commonly known as Asafetida is an herbaceous, perennial medicinal plant native to Iran [1]. Asafetida is very effective due to its culinary and medicinal qualifications including anti-spasmodic, aromatic, carminative, digestive, expectorant, laxative, sedative, nervine, analgesic, anthelmintic, aphrodisiac and antiseptic effects [2].

This is a monocarpic plant that produces the flower only once during life cycle (after 5 or 6 years). Formed seeds have long dormancy, low vigor and germination rates. Thus, plant propagation by seeds is labor and time-consuming.

Somatic embryogenesis has the potential to enable mass production of clonal plants, but low germination rate, unsuccessful conversion into normal plantlets, morphological and structural differences and non-synchronization between maturation and conversion have caused to reduce production of normal plants from these embryos [3]. Some of the reports have shown different morphologies of somatic embryos and mutuality between morphological abnormality and the lack of conversion of somatic embryos into plants [4].

By secondary somatic embryogenesis in *Ferula assa-foetida* new irregular embryos are initiated from all primary embryos [5] and abnormal plantlets with one or multiple cotyledonary structures can be produced [3].

The industrial use of somatic embryos can be limited by the low rates of conversion into plantlets [4]. Manipulation in nutrition medium, environmental factors and selection of optimum conditionsare necessary for having complete conversion [3]. Application of some stress during tissue culture, such as desiccation may increase the numbers of converted embryos [4]. Troch et al. [6] have reported that the application of osmotic stress can improve the quality of embryos, which is an important factor for directing embryo development and maturation.

Lee et al. [4] have found that high concentrations of sucrose have promoted the maturation. In the maturation medium, high sucrose concentration have result in high osmotic condition of the cells and tissues, which has been useful to prevent precocious embryo conversion and enhances embryo maturation [7]. Many authors have reported that various sources of carbon such as manitol and sorbitol have important effects in the germination of somatic embryos [8]. Troch et al. [6] have also reported that decrease in water content of the embryos is mainly achieved by use of polyethylene glycol (as non-plasmolyzing osmoticum). Anjaneyulu [7] has tested the activated charcoal (AC) and the polyethylene glycol (PEG) for the regeneration and maturation of somatic embryos. Burns et al. [9] have also used supplemented media with various concentrations of polyethylene glycol.

Activated charcoal is often used in tissue culture to darken the media and to absorb inhibitory or toxic substances and plant growth regulators [10]. Moreover, charcoal has been used in all stages of somatic embryogenesis to improve yield and quality of somatic embryos during maturation [11-13] and most frequently during germination [14-16].

According to Anjaneyulu [7], different strengths of MS nutrients such as full, one-half and one-quarter were evaluated for somatic embryo germination. In addition, MS basal medium supplemented with media additives such as glutamine, maltose, mannitol, sorbitol and polyethylene glycol have been tested for somatic embryo conversion [7].

In *Ferula assa-foetida* L., abnormal secondary somatic embryos are formed all over the surface of the primary somatic embryos and resulted to low germination and prevents production of normal plantlets from primary somatic embryos. To the best of our knowledge, a little information is available on investigation of problems mentioned above. Thus, the effects of some media additives and different strengths of MS medium on germination and plantlet formation of *in vitro* derived somatic embryos of *Ferula assa-foetida* were studied.

2. MATERIALS AND METHODS

The experiment was carried out at Tissue Culture Laboratory of Agricultural Biotechnology Research Institute of Iran in 2012.The seeds of Asafoetida were collected from the Research Center for Agriculture and Natural Resources, Isfahan, Iran and were sterilized according to Otroshy et al. [17]. Then, disinfected seeds were sown under in vitro culture as described by Roozbeh et al. [18].

2.1 Explant Preparation

2.1.1 Callus induction

The roots of *in vitro* derived plantlets of *Ferula assa-foetida* were excised to 1cm segments and considered as explants for callus induction stage. They were cultured in MS medium combined with 2,4-D (0.5mg/l) to produce embryogenic callus.

2.1.2 Somatic embryogenesis

The somatic embryos were obtained from suspension culture of embryogenic callus in the MS medium containing 2,4-D(0.5 mg/l) and Kin (0.5 mg/l).

2.1.3 Development of the embryos

To develop globular embryos which were derived from previous stage into cotiledonary embryos, they should be cultured in MS medium containing 15g/l sucrose and 15g/l maltose.

2.2 Maturation of Somatic Embryos

Among embryos in different stages (globular, heart shape, torpedo and cotiledonary) single cotiledonary somatic embryos were selected by using a binocular microscope and then cultured in media as mentioned below:

- MS medium containing various concentrations of sucrose (30, 50 or 70g/l) with and without activated charcoal (0.5% or 1%).
- MS medium containing various concentrations of manitol (30, 50 or 70g/l).
- MS medium containing 30 g/l sucrose with activated charcoal (0.5% or 1%) alone or in combination with 1% PEG (6000).

Each petri dish was cultured with 20 embryos for three replications. The lids of Petri dishes were sealed with household plastic foil and were randomly placed in a growth chamber set at 25ºC and 16/8h (light/dark) photoperiod and the light intensity of 3000lux for a period of 1 month.

2.3 Germination of Somatic Embryos and Plantlet Formation

Germination, conversion and plantlet formation were stimulated by transferring mature somatic embryos to regeneration media. These fully developed cotyledonary embryos from maturation experiments were conducted by seven experiment: different strengths of MS medium such as full, one-half and one-quarter; MS medium with 30g/l sorbitol or 30g/l maltose instead of sucrose; MS medium with 1% PEG (6000) or 500mg/l glutamine.

Each petri dish was cultured by 20 embryos with three replications. The Petri dishes were incubated in a growth chamber set at 25ºC and 16/8h (light/dark) photoperiod and the light intensity of 3000lux for a period of 1 month.

Before transferring regenerated plantlets to pots, the plantlets were subcultured on half-strength MS medium containing activated charcoal (1%), and preserved for 5 weeks. Then the plantlets were transferred to pots containing peat moss and perlite (1:1) for continuance of growth in phytotron and transferring to greenhouse.

2.4 Data Collection

The number of embryos that showed secondary somatic embryogenesis (SSE), those produced just root or shoot, length of roots and shoots and the number of failed growth and normal embryos were measured in maturation experiments. Those embryos were transferred to germination media and after one month the number of secondary somatic embryos, normal embryos and well converted embryos were measured. Germination was defined as emersion of root and epicotyl structures [19].

2.5 Experimental Design and Statistical Analysis

Each experiment was conducted as a completely randomized factorial design. Each treatment consisted of 20 explants for three replications. Data were analyzed using the SAS package (version 9.1) statistical computer program. When the ANOVA indicated significant treatment effects (5 or 1%) based on the F-test, the Duncan's Multiple Range Test ($P \leq 0.05$) was used to compare the mean values.

3. RESULTS

3.1 Development and Maturation of Somatic Embryos

The modified MS medium that included both sucrose and maltose (15g/l) showed positive effects on development of embryos. At the maturation experiment some of the somatic embryos developed as normal, just rooted or shooted embryos. No any of growth and maturation was observed in other cultured embryos.

All maturation experiments had highly significant effects on the measured factors. Treatments of 30g/l sucrose with 0.5 or 1% AC, 70g/l sucrose with 0.5% AC and PEG with 0.5% AC had the lowest level of failed growth embryos and all concentrations of manitol (30,

50 or 70g/l) showed the highest level of failed growth embryos. Thus, other results of manitol treatments were ignored (Table 1).

The lowest secondary somatic embryogenesis occurred in MS medium containing PEG with 0.5% or 1% AC, 50g/l sucrose with 1% AC and in 70g/l sucrose. The highest number of normal embryos (with both primary shoots and roots) was observed in MS medium containing 30g/l sucrose with 0.5% or 1% AC and in MS medium supplemented with PEG and 0.5% or 1% AC. The treatments containing all concentrations of sucrose without AC didn't show favorite normal growth (Table 1).

The number of just rooted embryos enhanced by application of PEG and MS medium containing 50g/l sucrose with 0.5% AC (Fig. 1a). MS medium containing 30g/l sucrose and 0.5% or 1% AC; and with PEG and 0.5% or 1% AC showed the minimum value of just shooted embryos (Fig. 1b). 70g/l sucrose without AC had maximum number of just shooted embryos (Table 1).

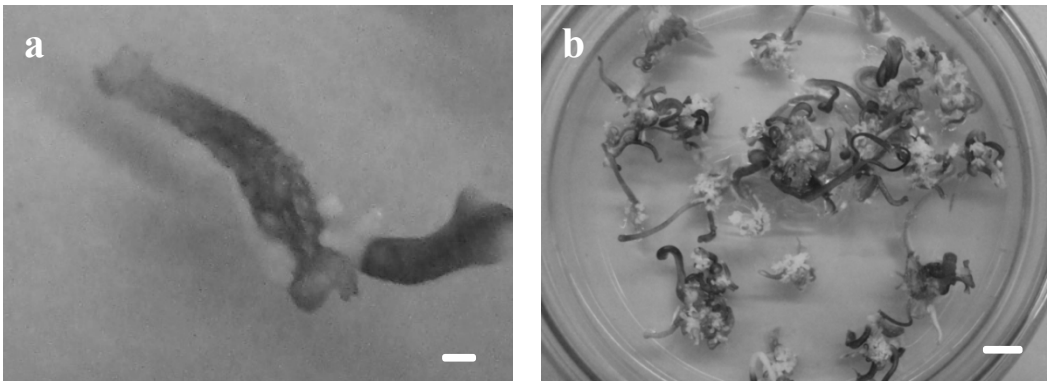

Fig. 1. Just rooted (a) and just shooted (b) embryos. Bar=1mm (a), Bar=1cm (b)

30 g/l sucrose without AC and 70g/l sucrose with 1% AC caused the highest level of shoot high and 30 g/l sucrose with 1% AC and PEG with 1% AC had maximum length of the roots. Apparently presence of 1% AC in the culture medium there seemed to be an optimum concentration for elongation of roots and shoots (Table 1).

3.2 Effects of Different Strengths of MS Medium and Some Media Additives on the Somatic Embryo Germination and Plantlet Formation

3.2.1 Experiment 1: Interaction effects of MS medium with different concentrations of sucrose and activated charcoal ×different strengths of MS medium and some media additives

The interaction effects of maturation and germination medium showed very significant effects in all measured factors. The maximum number of failed growth embryos was found in the embryos that cultured in MS medium with 50 g/l sucrose containing 1% AC and transferred to MS medium containing glutamine (Fig. 2). The lowest level of secondary somatic embryogenesis was observed in MS medium containing 30 g/l sucrose with 0.5 or 1% AC after transferring to MS medium with sorbitol or maltose; and MS medium containing 50g/l sucrose with 0.5% AC that sub cultured in MS medium with sorbitol. Treatments without AC showed highest number of secondary somatic embryos (Fig. 2).

Table 1. Maturation of the somatic embryos

Treatments	Failed growth embryos	SSE	Normal	Just rooted	Just shooted	Shoot length	Root length
30 g/l Sucrose (S30)+0.5% AC	0.24e	5.71c	19.00a	1.04cd	0.67e	0.86bc	1.23bc
30 g/l Sucrose (S30)+1% AC	0.38e	6.29bc	18.95a	1.09bcd	0.38e	0.74bcd	1.41a
50 g/l Sucrose (S50)+0.5% AC	0.86de	6.09c	16.38cde	1.17ab	2.48cd	0.71bcd	0.97de
50 g/l Sucrose (S50)+1% AC	4.62b	4.80 cd	16.95bcd	1.11bcd	1.28ed	0.44d	0.78f
70 g/l Sucrose (S70)+0.5% AC	0.43e	6.29bc	15.19e	1.02cd	4.28b	0.84bc	0.76f
70 g/l Sucrose (S70)+1% AC	0.57de	8.14ab	17.10bcd	1.05bcd	2.71c	0.97ab	1.07cd
30 g/l Sucrose (S30)	0.66de	8.29a	15.62de	1.00d	3.71bc	1.19a	0.80ef
50 g/l Sucrose (S50)	2.19c	8.62a	13.52f	1.02cd	4.71b	0.73bcd	0.65f
70 g/l Sucrose (S70)	1.95cd	4.48cd	5.57g	1.02cd	14.19a	0.62bcd	0.30g
30 g/l Manitol (M30)	20.00a	0.00e	0.00h	1.00d	0.00e	0.58cd	0.11g
50 g/l Manitol (M50)	20.00a	0.00e	0.00h	1.00d	0.00e	0.50cd	0.205g
70 g/l Manitol (M70)	20.00a	0.09e	0.00h	1.00d	0.00e	0.50cd	0.22g
PEG+0.5% AC	0.24e	3.29d	18.38ab	1.14abc	1.00e	0.69bcd	1.12cd
PEG+1% AC	0.76de	4.86cd	17.86abc	1.25a	0.52e	0.67bcd	1.32ab

*** Significant at p≤0.01, * Significant at p≤0.05. Values followed by a similar letter are not statistically significantly different*

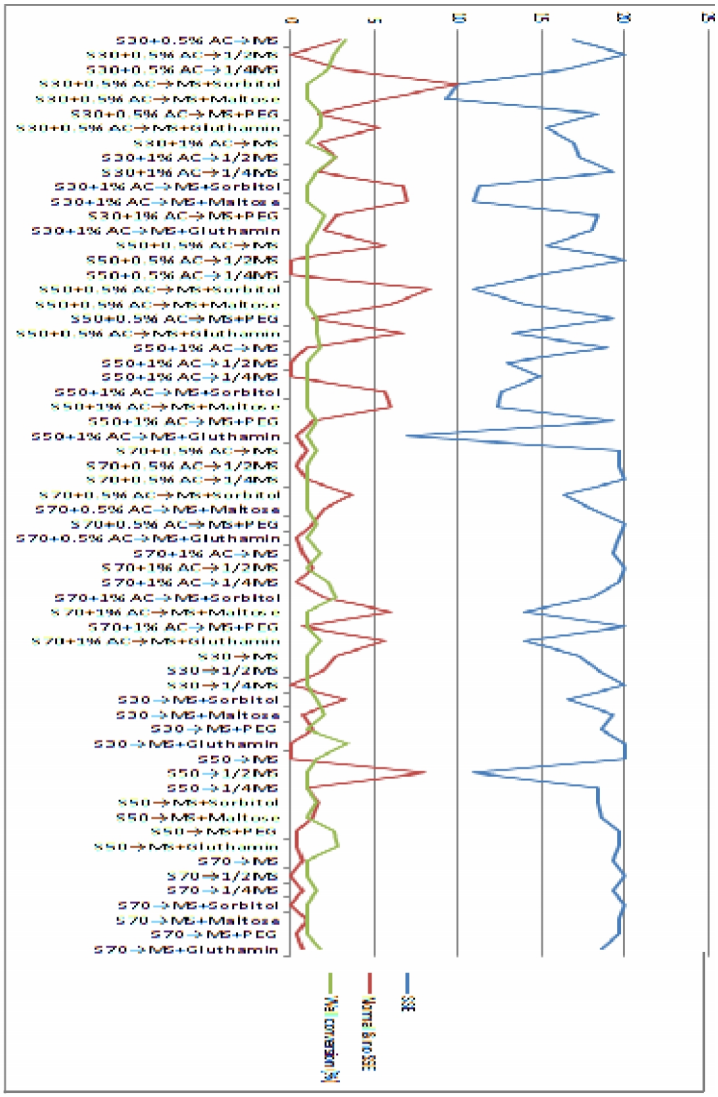

Fig. 2. Interaction effects of MS medium with different concentrations of sucrose and activated charcoal × different strengths of MS medium and some media additives (From maturation media → germination media)

The embryos matured in MS medium with 30g/l sucrose and 0.5% AC and germinated in MS medium combined with sorbitol had maximum number of normal germinated embryos without SSE (embryos germinated into both shoots and roots). Also the interaction effect of MS medium with 30g/l sucrose and 1% AC × MS medium supplemented by maltose; and MS medium with 50g/l sucrose and 0.5% AC × MS medium combined with sorbitol showed high level of normal germinated embryos without SSE (Fig. 2).

In some of the treatments the embryos were converted better than the others, such as; the interaction effect of MS medium with 30g/l sucrose and 0.5% AC× MS, ½ MS or ¼ MS and the same maturation medium with 1% AC × ½ MS; MS medium with 70 g/l sucrose with 1%

AC× ¼ MS or MS containing sorbitol; MS medium with 50 g/l sucrose × MS medium containing PEG or glutamine (Figs. 2 and 3).

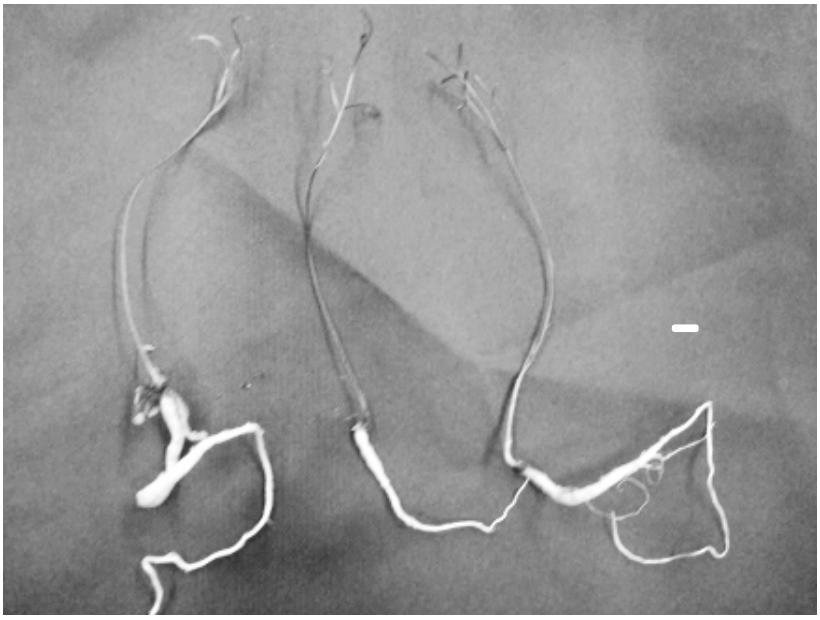

Fig. 3. Well converted embryos. Bar= 1cm

3.2.2 Experiment 2: Interaction effects of MS medium with different concentrations of sucrose and manitol × different strengths of MS medium and some media additives

The interaction effects of maturation and germination medium had significant effects in all factors. MS medium containing 70g/l manitol for maturation and MS medium supplemented with sorbitol or glutamine for germination showed the highest level of failed growth embryos (Fig. 4). The embryos cultured in MS medium containing 30, 50 or 70g/l manitol (for maturation) after transferring to sorbitol (for germination) and MS medium with 70g/l manitol cultured in MS medium containing maltose, showed the minimum number of secondary somatic embryos (Fig. 4).

Normal germination of the embryos without producing SSE was observed in some of the treatments such as; MS medium with 30g/l manitol × MS medium with sorbitol or ¼ MS; MS medium with 50g/l manitol × MS medium with or without maltose, PEG or glutamine; MS medium with 70g/l manitol × ½ MS (Fig. 4).

The best result for well conversion of the embryos was obtained from interaction effect of MS medium with 30g/l sucrose × MS medium with glutamine; MS medium with 50g/l sucrose × MS medium with PEG or glutamine (Fig. 4).

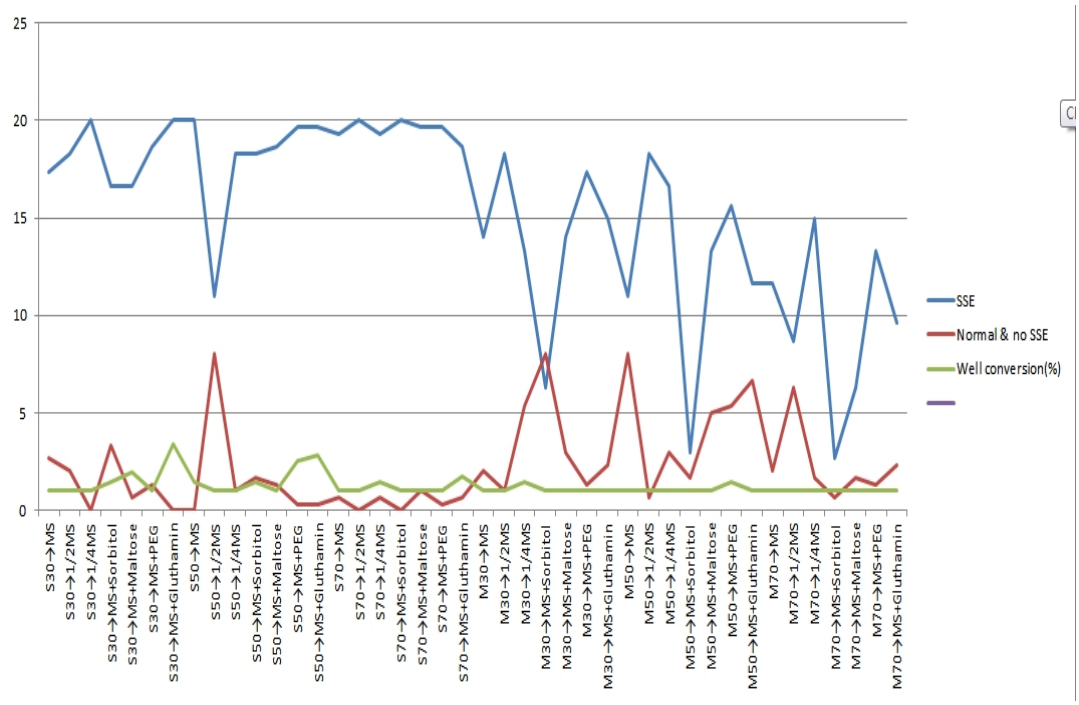

Fig. 4. Interaction effects of MS medium with different concentrations of sucrose and manitol × different strengths of MS medium and some media additives (From maturation media → germination media)

3.2.3 Experiment 3: Interaction effects of MS medium with PEG treatments with activated charcoal × different strengths of MS medium and some media additives

The interaction effects of maturation × germination medium on SSE and normal germination of the somatic embryos were highly significant but in other factors weren't observed any significant effect. MS medium with 30g/l sucrose and 0.5% AC × ½ MS showed the maximum value for secondary somatic embryogenesis, in comparison with other treatments in this experiment (Table 2). While MS medium in combination with PEG and 0.5% AC × ½ MS was the best treatment to reduce secondary somatic embryogenesis. Also, MS medium with 30 g/l sucrose and 0.5% AC × MS medium containing maltose; MS medium containing PEG with 0.5% AC × ¼ MS; MS medium supplemented with PEG and 1% AC × ½ MS or MS medium containing maltose had the minimum of secondary somatic embryogenesis (Table 2).

The interaction effects of MS medium containing PEG and 0.5% AC × ½ MS for maturation and germination respectively, showed the best result for production of normal germinated embryos without SSE. The treatments of MS medium with 30g/l sucrose and 0.5% AC × MS medium containing sorbitol; MS medium containing PEG with 0.5% AC × ¼ MS; MS medium containing PEG and 1% AC × ½ MS, MS or MS medium containing maltose had also the maximum number of normal embryos without SSE (Table 2).

Table 2. Interaction effects of MS medium with PEG treatments with activated charcoal × different strengths of MS medium and some media additives

Maturation treatments	Germination treatments	SSE	Normal & no SSE
S30+0.5%	MS	17.00abc	3.00ghij
S30+0.5%	1/2MS	20.00a	0.00j
S30+0.5%	1/4MS	16.67abcd	3.00ghij
S30+0.5%	MS+Sorbitol	10.00fgh	10.00a-d
S30+0.5%	MS+Maltose	9.33gh	5.67d-h
S30+0.5%	MS+PEG	18.33ab	1.67hij
S30+0.5%	MS+Gluthamin	15.33a-e	5.33efgh
S30+1%	MS	17.00abc	1.67hij
S30+1%	1/2MS	17.33ab	2.67ghij
S30+1%	1/4MS	19.33ab	0.66ij
S30+1%	MS+Sorbitol	11.33efg	6.67c-g
S30+1%	MS+Maltose	11.00efg	7.00c-g
S30+1%	MS+PEG	18.33ab	2.67ghij
S30+1%	MS+Gluthamin	18.00ab	2.00hij
PEG +0.5%	MS	14.67b-f	5.33efgh
PEG+0.5%	1/2MS	5.00h	13.67a
PEG+0.5%	1/4MS	8.67gh	10.33 abc
PEG+0.5%	MS+Sorbitol	15.66a-e	4.33f-j
PEG+0.5%	MS+Maltose	10.67efg	7.67cdef
PEG+0.5%	MS+PEG	14.67efg	5.00efghi
PEG+0.5%	MS+Gluthamin	12.00c-g	7.67cdef
PEG+1%	MS	10.67efg	9.33a-e
PEG+1%	1/2MS	7.66gh	12.33ab
PEG+1%	1/4MS	14.67b-f	5.33efgh
PEG+1%	MS+Sorbitol	11.67defg	8.00b-f
PEG+1%	MS+Maltose	7.00gh	10.00abcd
PEG+1%	MS+PEG	15.67a-e	3.67f-j
PEG+1%	MS+Gluthamin	11.67defg	6.67c-g

*** Significant at p≤0.01, * Significant at p≤0.05. Values followed by a similar letter are not statistically significantly different*

4. DISCUSSION

4.1 Development and Maturation Stage

Based on our preliminary observations the favorite development of the embryos was occurred in modified MS medium with 1.5% sucrose and 1.5% maltose. Lee et al. [4] have also reported that in MS medium without sucrose or with 1.5% sucrose, the embryos matured more quickly to the cotyledonary stage than those of the control (3% sucrose). Tang et al. [20] have also reported that plantlet development will be improved by using lower sucrose concentrations. Based on Strickland et al. [21] maltose improved the development of alfalfa somatic embryos, concluded that the primary effects of maltose caused by its nutritional and not osmotic influence.

Our results showed that, the presence of PEG and AC (0.5%) can reduce the undesirable traits such as; the rate of failed growth embryos and just shooted embryos and SSE.

Stasolla and Yeung [22] reported that the effect of PEG behavior the naturally occurring water stress on seeds during the late stages of maturation. Maruyama [23] also reported that the beneficial effect of PEG on embryo maturation may be related to its giving rise to water stress and inducing storage reserve synthesis. Based on results of Capuana and Deberg [24] cultures on media containing PEG in combination with AC showed a better maturation. Capuana and Deberg [24] reported lower frequency of secondary embryogenesis, which can be considered as an expression of the maturation of somatic embryos that observed in embryos cultured on media containing PEG. These results confirm the role of PEG in improving maturation and post-maturation phases and increasing somatic embryo germination and conversion [25].

According to Capuana and Deberg [24] in media containing PEG and manitol maturation was higher. But in our experiment, manitol showed the highest number of aborted embryos and didn't have proper effect on maturation.

MS medium with 30g/l sucrose and 0.5% or 1% AC was also the appropriate medium for maturation of somatic embryos; because of low content of failed growth embryos and just shooted embryos and high number of normal matured embryos and maximum of root length. Our results showed that presence of AC in the culture medium is necessary for normal growth of the embryos and it is useful for elongation of roots and shoots. Buchheim [26] obtained that maturation of somatic embryos occurred on MS basal medium supplemented with 0.5% (w/v) activated charcoal. Tang et al. [20] reported that the addition of activated charcoal enhanced root development. The highest concentration of sucrose (70 g/l) reduced SSE but didn't show normal embryos. It has been observed that elevated concentrations of carbohydrates create osmotic stress and improve embryogenesis [27].

4.2 Germination Stage

In experiment 1 the interaction effect of different concentrations of sucrose and activated charcoal × different strengths of MS medium and some media additives were investigated. The treatments of 30g/l sucrose with 0.5% or 1% AC× MS medium containing sorbitol or maltose were the favorite treatments for embryo maturation and germination because of maximum number of normal germinated embryos and minimum number of SSE. The addition of sucrose, maltose or sorbitol as osmoticum in the culture media has been frequently reported to effectively promote somatic embryo maturation and germination in a number of species [28,29]. Agarwal et al. [27] reported that the percentage of secondary somatic embryogenesis at 3% sucrose was lower than 6% sucrose, and in the presence of maltose SSE was lower. Corredoira et al. [29] observed that a few secondary embryos occasionally were differentiated in maltose-supplemented media. Zouine et al. [30] also obtained that the addition of activated charcoal maybe useful for enhancing the germination of somatic embryos.

The treatments without AC had the highest rate of SSE. Pinto et al. [31] reported that activated charcoal promoted a significant increase in growth in terms of relative size and weight of somatic embryos, as well as a more efficient control of secondary somatic embryogenesis. Charcoal is generally used to absorb hormones [32]. According to Daigny et al. [33], moderate concentrations of 2,4-D induce secondary somatic embryogenesis. Aderkas [34] states that, 2,4-D promotes cleavage poly-embryony, they also reported that Charcoal reduces 2,4-D concentrations of embryos by an order of magnitude greater than PGR-free medium alone. Thus we can conclude that Activated charcoal absorbs internal

2,4-D of the somatic embryos (in our experiment 2,4-D used in callus induction and somatic embryogenesis stage) and reduces secondary somatic embryogenesis.

From treatments that had the maximum of conversion, the treatment of MS with 30g/l sucrose and 0.5% AC × MS was the best treatment. Anjaneyulu [7] also reported that among different strengths of MS nutrients used for conversion of somatic embryos, full-strength MS basal medium showed the best response compared to half-strength MS and quarter-strength MS nutrients.

In experiment 2 we observed the interaction effect of different concentrations of sucrose and manitol × different strengths of MS medium and some media additives. MS medium with 70g/l manitol × MS medium containing sorbitol was appropriate medium for reduce failed growth embryo and SSE. All concentrations of manitol after transfer to MS medium containing sorbitol reduced SSE. According to Tang et al. [20] secondary somatic embryo production depends on the presence of sucrose; they stated that manitol and sorbitol strongly suppress somatic embryo production when compared with sucrose. MS medium containing 30g/l manitol × MS medium containing sorbitol or ¼ MS and MS medium with 50 g/l manitol × MS medium with or without maltose, PEG or glutamine showed the maximum number of normal germinated embryos. Maruyama [23] obtained that the addition of PEG to the medium stimulated maturation of somatic embryos and subsequently enhanced germination and conversion efficiency. Hilae and Te-chato [35] found that sorbitol is a suitable osmoticum for induction of shoot and root and production of normal plantlet. Lee et al. [4] obtained that changes in the chemical conditions during the tissue culture of embryogenic callus increased the induction, maturation and germination levels of somatic embryos.

As already mentioned the best conversion of the embryos took place from interaction effect of MS medium with 30g/l sucrose × MS medium with glutamine, MS medium with 50g/l sucrose × MS medium with PEG or glutamine. Findings of Anjaneyulu [7] indicated that MS medium supplemented with glutamine showed the highest frequency of somatic embryo conversion.

Experiment 3 showed interaction effects of PEG treatments with activated charcoal × different strengths of MS medium and some media additives. In this experiment the treatment of MS medium in combination with PEG and 0.5% AC ×½ MS was the best treatment for reduce SSE and enhance normal germination of the somatic embryos. Maturation of somatic embryos on PEG-supplemented media subsequently increased both the frequency of germination and the number of embryos converted into plantlets. PEG is a potent osmoticum induces stress conditions equivalent to drought; when added to the medium the cells adapt to PEG accumulate additional sugars and amino acids [36]. Lee et al. [4] reported that somatic embryos were cultured with low concentrations of MS salts and 3% sucrose showed increase in germination rate.

The results indicated that for well conversion of the embryos 0.5g/l AC is better that 1%. Anjaneyulu [7] also reported that 0.5g/l charcoal concentration showed the best response of conversion frequency.

5. CONCLUSIONS

The results obtained in this study show that using different strength of MS medium and presence of some media additives particularly sorbitol, PEG and glutamine are effective on

germination and conversion of somatic embryos into normal plantlet. Presence of Activated Charcoal in the culture medium can reduce secondary somatic embryogenesis and enhances development of somatic embryos´ roots and shoots.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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