USE OF SOMATIC EMBRYOGENESIS AS AN EFFECTIVE APPROACH FOR DEVELOPING NEW STRAINS OF HEIRLOOM FOREIGN CARROT (*DAUCUS CAROTA* L.) CULTIVARS TO PRODUCE BOLTING-TOLERANT CULTIVARS UNDER EGYPTIAN CONDITIONS

ABSTRACT

The process of induction of tissues in the laboratory and inducing the formation of somatic embryos requires pushing the somatic cells to divide and form a new polarity. It was found that some auxins have an important role, especially 2, 4-D (2,4-dichlorophenoxy acetic acid), which an important role in the formation of these embryos. This experiment was carried out to investigate the effect of five procedures in a tissue culture laboratory using two concentrations of 2, 4-D at a concentration of 2.5 and 5 mg/l for indirect somatic embryos induction from sexual embryos produced from culturing carrot (*Daucus carota* L.) seeds of Carrot cv. Kuroda (an heirloom variety). The results confirmed by scanning electron microscopy showed that it is crucial to compare the various processes used to create somatic Embryogenesis to assess their effectiveness. The four stages of somatic embryos, globular, heart, torpedo, and cotyledon form, were observed on the formed callus. The highest amounts of callus fresh weight, the number of globular-shaped stage embryos, the number of heart-shaped stage embryos, the number of torpedo-shaped stage embryos, the number of cotyledon-shaped stage embryos, and the number of seedlings had been recorded in the media supplemented with 2, 4-D at a concentration of 5 mg. These results constitute a step in the way in the field of developing new strains of heirloom foreign carrot (Daucus carota l.) cultivars to produce bolting-tolerant cultivars under Egyptian conditions; this study was able to produce somatic embryos of the foreign carrot cv. Kuroda. However, since selected strains' production relies on the development of somatic embryos, more work and research are required to address some of the issues that have been noted and will be of interest to the research team in subsequent investigations.

Keywords: Carrot, *Daucus carota* L., somatic embryogenesis, seed production, 2,4-D.

INTRODUCTION

A rich source of essential vitamins and minerals is carrots. According to 73% of your daily vitamin A needs, 9% of your daily vitamin K needs, 8% of your daily potassium and fiber needs, 5% of your daily vitamin C needs, and 2% of your daily calcium and iron needs can all be met by a half-cup. Antioxidants are abundant in carrots, which have several health benefits. The main ideas are as follows: They are healthy for your eyes, can reduce your risk of cancer, are good for your heart, can strengthen your bones, can improve your immune system, can ease constipation, can help you manage diabetes, and can be beneficial for your heart **[1]**.

One of the top 10 vegetables farmed worldwide is the carrot. On an annual basis, 1,137,738 hectares throughout the world produce an average of 42,158,403 tons of carrots and turnips, with an average yield of 37 tons/ha. The production of carrots worldwide increased from 40, 265, 109 tons in 2015 to 44, 762, 859 tons in 2019, and future increases are anticipated. The greatest carrot producer in the world is China, whereas the major carrot markets are in North America, specifically Canada and the United States of America. Europe is reportedly the region with the quickest rate of carrot market growth. Worldwide consumption of carrots and turnips in 2020 was 46.3 million metric tons. Also, FAO reports showed that the production of carrots increased from 40,265,109 tons in 2015 to 44,762,859 tons in 2019. Annual Egypt production (2021) is about 28.7681 tons of carrot from 7694 hectares; while, annual world production (2021) is about 38.016 tons of carrot from 1096007 hectares **[2]**.

Many factors affect the induction of embryogenesis in vitro. The sample cultivation conditions have a significant impact on the process of producing haploid and doubled haploid carrot plants, despite the protocol's existence. As a result, this technique must be enhanced for particular genotypes **[3]**. Global climatic shifts and increasing population pressure have increased the demand for agronomic resources, resulting in a shortage of arable land. They have also sparked several abiotic stresses that, when combined with biotic ones, cause physiological and metabolic disorders that ultimately have an impact on yield at the exact time that it needs to be improved. The influence of stress on yield is a significant scientific and agronomic problem, and biotechnology breeding would be a useful solution. Therefore, it is preferable to create regenerates and transformants by somatic

embryogenesis to limit the hazards of soma clonal variants. A somatic cell can be used by plants to reproduce bipolar structures through a process known as somatic embryogenesis. Explants can be treated with growth regulators or stressors to in-vitro promote somatic embryogenesis. Just a specific subset of cells can alter their cell fate and produce somatic embryos. Somatic embryogenesis is a complicated and poorly understood phenomenon. Differential responses among cells can be sparked by a variety of circumstances, with the genotype, physiological condition, and explant origin being particularly important. Other elements to consider include the culture media or plant growth regulators utilized for in-vitro culture **[4].**

When a plant cell generates embryos without callus development, this is known as the direct pathway. Before embryo development, the indirect pathway requires an additional stage for callus formation **[5]**. Somatic embryogenesis has seven steps: induction, expression, multiplication, development, maturation, germination, and plant conversion. These phases are defined by many physiological, morphological, and molecular phenomena. **[6]**. It is known that auxins, notably 2,4-dichloro phenoxy acetic acid (2,4-D), are required for somatic embryogenesis induction and embryo multiplication, which increases the number of embryos that may be created by indirect somatic embryos. **[7]**. But, at the following expression, this plant growth regulator (PGR) must be removed. The globular, heart, torpedo, and cotyledon stages in dicotyledonous species, the globular, scutellar, and coleoptile stages in monocotyledonous species, and the early and late embryogenesis stages in gymnosperm species are examples of equivalent zygotic embryos that display the same developmental stages as embryogenic. However, zygotic embryos mature and grow inside the endosperm while somatic embryos arise from somatic cells without endosperm, therefore they differ in the stages of maturation and germination. **[8]**. somatic embryogenesis technique uses in agriculture have been produced by utilizing the advantages of a vegetative regeneration system with the capacity for long-term preservation **[9]**. Two types of crops are grown for the manufacturing of artificial seeds: the first, those with somatic embryos of excellent quality; the second, those that have a strong commercial foundation **[10]**. Somatic embryogenesis may be a useful tool for growing plants and preserving their propagules for a suitable amount of time **[11]**. The growth of plant species that are unable to generate seeds, such as seedless watermelons and grapes, depends on the manufacture of artificial seeds **[12]**. Somatic embryogenesis technique can be used to create polyploids with superior features, preventing genetic recombination that occurs when these plants are reproduced using traditional plant breeding techniques. This can save time and money. To produce hybrid seeds, Somatic embryogenesis technique can be used to create polyploids with superior features, preventing genetic recombination that occurs when these plants are reproduced using traditional plant breeding techniques. This can save time and money. To produce hybrid seeds, artificial seeds may also be employed to increase the number of male or female sterile plants may also be employed to increase the number of male or female sterile plants **[13]**. An essential method for transgenic plants is the use of somatic embryos, in which a single gene can be inserted into a somatic cell, where it will then be found in all the plants derived from this cell **[14]**. The substantial scale-up necessary for multi-clone commercial production may be addressed by somatic embryogenesis technique **[15]**. Moreover, using this technique reduces the amount of time, space, and media needed for typical tissue culture methods **[16]**. Comparing somatic embryogenesis generation to conventional tissue culture techniques has many benefits. somatic embryogenesis are simple to handle, plant, and transport, and they may be produced at a reasonable cost. Dehydration and cryopreservation methods can also be used to store them for a long time **[17]**.

This research aims to determine the appropriate focus in inducing somatic embryos of carrots cv. Kuroda (an heirloom variety) for developing new strains of heirloom foreign carrot (*Daucus carota* l.) cultivars to produce bolting-tolerant cultivars under Egyptian conditions, as well as conducts an electronic scan study for the stages of the indirect development of somatic embryos.

MATERIALS AND METHODS

Plant Material and Germination

This study was conducted in the tissue culture laboratory of the Potato and Vegetatively Propagated Vegetables Department, Horticulture Research Institute, Agricultural Research Centre, Egypt, In the period from 2017 – 2019, to propagate carrot (*Daucus carota* L.) cv. Kuroda (an heirloom variety). Several preliminary experiments were conducted to overcome some technical problems such as contamination, deformed embryos, and choosing the appropriate range of hormone concentration in the media used. Seeds were obtained from the Vegetable Seed Production Unit, Vegetable Research Departments, Dokki, Giza, Egypt. The seeds were washed in detergent and running water, surface sterilized for 30 seconds with 70% ethanol and then exposed to 1% sodium hypochlorite for 10 minutes. To reduce the surface tension of the Na-Hypochlorite solution, one drop of Tween 80 was added. The seeds were then three times washed with sterile

water. The Murashige and Skoog (MS) medium **[18]** was then saturated on filter paper (Whatman No. 1), and the individual seeds were then inoculated in the jars for germination. Following the germination of five seeds per jar, they were incubated at 25±1 °C with lights on and a photoperiod of 16 h/8 h (light/dark), at a photo mole concentration of μmol/m^2/s. Once germination has been completed for two to three weeks, the single cotyledonous leaf is separated into two parts, leaving the ends off to increase the wound surface and promote callus formation.

Embryogenic Callus Induction

At dosages of 2.5 and 5.0 mg/L, 2,4-D ((2,4-Dichlorophenoxyacetic acid)) was added to the MS medium containing 3% (w/v) sucrose and the sterilized explants. The containers for the solid cultures were tubes with a 25 ml capacity and jars with a 30 ml capacity of the basic nutritional medium, respectively. Agar made up 0.8% of the solid medium. The pH was adjusted to 5.7 ± 0.1 before autoclaving at 121°C for 15 minutes. at a media incubation temperature of 25°C and a constant illumination of 60 μmol/m^2/s. About six weeks passed during this stage.

Embryogenesis and Maturation

The ability of the carrot to undergo somatic embryogenesis was investigated using two different 2,4-D concentrations (2.5 and 5.0 mg/L) in MS medium in solid media. Three pieces of the callus from the previous phase were placed in each jar, which was subsequently incubated at 25± 1°C with continuous light at 60 μmol/m^2/s. The somatic embryo's induced organs were created after six weeks, and they were then repeatedly rinsed with a hormone-free basal medium before being placed there. Embryo counting began six weeks after conception when they began to form globular, heart-shaped, and torpedo-shaped embryos that later gave rise to tiny plantlets.

Germination of Embryos

As the embryos had grown and reached the cotyledonary stage, they were transferred to the germination DCR media (Douglas-fir Cotyledon Revised medium) without growth regulators. according to descriptions of **[19]**, contains both macro- and micronutrients (1985). This form of media was developed to promote the development of fresh shoots. This formulation includes NH_4NO_3 and KNO_3 concentrations that are roughly 1/4 higher than MS. More Ca^{+2} and NO_3- ions can be found in $Ca(NO_3)_2$. Embryos were extracted using sterile forceps and put in Petri plates with DCR media. Once each plantlet had grown into a plant, it was transferred to the culture jar (PhytoTechnology) containing the MSO medium.

Somatic Embryogenesis pathways Studied

In this paper, we studied three somatic embryogenesis pathways. Callus induction and embryo development were included in each pathway, as maturation, and germination on solidified medium in all pathways.

In the first pathway, Three steps are involved in plant regeneration by somatic embryogenesis:

1. Initiation of callus by growing the first explants (Cotyledonous leaf slices obtained from carrot seed germination under sterile conditions) on a Hormone-free medium (solidified medium).
2. On solidified media supplemented with 2,4-D (2.5 and 5.0 mg/L), embryogenic colonies proliferate similarly as under initiation.
3. Maturation of somatic embryos by culturing on a Hormone-free medium reduced osmotic potential. The two cotyledonous leaves obtained from one seed in this procedure produced 18 jars, with an interval of transfer to the next medium at 45 days interval.

In the second pathway, Three steps are involved in plant regeneration by somatic embryogenesis:

1. Growing embryogenic cultures by using the primary explants (Cotyledonous leaf slices obtained from carrot seed germination under sterile conditions) on a medium supplemented with 2,4-D (2.5 and 5.0 mg/L), as auxin.
2. The growth of embryogenic cultures on supplemented solidified media without hormones is comparable to under initiation.
3. Maturation of somatic embryos by culturing on a Hormone-free medium reduced osmotic potential. The 2,4-D concentration of 2.5 mg/L in this pathway has been called procedure 2; while the 2,4-D concentration of 5 mg/L in this pathway has been called procedure 4. The two cotyledonous leaves obtained from one seed in this procedure produce 18 jars, with an interval of transfer to the next medium 45 days.

In the third pathway, Three steps are involved in plant regeneration by somatic embryogenesis:

1. Growing embryogenic cultures by using the primary explants (Cotyledonous leaf slices obtained from carrot seed germination under sterile conditions) on a medium supplemented with 2,4-D (2.5 and 5.0 mg/L), as auxin.

2. The proliferation of embryogenic cultures on solidified medium supplemented with 2,4-D (2.5 and 5.0 mg/L) is similar to under initiation.
3. Maturation of somatic embryos by culturing on a Hormone-free medium reduced osmotic potential. The 2,4-D concentration of 2.5 mg/L in this pathway has been called procedure 3; while the 2,4-D concentration of 5 mg/L in this pathway has been called procedure 5. The two cotyledonous leaves obtained from one seed in this procedure produce 54 jars, with an interval of transfer to the next medium 45 days.

Studied traits

Callus fresh weight, number of globular-shaped stage embryos, number of heart-shaped stage embryos, number of torpedo-shaped stage embryos, number of cotyledon-shaped stage embryos, and number of seedlings were measured. These measurements were obtained as an average of the number per jar produced from one germinated seed. Three jars were taken at random, representing one replicate of each procedure.

Scanning Electron Microscopy

To prepare specimens for scanning electron microscopy, they were fixed for 4 hours at 4 °C in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0), dehydrated in ethanol using a critical point drier, and then coated with gold in an ion coater **[20]**. The specimen was examined under a scanning electron microscope, and photos of it were taken.

Counting the somatic embryo-stages

When embryo tissue (ET) was created by prior reports, the embryonic callus that was growing on a solid medium was transferred to a suspension culture **[21]**. The suspension cultures were initiated shortly after 1 g of embryo tissue (ET) was added to 30 mL of liquid proliferative medium in 100 mL Erlenmeyer flasks, which were then set on an orbital shaker at 90 rpm. After 7 days of subculturing, the suspension cultures (2 mL) were dispersed on top of a stack of five sterile filter paper sheets. The cultures were kept in the filter paper after the liquid media was absorbed. Using a stereo microscope, the evaluation involved counting the somatic embryos that had formed in each plot during the globular, heart, torpedo, and cotyledonary stages. In the current investigations, carrot somatic embryos were divided into four categories: globular embryos, heart-shaped embryos, torpedo-shaped embryos, and embryos in the cotyledonary stage. Globular embryos were those that lacked any indication of cotyledon commencement and had an axial length of between 100 and 300 mm. Heart-shaped embryos were those with cotyledon emergence and an axial length of roughly 300-900 mm. Embryos with an axial length higher than 900 mm and no root development were referred to as torpedo-shaped. Embryos in the cotyledonary stage are those that have roots. Because they were seen to develop into plants, twin embryos (those with two cotyledonary sections), which are often categorized as defective embryos, were counted as normal in the current study **[22]**.

Experimental design:

The experiment followed a completely randomized design, with three replications per procedure. Experimental data were statistically analyzed using analysis of variance (ANOVA). Obtained data of the different procedures for producing Somatic Embryogenesis were separated by using Duncan's multiple range tests, a least significant difference (LSD) at $p \leq 5\%$ level. The statistical analysis consisted of a two-factor; the first factor; is the concentration of (2,4-Dichlorophenoxyacetic acid) 2,4-D (2.5 and 5.0 mg/L of medium), and the second factor; is the proposed procedure for producing embryo tissue stages. The data were statistically analyzed using the CoStat package program, version 6.4 **[23]**. The differences among the means of treatments were tested using the least significant differences (LSD) at 0.05 level of probability according to the method described by **[24]**; and **[25]**. The experiment was done twice, and both times the findings revealed the same pattern.

RESULTS

The results obtained from the first procedure (after 45 days) of cultivation on the medium showed that the embryonic callus did not form, and therefore this procedure was not included in the statistical analysis process.

Mean squares and probabilities for combined ANOVA over two experiments and the different procedures for producing Somatic Embryogenesis in carrots are presented in Table (1) showing that all the traits of embryonic callus and somatic embryos formation varied significantly among the procedures for producing embryonic callus and somatic embryos formation. There was no significant difference among the experiments for all the traits. There were no significant differences among interactions of Procedures X Experiments. The absence of significant differences among interactions of Procedures X Experiments indicated that results were similar in both experiments.

Considerable significant variations were observed in callus fresh weight, number of globular-shaped stage embryos, number of heart-shaped stage embryos, number of torpedo-shaped stage embryos,

number of cotyledon-shaped stage embryos, and number of seedlings for the different procedures for producing somatic embryogenesis (Table 2). As shown in table 2, the significantly highest callus fresh weight was obtained in the fifth procedure.

As shown in Table 2, a statistically significant difference exists between the different procedures for producing somatic embryogenesis for the number of globular-shaped stage embryos, number of heart-shaped stage embryos, number of torpedo-shaped stage embryos, number of cotyledon-shaped stage embryos, and number of seedlings. Based on the comparison of the mean value, the statistically highest number of globular-shaped stage embryos (355), heart-shaped stage embryos (1133), torpedo-shaped stage embryos (1570), cotyledon-shaped stage embryos (233), and the number of seedlings (201) were counted in the fifth procedure.

As shown in Table 2, any number between 0 and 1 can be used for R-squared. R-squared is most frequently used to determine how well a regression model explains observed data. For instance, an r-squared of 60% indicates that the regression model accounts for 60% of the variability seen in the target variable. A greater r-squared typically means the model is better at explaining the variability. The coefficient of variation demonstrates the degree of data variability relative to the population means in a sample.

Scanning electron microscopy was utilized to analyze the morphology and induction of somatic embryogenesis. In its early phases, the callus appeared as a scattering of elongated cells. And after being transferred to the DCR medium, the cells became tightly packed together, and globular-shaped embryos began to emerge between the tightly packed together cells (Figure 1). Figure 1 displays the many somatic embryo types that were produced. The mature embryos that were taken out of the cultures varied significantly. The mature somatic embryos that were harvested ranged in size from 1.00 mm to 200 µm. Four different types of somatic embryos were identified based on the variance in their morphology (Figure 1).

DISCUSSION

To substantiate these results, we listed certain studies. We examined the findings in highlights of five primary topics. These five topics were: the beginning of somatic embryogenesis, Embryo development stages, operations for somatic embryogenesis (SE), the impact of plant growth regulators on somatic embryogenesis introduction, and the development of SE and plant embryos is significantly influenced by the leafy cotyledon (LEC) family of transcription factors (TFs).

1. Beginning of somatic embryogenesis:

How cells begin the development of an embryo is unknown. Yet, it has been determined that to begin embryo production, an uneven distribution of auxins must be produced. Due to differential transport, auxin is distributed asymmetrically **[26]**. Asymmetric cell division happens in zygotic embryogenesis (ZE), however, in somatic embryogenesis (SE) this is frequently not seen. The zygote undergoes an asymmetric mitotic division, resulting in the formation of two distinct cells: the suspensor and the embryo. Protoderm development and primordial initiation occur at the octant and globular stages. Throughout these phases, auxin is distributed asymmetrically and differentially, resulting in the development of the many tissues that make up the embryo. Certain genes are expressed as a result of the interplay between the transport and accumulation of auxin and other factors, such as cytokinins (CKs) **[27].**

2. Embryo development stages:

Despite some anatomical similarities between somatic and stage of the cycle embryos, the development of each differs based on the classification of the plants (angiosperms and gymnosperms). Zygotic embryos are assumed to be fed by the phloem tissue, as opposed to somatic embryos, which use an exogenous source of carbohydrates to undergo their morphological processes without a relationship to vascular tissue **[28]**.

The globular, scutellar, and coleoptiles stages in monocotyledonous species and the globular, heart, torpedo, and cotyledonal stages in dicotyledonous species are phases similar to ZE that are involved in somatic embryo development **[29]**. When the somatic embryos reach the cotyledonary stage, they begin to form a shoot meristem, and seedling growth then begins **[30].**

3. operations for somatic embryogenesis:

A critical first step in manipulating SE is to comprehend the physiological and molecular mechanisms by which its induction (direct or indirect) takes place **[31]**. Several things can cause SE. Other sources of stress that can alter plant cells' cellular and molecular processes include the characteristics of the culture medium, high concentrations of PGRs, and wounding of explants. To achieve the acquisition of

embryogenic potentials, factors such as the type of explant, the age and genotype of the mother plant, the physiological incubation conditions, the cellular density in suspension cultures, the formation of homogeneous cell aggregates, and the physiological incubation conditions must be taken into account **[32]**.

4. The impact of plant growth regulators on somatic embryogenesis introduction:

When SE is induced in plant culture systems, the addition of PGR to the culture media is crucial for promoting cell differentiation. The type and concentration of PGR employed for each culture affect the SE process most. Using various explants, circumstances, and PGR concentrations, various plant species, including C. canephora **[26]**, A. thaliana **[33]**, and Musa spp. **[34]**, successfully responded to the SE induction. Auxins must be added to the culture medium for many species that may develop somatic embryos from cell suspension cultures. Inducing SE and the early stages of somatic embryo development requires the use of 2, 4-dichloroacetic acid (2, 4-D) **[35]**. For instance, by applying a low concentration of 2, 4-D, the production for embryogenic date palm harvests improved 20 times **[36]**. Other PGRs, like CKs, also contribute to the growth of the plants by encouraging the development of buds, delaying the aging of the leaves, and boosting cell division in conjunction with auxins; these two regulators are known to work in concert **[37]**.

5. The development of SE and plant embryos is significantly influenced by the leafy cotyledon (LEC) family of transcription factors (TFs):

The leafy cotyledon (LEC) family of transcription factors (TFs) is a significant player in the control of SE and plant embryo development **[38]**. The LEC1 gene plays a crucial role in zygotic embryogenesis (ZE) and has been proposed to regulate several seed development processes, including embryo morphogenesis, maturation phases, germination, and early and late embryogenesis **[39]**. It also appears to allow the formation of the embryo by creating an embryonic environment**.** LEC1 is also involved in seed maturation, early seed development processes including photosynthesis and chloroplast biogenesis, and late zygotic embryonic development processes like seed maturation **[40]**. We are making great progress in our understanding of how stress, other PGRs, and auxins affect the induction of SE **[35]**. We now have a group of genes that, in some circumstances, can be utilized as indicators of SE onset. The signaling channel from the first signal to the initial stages of the somatic embryo's development, however, is essentially unknown.

CONCLUSION

It is crucial to compare the various processes used to create somatic Embryogenesis to assess their effectiveness. The four stages of somatic embryos, globular, heart, torpedo, and cotyledon form, were observed on the formed callus. The highest amounts of Callus fresh weight, the number of globular-shaped stage embryos, the number of heart-shaped stage embryos, the number of torpedo-shaped stage embryos, the number of cotyledon-shaped stage embryos, and the number of seedlings recorded in the media supplemented with 2,4-D at a concentration of 5 mg, using the steps of the fifth procedure. These results constitute a step in the way in the field of developing new strains of heirloom foreign carrot (Daucus carota l.) cultivars to produce bolting-tolerant cultivars under Egyptian conditions; this study was able to produce somatic embryos of the foreign carrot cv. Kuroda. However, since artificial seed production relies on the development of somatic embryos, more work and research are required to address some of the issues that have been noted and will be of interest to the research team in subsequent investigations.

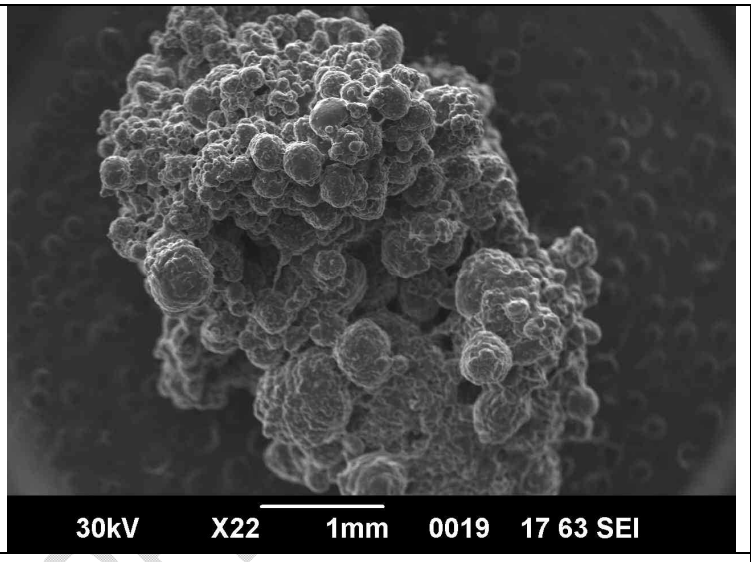
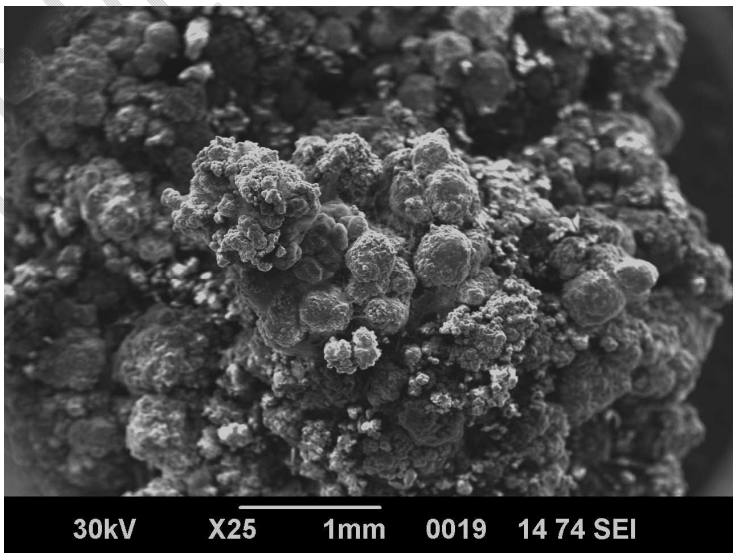
Table 1. Mean squares and probabilities for combined ANOVA over two experiments and the different procedures for producing Somatic Embryogenesis in carrot.

Traits	Sources of variation	df	MS	F	P
Callus fresh weight (mg)	Main Effects				
	Procedures	3	5482079.9	103.74066	***
	Experiments (Trails)	1	70601.605	1.3360361	.2647 ns
	Interaction				
	Procedures * Experiments	3	4099.1122	0.0775699	.9712 ns
	Error	16	52844.083<-		
Number of globular-shaped stage embryos	Sources of variation	df	MS	F	P
	Main Effects				
	Procedures	3	5436.9306	13.136649	***
	Experiments (Trails)	1	1001.0417	2.4187053	.1395 ns
	Interaction				
	Procedures * Experiments	3	10.708333	0.0258734	.9941 ns
	Error	16	413.875<-		
Number of heart-shaped stage embryos	Sources of variation	df	MS	F	P
	Main Effects				
	Procedures	3	112182.56	19.559617	***
	(Experiments (Trails)	1	7210.6667	1.2572176	.2787 ns
	Interaction				
	Procedures * Experiments	3	40.777778	0.0071098	.9991 ns
	Error	16	5735.4167<-		
Number of torpedo-shaped stage embryos	Sources of variation	df	MS	F	P
	Main Effects				
	Procedures	3	64913.611	7.9157309	**
	Experiments (Trails)	1	8740.1667	1.0657982	.3173 ns
	Interaction				
	Procedures * Experiments	3	793.83333	0.0968021	.9607 ns
	Error	16	8200.5833<-		
Number of cotyledon-shaped stage embryos	Sources of variation	df	MS	F	P
	Main Effects				
	Procedures	3	26008.722	41.2564	***
	Experiments (Trails)	1	864	1.3705221	.2589 ns
	Interaction				
	Procedures * Experiments	3	511	0.810575	.5064 ns
	Error	16	630.41667<-		
	Sources of variation	df	MS	F	P
	Main Effects				
	Procedures	3	28802.264	1019.5492	***

Table 2. Comparison of mean values for the different procedures for producing Somatic Embryogenesis in carrot.

	Procedures	Mean	Coefficient of Variation (CV)	Coefficient of determination (R^2)
Callus fresh weight (mg)	The second procedure	917.460[c]	13.912%	0.951
	The third procedure	1289.311[b]		
	The fourth procedure	1344.940[b]		
	The fifth procedure	3057.583[a]		
	LSD 0.05	198.947		
Number of globular-shaped stage embryos	The second procedure	286[c]	6.187%	0.723
	The third procedure	342a[b]		
	The fourth procedure	330[b]		
	The fifth procedure	355[a]		
	LSD 0.05	17.606		
Number of heart-shaped stage embryos	The second procedure	813[c]	7.521%	0.7893
	The third procedure	1054[ab]		
	The fourth procedure	1025[b]		
	The fifth procedure	1133[a]		
	LSD 0.05	65.542		
Number of torpedo-shaped stage embryos	The second procedure	1336[c]	6.172%	0.610
	The third procedure	1434b[c]		
	The fourth procedure	1526a[b]		
	The fifth procedure	1570[a]		
	LSD 0.05	78.372		
Number of cotyledon-shaped stage embryos	The second procedure	105[b]	14.820%	0.888
	The third procedure	218[a]		
	The fourth procedure	120[b]		
	The fifth procedure	233[a]		
	LSD 0.05	21.729		
number of seedlings	The second procedure	68[d]	3.926	0.994
	The third procedure	188[b]		
	The fourth procedure	82[c]		
	The fifth procedure	201[a]		
	LSD 0.05	6.505		

Means followed by the same letter are not significantly different at 5% by LSD.

Picture (1): embryonic callus clumps (A), bar = 1.00 mm.	
Picture (2): embryonic callus clumps (A), bar = 1.00 mm.	

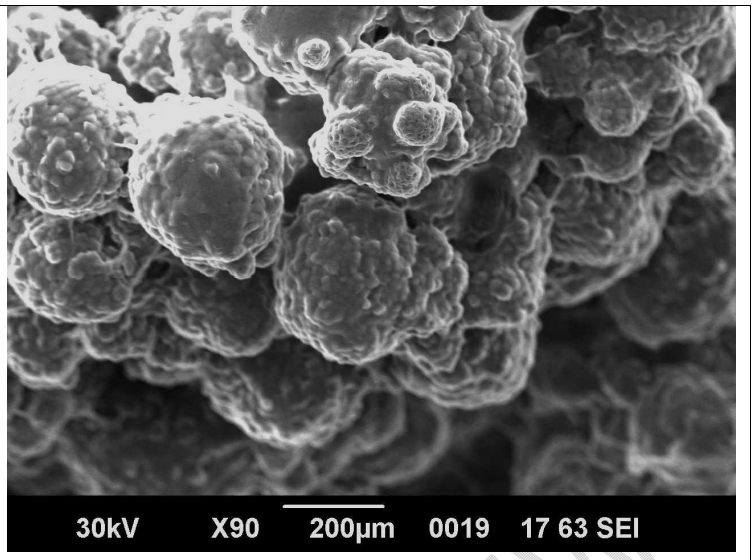
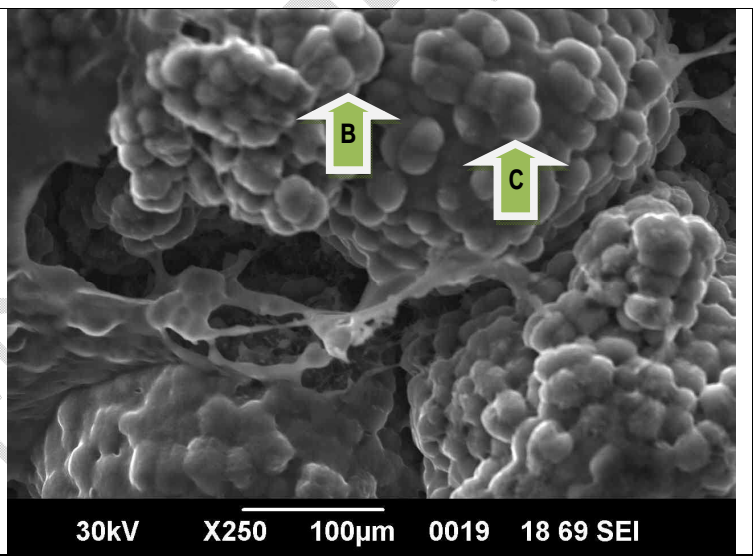
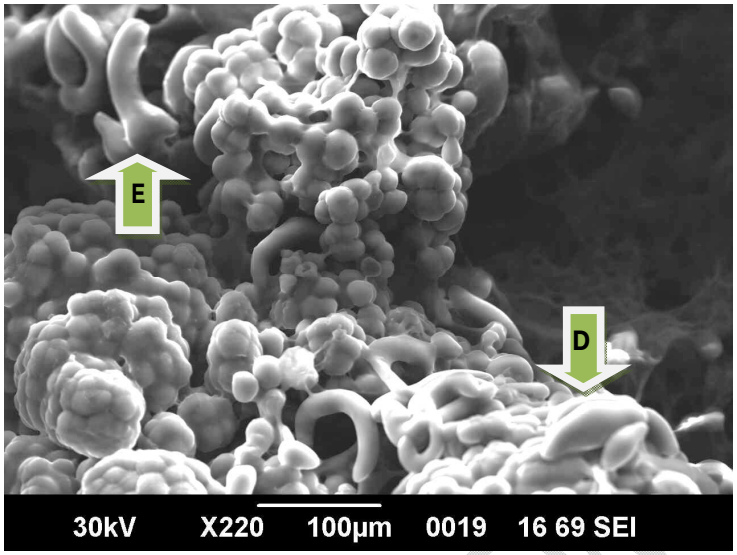
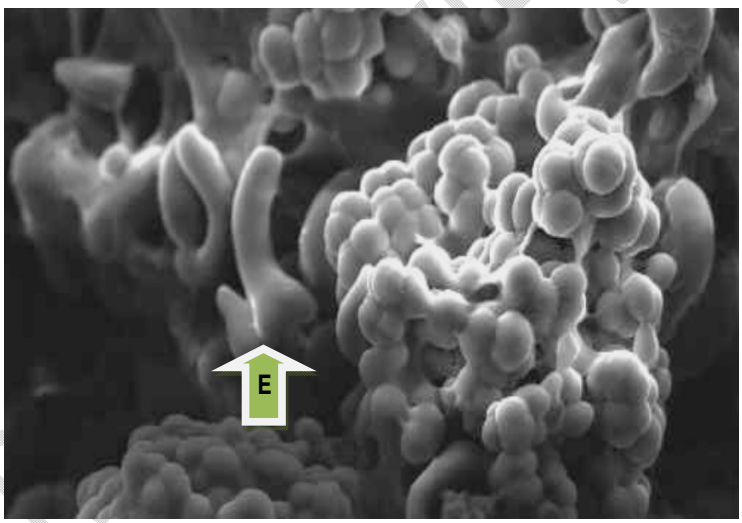
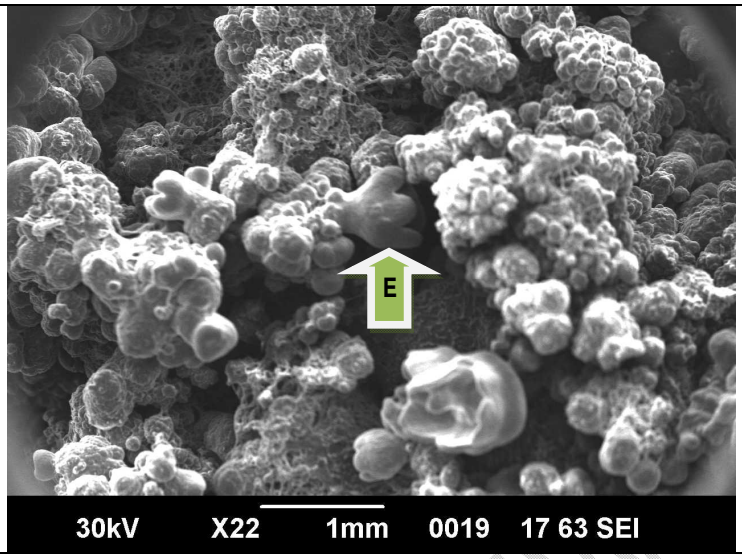
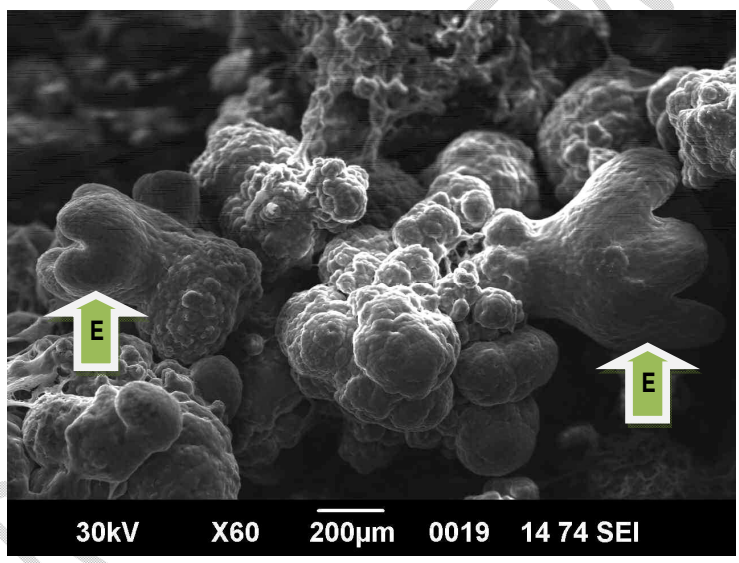
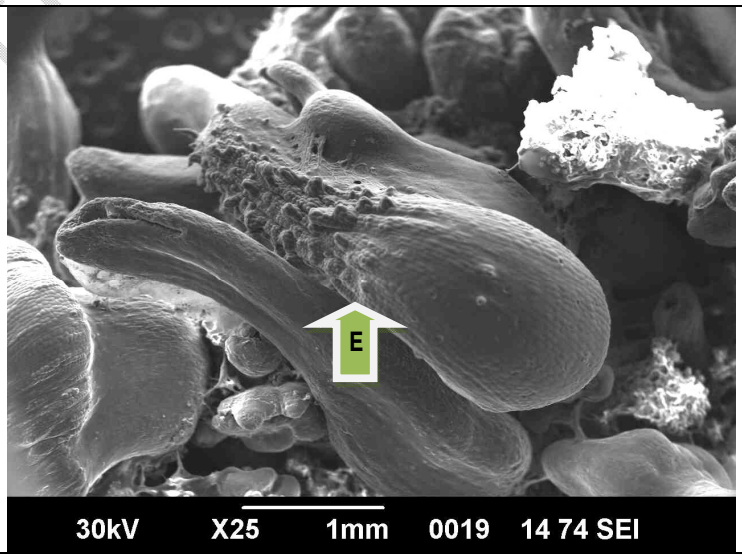
Picture (3): embryonic callus clumps (A), bar = 200 µm.	

Figure 1. Different stages of somatic embryogenesis as seen under scanning electron microscope (A–E). (A) embryonic callus clumps, (B) Globular shaped, (C) heart shaped, (D) torpedo shaped and (E) cotyledonary staged. All stages were taken from the procedure 5 pathway.

Picture (4): Globular (B) and heart-shaped (C) stage embryos, bar = 100 µm.	

Picture (5): Torpedo (D) and cotyledon-shaped (E) stage embryos, bar = 100 µm.	
Picture (6): cotyledon-shaped (E) stage embryos (this image has been manually enlarged)	

Cont. Figure 2. Different stages of somatic embryogenesis as seen under scanning electron microscope (A–E). (A) embryonic callus clumps, (B) Globular shaped, (C) heart shaped, (D) torpedo shaped and (E) cotyledonary staged. All stages were taken from the procedure 5 pathway.

Picture (7): Cotyledon-shaped (E) stage embryos, bar = 1.00 mm.	
Picture (8): Cotyledon-shaped (E) stage embryos, bar = 200 µm	
Picture (9): Cotyledon-shaped (E) stage embryos, bar = 1.00 mm	

Cont. Figure 3. Different stages of somatic embryogenesis as seen under scanning electron microscope (A–E). (A) embryonic callus clumps, (B) Globular shaped, (C) heart shaped, (D) torpedo shaped and (E) cotyledonary staged. All stages were taken from the procedure 5 pathway.

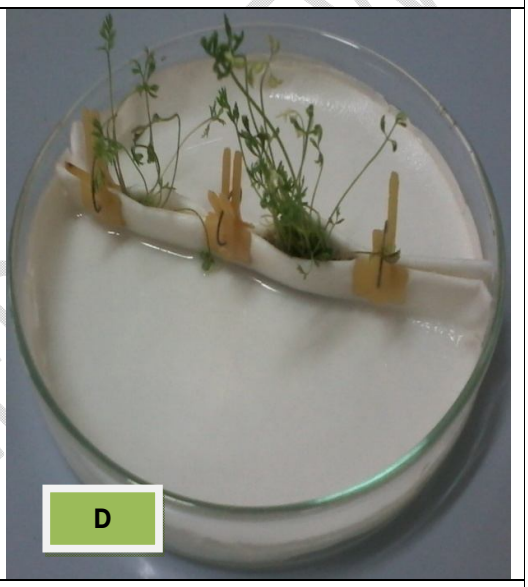


Figure 4. (**A**) somatic embryos, (**B**) Embryo germination, (**C**) Mature embryos picked and germinated separately, (**C** and **D**) embryos forming plantlets after germination, (**E)** root formation after somatic embryo germination, plantlets ready to be transferred.

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