International Journal of Agriculture Innovations and Research
Volume 7, Issue 2, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 12/09/2018 | Accepted on: 20/09/2018 | Published: 27/09/2018

Phytohormones Action on *Fouquieria Splendens* Callogenesis and Organogenesis Processes

SALINAS-PATINO V.A.[1], ESPINOZA-MELLADO M.R.[2], HERNÁNDEZ-PIMENTEL M.V.[1], GARCÍA-PINEDA M.[3], MONTES-VILLAFÁN S.[1] and RODRÍGUEZ-DORANTES A.[1]*

[1] Departament of Botany, National Biological Sciences School, Instituto Politecnico Nacional, Mexico City, Mexico
[2] Central of Microscopy, National Biological Sciences School, Instituto Politecnico Nacional, Mexico City, Mexico
[3] Botanical Garden, Faculty of Higher Studies, Iztacala, Universidad Autonoma de Mexico, Mexico City, Mexico
*Corresponding author email id: rodorantes@yahoo.com.mx

***Abstract* – It is known that callus can be produced from a single differentiated cell and many callus cells could be totipotent and able to regenerate a whole plant considering that high plasticity is the principal characteristic for their differentiation as a product of the particularly gene expression profiles; produced when the balance between cytokinins and auxins determines the state of differentiation and dedifferentiation. This work analyzed the effect of different actions of two cytokinins: bencil amino purine (BAP) and kinetin (KIN) and the naphthalene acetic acid (NAA) auxin on growth and morphological changes in the desert plant *Fouquieria splendens* callus. There were two evident morphological process determined not only by the Callus Induction Frequency (CIF) obtained, showing a great initial callus induction and after a good callogenesis and organogenesis processes; it also implies an active role in plant morphogenesis of *F. splendens*, by the cytokinin/auxin combination tested, showed by the scanning electron microscopy analysis, where a particularly presence of extracellular matrix surface network was present considering an important morphoregulatory structure during organogenesis process; showing not only a fast induction of calli and also a remarkably effect of growth and differentiation response of this interesting desert plant species.**

***Keywords* – Auxins, Callus Culture, Cytokinins, Fouquieriaceae.**

I. Introduction

Ikeuchi *et al.* [1] defined "callus" to the massive growth of cells and also applied to the disorganized plant cell masses (calli). Steward *et al.* [2] and Nagata and Takebe [3] described that callus can be produced from a single differentiated cell and many callus cells could be totipotent and able to regenerate a whole plant; considering that high plasticity is the principal characteristic for their differentiation. Ikeuchi *et al.* [1] also established that calli are diverse and could be particularly classified based on their macroscopic characteristics. Zimmerman [4] and Frank *et al.* [5] described two principal kinds of calli; one with no apparent organ regeneration called as friable or compact callus and the other that shows some degrees of organ regeneration called rooty, shooty, or embryonic callus. Taking the consideration of Iwase *et al.* [6] about the different types of callus formed in *Arabidopsis thaliana* as a product of the particularly gene expression profiles; this term must include cells with various degrees of differentiation when the balance between auxins and cytokinins determines the state of differentiation and dedifferentiation. Is important to note here the mention of Skoog and Miller [7] whose conclude: "an intermediate ratio of auxin and cytokinin promotes callus induction; while a high ratio of auxin-to-cytokinin or cytokinin-to-auxin induces root and shoot regeneration, respectively". Some authors as Jehan *et al.* [8], Shimizu *et al.* [9] and Wang *et al.* [10] reported that the phytohormonal composition of the medium is the most important factor for *in vitro* regeneration; Boltenkov *et al.* [11] also noted that the morphogenetic capacity of explant tissues can be induced by varying the composition of phytohormones in the medium for the production of organogenic callus and differentiation via organogenesis or embryoidogenesis. Based on it finally, phytohormone concentrations in culture medium will be the critical point for the control of growth and morphogenesis [12], [13], [14]. This work analyzed the effect of different phytohormone actions on growth and morphological changes in the desert plant *Fouquieria splendens* callus.

II. Material and Methods

A. *Fouquieria Splendens Callus Culture and Development*

Branches of the desert plant *Fouquieria splendens*, were collected from the Botanical Garden of the Facultad de Estudios Superiores, Iztacala (FES)-UNAM, these were maintained in water 15 days for the development of leaves. Leaves were surface-sterilized with sodium hypochlorite solution (10%) for 90 seconds, rinsed three times in sterile distilled water and leaf explants were obtained aseptically cutting fractions of $1cm^2$. Five explants were placed separately in baby food flasks with Magenta SIGMA caps containing 25mL of Murashige and Skoog (MS) ¼ salts medium [15] supplemented with 30 g/L of sucrose and 3 g/L phytagel. The treatments consider the presence or absence of phosphate salt and different phytohormones combination as follows: "MSA" Medium: 42.5mg/L NaH_2PO_4 + 10mg/L BAP (Bencil Amino Purine) + 1mg/L NAA (Naphthalene Acetic Acid), "MSB" Medium: 42.5mg/L NaH_2PO_4 + 1mg/L KIN (Kinetin) + 5mg/L NAA), "MSC" Medium: 10mg/L BAP + 1mg/L NAA, "MSD" Medium: 1mg/L KIN + 5mg/L NAA and "MSE" Medium: 1.5mg/L KIN + 1mg/L NAA. Incubated at 28°C with photoperiod of 16 h light /8 h dark with a fluorescent Phillips T8 32 Watts 5000ºK lamp, all the experiments were performed by 36 replicates and growth was analyzed at 35 days.

B. *Analysis of Growth and Morphological Changes of*

International Journal of Agriculture Innovations and Research
Volume 7, Issue 2, ISSN (Online) 2319-1473

Fouquieria Splendens Callus

As Visarada *et al.* [16] mention that calli variation is based on their morphology and could be classified to facilitate the identification of changes that conduce their development or plants regeneration; in this study, explants modified or with callus development were recovered at the final time and analyzed by visual classification according to the next code: without response (WR), chlorotic and without response (CL/WR), initial callus and chlorotic (IC/CL), initial callus (IC), callus (C), abundant callus (AC), green callus (GC) and abundant green callus (AGC). Also, the Callus Induction Frequency (CIF), was determined according to Ali *et al.* [17] as follows: CIF = Number of total calli produced / Total number of explants x 100.

Selected fractions were prepared for scanning electron microscopy (SEM) for the analysis of morphological changes induced in *F. splendens* callus according to Corona-Álvarez *et al.* [18] as follows: samples were cut into 4 or 5mm pieces, fixed in 2.5% gluataraldehyde for 1h at room temperature (27°C), then, washed three times in a phosphate buffer, postfixed in 1% OsO_4 for 1h at room temperature, washed and dipped into distilled water for three times. The samples were undergone a series of dehydration processes: 30 % ethanol for 10 min; 40 % ethanol for 10 min; 50 % ethanol for 10 min; 60 % ethanol for 10 min; 70 % ethanol for 10 min; 80 % ethanol for 10 min; 90 % ethanol for 10 min and finally 100 % ethanol for 10 min (three times). The material was dried in the critical point dryer apparatus, mounted and sputter-coated with gold in an ion coater for 60 seconds. Finally, the samples were ready for viewing under field scanning electron microscope JSM 5800 LV for their examination and photography.

C. *Statistical Analysis*

All data obtained were analyzed by one-way analysis of variance and the mean differences were compared applying a Tukey-Kramer Method using the statistics program Graph Pad Instat Ver. 2.03. Principal Component Analysis (PCA) was done with matrix data set of all the experimental conditions and callus categories; employing the Pearson correlation, with PAST (Paleontological Statistics Software Package) Ver. 2.17b.

III. Results and Discussion

A. *Growth and Efficiency of F. Splendens Callus*

Is important to note according to Dodds and Robert [19] and Aitchison *et al.* [20] that the response of plants in culture from explants, proliferate as a mass of relatively undifferentiated tissue called callus, forming by an amorphous mass of loosely arranged thin walled parenchyma cells from the parent tissue as a result of dramatic changes in the appearance and metabolism of the plant cells. Table 1 shows the total evaluation of *F. splendens* explants response regarding to the medium tested; where the number of WR explants was less in all the conditions MSA: 17±1.16, MSB: 4±0.57, MSC: 22±0.81 and MSE: 21±1.11, with a statistical significance only between MSB and MSC ($p<0.05$), giving a good response of this plant species in this culture.

Table 1. Total evaluation of *Fouquieria splendens* explants development

MS	WR	CL/WR	IC/CL	IC	C	AC	GC	AGC	Total
A	8	9	10	3	0	0	11	0	41
B	4	0	0	6	4	11	0	13	38
C	16	6	14	15	0	0	0	0	51
D	1	0	0	10	0	3	0	16	30
E	21	0	0	21	0	2	8	20	72

Where: without response (WR), chlorotic and without response (CL/WR), initial callus and chlorotic (IC/CL), initial callus (IC), callus (C), abundant callus (AC), green callus (GC) and abundant green callus (AGC).

Is important to note as Slesak *et al.* [21] mention that the intensity and frequency of callogenesis depends on the media culture; thus: "calli obtained on media supplied with different phytohormones were morphologically different, light green, soft, well hydrated, semi-translucent and mucilaginous and consisted of loose, large and elongate cells and/or dry compact friable calli that were of different colors". Figure 1 shows the appearance of callus grown in MSD (Figure 1a) and in MSE (Figure 1b) media, showing some characteristics as these authors noted. Table 2 resumes the response of CIF for the *in vitro* callogenesis of *F. splendens*, focusing only this response considering the callus classification of: IC (MSA: 13±0.89, MSB: 10±1.73, MSC: 29±1.28 and MSE: 29±0.70, without statistical significance between experimental conditions), AC (without statistical significance between experimental conditions) and GAC (MSB: 13±2.08, MSD: 27±1.14 and MSE: 20±1.24, with a statistical significance between MSE medium to the others, at $p<0.01$). IC obtained a good percentage of CIF in all medium culture tested: MSC (56.9%) > MSE (40.2%) > MSD (33.3%) > MSB (26%) > MSA (24.3%); even MSC medium showed a great initial callus induction; calli do not growth and turning brown, dark and necrotic at the final time (35 days).

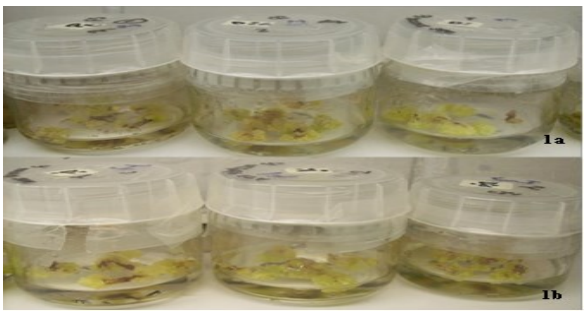


Fig. 1. *In vitro Fouquieria splendens* callus development: 1a) grown under MSD medium and 1b) grown under MSE medium.

Table 2. CIF (%) obtained for each *F. splendens* calli classification

MS	IC	AC	GAC
A	24.4	26.8	0
B	26.3	28.9	34.2
C	56.9	0	0
D	33.3	10	90
E	40.3	2.77	27.8

MSA medium has the same phytohormones concentra-tion plus phosphate salt and promote the production of

abundant calli. Comparing MSB and MSD media with the same phytohormones but phosphate salt only in MSB; even both conditions showed high values of CIF of the three callogenesis classification, the best response with green abundant calli was obtained in MSD medium (90%). MSE medium that contains 1.5mg/L KIN + 1mg/L NAA showed a discrete callogenesis response according to the CIF obtained between IC, AC and GAC.

Figure 2 analyzed the callus classification and culture media tested by PCA, were negative Component 1 values (65.6%) indicates a particular relationship between GAC and "MSB and MSD" media. In this figure there is also a particularly positive association between IC and "MSC and MSE" media and a negative response between WR explants and MSA medium, these associations were agree with ICF values noted in Table 2. Smolenskaya *et al.* [22] reported that the presence of particularly growth regulators and their concentrations are important factors for the maintaining of callus lines in cultures; which is agree with Lakshmanan *et al.* [23] showing that the effect of growth regulator on tissue cultures can vary according to the chemical nature of the compound, plant species, type of culture and even the developmental state of the explant.

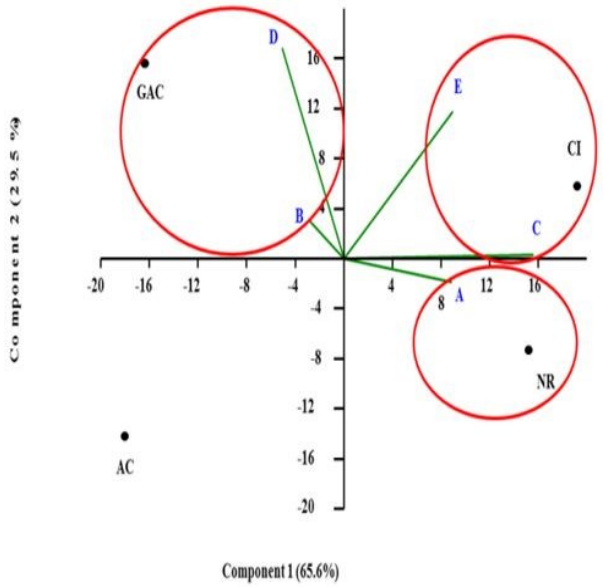


Fig. 2. Principal componente analysis grouping the *Fouquieria splendens* callus categories and experimental conditions.

In this study, the nature of phytohormones assayed in *F. splendens* cultures in the media tested: MSD and MSE the presence of NAA and KIN was determinant. Boltenkov *et al.* [11] commentaries about the suggestion that different morphogenetic capacity of callus tissues induced on different media was determined by their "hormonal prehistory," were agree to Burgess [24] considering that initial culture conditions defining the morphogenetic response of tissues depending on the phytohormonal composition of the medium. It is important to note that as Howell *et al.* [25] established, the presence of cytokinins in medium is a prerequisite for plant regeneration in tissue culture, since these phytohormones are required for the expression of genes, that is agree with the explanation of Barrueto *et al.* [26], Echeverrigary and Fracaro [27] and Meftahizade *et al.* [28] considering that cytokinins especially at the high concentration promotes apical dominance thus shoot formation and auxins promotes also shoot elongation.

In this study, the appearance and growth of calli was agreed with Meftahizade *et al.* [28], whose mention that the increase of cytokinins like kinetin, induced calli with hyperhydricity; property that is characterized by a "slightly swollen, lighter green and translucent tissue" and according to Gopi and Vatsala [29] regarding to the note that exclusive presence of auxins in the medium, even which was its concentration, are not enough to induce callus formation. Thus both phytohormones combination with synergistic effects could induce the callus development and as these authors noted, they induce more friable callus and reduce the number of days taken for callus induction, favoring the fast growth of them. Shamsardakani *et al.* [30] reported that the combination of auxins and cytokinins can induce compact callus and Kitamura *et al.* [31], also mention that usually roots that formed from calli supplied with NAA were hairy, but those directly formed from leaf explants without NAA were thick.

B. *Induced Morphogenesis in F. Splendens Callus*

Two considerations must note at first; one of them is that finally according to Rueb *et al.* [32] and Lee *et al.* [33], many auxins and cytokinins present in media promoted highest regeneration frequency on MS medium containing 2 mg/L NAA and KIN between 1 to 4 mg/L for rice plants; meanwhile Din *et al.* [34] concluded about it that these phytohormones could interact through synergistic, antagonistic and additive mechanisms of tissue cultures, promoting the development of cells to callogenesis and/ or organogenesis. The second consideration is regarding to Delporte *et al.* [35], notation; these authors established with good genotypes competence for "callogenesis" characterized by undifferentiated cellular proliferation, considering calli as "non-morphogenetic" with white, limpid, watery and friable appearance and for "morphogenesis" considering the calli white to greenish-white, compact and smooth, plane or dome-shaped or nodular appearance with the formation of densely proliferating cells, as in this study, where Figures 3 to 6 show the appearance of calli developed in MSE and MSD media tested. For both media culture, there was an obvious development as SEM observations by Konieczny *et al.* [36] in *Triticum aestivum* where at the beginning of its culture, calli presented a smooth surface and were covered solely with large oval or oblong cells of parenchymatous nature and also some of them were nodular in appearance, forming numerous clusters of small, tightly packed meristematic cells; also Popielarska-Konieczna *et al.* [37] mention with the appearance of a discontinuous amorphous layer of complex non-cellular material outside the outer cell wall of the cells on the callus surface; similarly to the results obtained by Popielarska *et al.* [38] and Popielarska - Konieczna *et al.* [39], [40], on kiwifruit organogenesis with the presence of a membranous layer covering the outer cell wall.

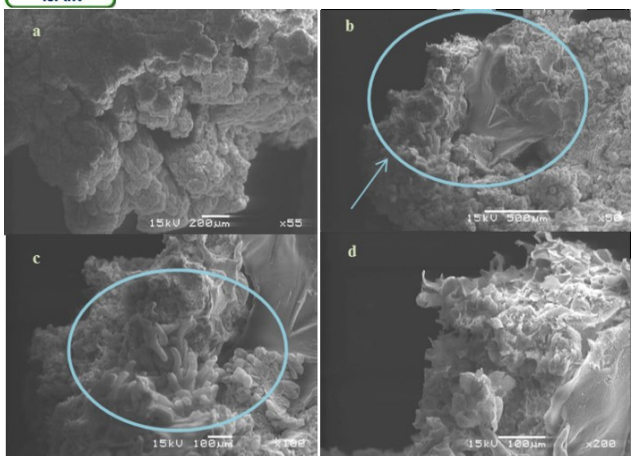
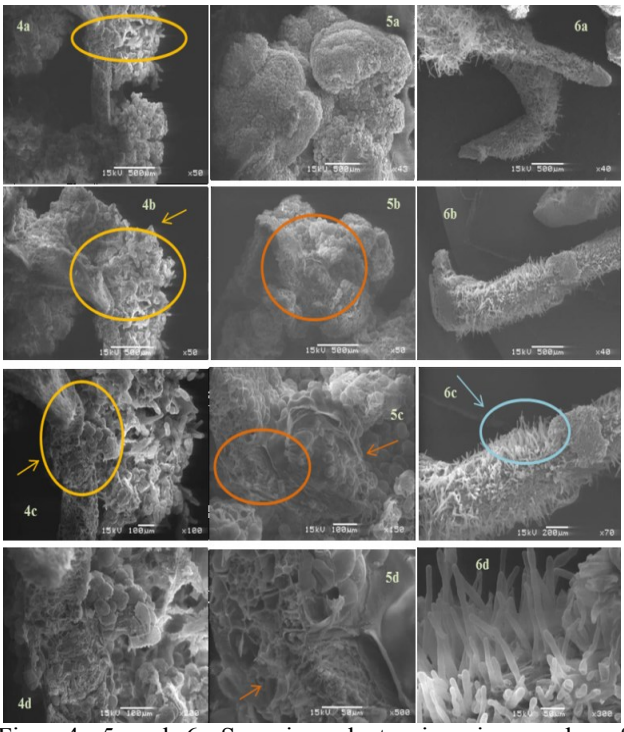


Fig. 3. Scanning electronic micrographs of selected *Fouquieria splendens* callus grown in MSE medium; where sequence 3a to 3d shows: a) general observation of non-morphogenic callus (x55); b) appearance of the extracellular matrix surface network covering some cell aggregates (x50); c) appearance of large cells near the extracellular matrix surface network (x100); d) massive surface area (x200).

These authors noted the formation of extracellular strands, fibrils and/or a continuous layer over callus cells accompanies the induction of morphogenesis in many plants cultured *in vitro*. This structure, which is referred to as extracellular matrix (ECM) established by Samaj *et al.* [41] or extracellular matrix surface network (ECMSN) [42], has been found during the early stages of somatic embryogenesis and organogenesis. The exact role of this structure is still not known, but its regulatory and coordinating functions during the early stages of morphogenesis have been suggested [36], [39], [43], [44], [45]. These authors noted that the surface of non-regenerative callus derived from the cultivar of *Helianthus tuberosus* var. Albik was covered with a membranous structure, similar to the ECMSN that has been reported in plant tissue cultures of different species. Is important to note, although the precise function of the ECMSN has not been established as Chapman *et al.* [46] mention; significant roles are reported for this structure, it participates in coordination of early developmental stages of somatic embryogenesis because its formation could be produced by stress response of plant tissues that is triggered by specific culture conditions and that covering callus with extracellular material could provide protection against external factors, thus, its role cannot be generalized but authors concluded that it is evident that is presence on the surface of cultured *in vitro* tissues possess a morphogenetic competence [37],[41], [47], [48].

Finally, as Popielarska - Konieczna *et al.* [40] reported the ECMSN plays an important morphoregulatory role during organogenesis, implying an active role in plant morphogenesis, for *F. splendens*, the cytokinin/auxin combination, defined the organogenesis process at 35 days of callus growth, with the evidence showed in Figure 4, where the differentiation of mesophyll cells from the original leaf explants in the sequence of Figures 4a to 4d, was similar to the reported by Konieczny *et al.* [36] in *Triticum aestivum*. In *F. splendens* explants cultured in MSD medium, callus morphogenesis derived to early shoots showed in the sequence in Figures 5a to 5d and hairy roots presented in the sequence in Figures 6a to 6d as an evidence of direct organogenesis.



Figs. 4, 5 and 6. Scanning electronic micrographs of selected *Fouquieria splendens* callus grown in MSD medium; where sequences 4a to 4d shows: 4a) a general view of the original explant (arrow) and at the upper extreme the initial dedifferentiation of cells (x50); 4b) shows a detail of the upper zone with a differentiation of mass cells (x50); 4c) a distinguish differentiation zone of mass cells more closely (arrow, x100); 4d) mass aggregates totally differentiated. Sequence of figures 5a to 5d shows: 5a) a general view of a shoot derived by organogenesis process (x43); 5b) particularly detail of the extracellular matrix surface network covering the cell mass forming a plate (x50); 5c) transversal section of parenchyma cells conforming the early shoot of *F. splendens* (x150); 5d) parenchyma cells detail with their tinny cell walls (x500). Sequences of figures 6a to 6d shows: 6a) a general view of a root derived by organogenesis process (x40); 6b) root zones clearly showed with a membranous material covering cells of root tip (x40); 6c) details of the root hairs zone (x70); 6d) large root hairs (x300).

IV. Conclusion

In this work, the morphological changes of *Fouquieria splendens* callus were determined and showed that there was a complex response by the active participation of kinetin and naphthalene acetic acid at first, giving a callogenesis response and finally a direct organogenesis process with a fast induction of them in 35 days and a remarkably effect on the growth and differentiation response of this interesting desert plant species.

ACKNOWLEDGMENT

Authors are grateful to the Research Projects SIP-IPN: 20171598 and SIP-IPN: 20181504 of the Secretaría de Investigacion y Posgrado, Instituto Politecnico Nacional, for providing the facilities to carry out this work and also wish to thank for the fellowships from Comisión de Operación y Fomento de Actividades Académicas (COFAA, I.P.N.), EDI (Estímulo al Desempeño de los Investigadores, I.P.N.) and SNI-CONACYT (Sistema Nacional de Investigadores-Consejo Nacional de Ciencia y Tecnologia).

REFERENCES

[1] M. Ikeuchi, K. Sugimoto and A. Iwase (2013). "Plant callus: mechanisms of induction and repression". *Plant Cell*: 25: 3159-3173.

[2] F.C. Steward, M.O. Mapes and K. Mears (1958). "Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells". *Am. J. Bot.*: 45: 705-708.

[3] T. Nagata and I. Takebe (1971). "Plating of isolated tobacco mesophyll protoplasts on agar medium". *Planta*: 99: 12-20.

[4] J.L. Zimmerman (1993). "Somatic embryogenesis: a model for early development in higher plants". *Plant Cell*: 5: 1411-1423.

[5] M. Frank, H.M. Rupp, E. Prinsen, V. Motyka, H. Van Onckelen H and T. Schmülling (2000). "Hormone autotrophic growth and differentiation identifies mutant lines of *Arabidopsis* with altered cytokinin and auxin content or signaling". *Plant Physiol.*: 122: 721-729.

[6] A. Iwase, N. Mitsuda, T. Koyama, K. Hiratsu, M. Kojima, T. Arai, Y. Inoue, M. Seki, H. Sakakibara, K. Sugimoto and M. Ohme-Takagi (2011). "The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in *Arabidopsis*". *Curr. Biol.*: 21: 508-514.

[7] F. Skoog and C.O. Miller (1957). "Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*". *Symp. Soc. Exp. Biol.*: 11: 118-130.

[8] H. Jéhan, D. Courtois, C. Ehret, K. Lerch and V. Pttiard (1994). "Plant regeneration of *Iris pallida* Lam. and *Iris germánica* L. via somatic embryogenesis from leaves, apices and young flowers". *Plant Cell Rep.*: 13: 671-675.

[9] K. Shimizu, T. Yabuya and T. Adachi (1996). Plant regeneration from protoplasts of *Iris germanica* L.". *Euphytica*: 89: 223-227.

[10] Y. Wang, Z. Jeknic, R.C. Ernst and T.H.H. Chen (1999). "Improved plant regeneration from suspension-cultured cell of *Iris germanica* L. "skating party"". *Hortscience*: 34: 1271-1276.

[11] E.V. Boltenkov, L.N. Mironova and E.V. Zarembo (2007). "Effect of phytohormones on plant regeneration in callus culture of *Iris ensata* Thunb". *Biology Bulletin*: 34: 446-450.

[12] F. Skoog and D.J. Armstrong (1970). "Cytokinin". *Annu. Rev. J. Plant Physiol.*: 21: 359-384.

[13] D.S. Letham DS. (1974). "Regulators of cell division in plant tissues. XX the cytokinins of coconut milk". *J. Plant Physiol.*: 32: 66-70.

[14] D.E. Akiyoshi, R.O. Morris, R. Hinz, B.S. Mischke, T. Kosuge, D.J. Garfinkel, M.P. Gordon and E.W. Nester (1983). "Cytokinin /auxin balance in crown gall tumors is regulated by specific loci in the T-DNA". *J. Proc. Natl. Acad. Sci.*: 80: 407-411.

[15] T. Murashige and F. Skoog (1962). "A revised medium for rapid growth and bioassays with tobacco tissues cultures". *Physiol Plant.*: 15: 473-497.

[16] K.B.R.S. Visarada, M. Sailaja and N.P. Sarma (2002). "Effect of callus induction media on morphology of embryogenic calli in rice genotypes". *Biologia Plantarum* 45: 495-502.

[17] G. Ali, F. Hadi, Z. Ali, M. Tariq and M.A. Khan (2007). "Callus induction and *in vitro* complete regeneration of diferent cultivars of tobacco (*Nicotiana tabacum* L.) on media of different hormonal concentrations". *Biotechnology*: 6: 561-566.

[18] D. Corona - Alvarez, V.A. Salinas-Patino, M.V. Hernández – Pimentel, S. Montes-Villafan, M. García-Pineda, L.A. Guerrero-Zúñiga and A. Rodríguez-Dorantes (2018). "Morphological changes in *Fouquieria splendens* callus cocultivated with endophyte bacteria. *Int. J. Curr. Microbiol. App. Sci.*: 7: 1577-1586.

[19] J.H. Dods and L.W. Robert. *Experiment in Plant Tissue Culture*. 2nd. ed. Cambridge, University Press, Cambridge, 1985.

[20] P.A. Aitchison, J.A. MacLeod and M.M. Yeoman, "Growth patterns in tissue (callus) cultures", in: *Plant Cell Culture*, H.E. Street, Ed. Blackwell Sci. Publ. Oxford; 1978, pp. 267-306.

[21] H. Ślesak, M. Liszniańska, M. Popielarska - Konieczna, G. Góralski, E. Sliwinska and A.J. Joachimiak (2014). "Micro propagation protocol for the hybrid sorrel *Rumex tianschanicus* x *Rumex patientia*, an energy plant. Histological, SEM and flow cytometric analyses". *Ind. Crops Prod.*: 62: 156-165.

[22] I.N. Smolenskaya, O.V. Reshetnyak, Y.N. Smirnova, N.D. Chernyak, A.M. Nosov and A.V. Nosov (2007). "Opposite effects of synthetic auxins, 2, 4-dichlorophenoxyacetic acid and 1-naphthalene acetic acid on growth of true ginseng cell culture and synthesis of ginsenosides". *Russian J. Plant Physiol.*: 54: 215-223.

[23] P. Lakshmanan, M. Danesh and A. Taji (2000). "Production of four commercially cultivated *Echinacea* species by different methods of *in vitro* regeneration". J. *Hort. Sci. Biotech.*: 77: 58-163.

[24] J. Burgess. *An Introduction to Plant Cell Development*. Cambridge: Univ. Press.

[25] S.H. Howell, S. Lall and P. Che (2003). "Cytokinins and shoot development". *Trends Plant Sci.*: 8: 453-459.

[26] C.L.P. Barrueto, A.C.M.G. Machado, S.B.R.C. Carvalheira and A.C.M. Brasileiro (1999). "Plant regeneration from seedling explants of *Eucalyptus grandis Europhylla*". *Plant Cell Tissue Organ. Cult.*: 56: 17-23.

[27] S. Echeverrigary and F. Fracaro (2001). "Micropropagation of *Cunila galioides*, a popular medicinal plant of south Brazil". *Plant Cell Tissue Organ. Cult.*: 64: 1-4.

[28] H. Meftahizade, M. Lotfi and H. Moradkhani (2000). "Optimization of micropropagation and establishment of cell suspension culture in *Melissa officinalis* L. *Afr. J. Biotech.*: 9: 4314-4321.

[29] C. Gopi and C. Vatsala (2006). "*In vitro* studies on effects of plant growth regulators on callus and suspension culture biomass yield from *Gymnema sylvestre*". *Afr. J. Biotechnol.*: 5: 1215-1219.

[30] M. Shamsardakani, Y. Amnzade, F. Jahanshir and A. Jamshidi (2005). "Production of suspension cell culture in *Melissa officinalis* and comparison of produced secondary metabolites in callus versus whole plant". *J. Med. Plant.*: 13: 68-71.

[31] Y. Kitamura, K. Kubo, L. Rahman and T. Ikenaga (2002). "Reproduction of *Sedum drymarioides*, an endangered rare species, by micropropagation". *Plant Biothecnology.*: 19: 303-309.

[32] S. Rueb, M. Leneman, R.A. Schilperoort and L.A.M. Hensgens (1994). "Efficient plant regeneration through somatic embryogenesis from callus induced on mature rice embryos (*Oryza sativa* L.)". *Plant Cell Tissue Organ. Cult.*: 36: 259-264.

[33] K.S. Lee, H.S. Jeon and M.Y. Kim (2002). "Optimization of a mature embryos-based *in vitro* culture system for high frequency somatic embryogenic callus induction and plant regeneration form japonica rice cultivar". *Plant Cell Tissue Organ. Cult.*: 71: 9-13.

[34] R.A.J.M. Din, F.I. Ahmad, A. Wagiran, A.A. Samad, Z. Rahmat and M.J. Sarmidi (2016). "Improvement of efficient *in vitro* regeneration potential of mature callus induced from Malaysian upland rice seed (*Oryza sativa* cv. Panderas). *Saudi J. Biol. Sci.*: 23: S69-S77.

[35] F. Delporte, A. Pretova, P. du Jardin and B. Watillon (2014). "Morpho-histology and genotype dependence of *in vitro* morphogenesis in mature embryo cultures of wheat". *Protoplasma*:: 251: 1455-1470.

[36] R. Konieczny, J. Bohdanowicz, A.J. Czaplicki and L. Przywara (2005). "Extracellular matrix surface network during plant regeneration in wheat anther culture". Plant *Cell Tissue Organ. Cult.*: 83: 201-208.

[37] M. Popielarska - Konieczna, J. Bohdanowicz and E. Starnawska (2010). "Extracellular matrix of plant callus tissue visualized by ESEM and SEM". *Protoplasma.*: 247:121-125.

[38] M. Popielarska, H. Ślesak and G. Góralski (2006). "Histological and SEM studies on organogenesis in endosperm-derived callus

of kiwifruit (*Actinidia deliciosa* cv. Hayward)". *Acta Biol. Cracov. Ser. Bot.*: 48: 97-104.

[39] M. Popielarska - Konieczna, M. Kozieradzka - Kiszkurno, J. Świerczyńska, G. Góralski, H. Ślesak and J. Bohdanowicz (2008). "Ultrastructure and histochemical analysis of extracellular matrix surface network in kiwifruit endosperm-derived callus culture". *Plant Cell Rep.*: 27: 1137-1145.

[40] M. Popielarska - Konieczna, M. Kozieradzka-Kiszkurno, J. Świerczyńska, G. Góralski, H. Ślesak and J. Bohdanowicz (2008). "Are extracelular matrix surface network components involved in signalling and protective function?". *Plant Signal Behav.*: 3:707-709.

[41] J. Šamaj, M. Bobák, A. Blehová, J. Krištin and O. Auxtová-Šamajová (1995). "Developmental SEM observations on an extracellular matrix in embryogenic calli of *Drosera rotundifolia* and *Zea mays*". *Protoplasma.*: 186:45-49.

[42] M. Pilarska, M. Popielarska - Konieczna, H. Slesak, M. Kozieradzka - Kiszkurno, G. Góralski, R. Konieczny, J. Bohdanowicz and E. Kuta (2014). "Extracellular matrix surface network is associated with nonmorphogenic calli of *Helianthus tuberosus* cv. Albik produced from various explants". *Acta Soc. Bot. Pol.*: 83:67-73.

[43] J.L. Verdeil, V. Hocher, C. Huet, F. Grosdemange, J. Escoute, N. Ferriere and M. Nicole (2001). "Ultrastructural changes in coconut calli associated with the acquisition of embryogenic competence". *Ann. Bot.*: 88: 9-18.

[44] M. Bobák, J. Šamaj, E. Hlinková, A. Hlavačka and M. Ovečka (2003). "Extracellular matrix in early stages of direct somatic embryogenesis in leaves of *Drosera spathulata*". *Biol. Plant.*: 47:161-166.

[45] A. Blehová, M. Bobák, J. Šamaj and E. Hlinková (2010). "Changes in the formation of an extracellular matrix surface network during early stages of indirect somatic embryogenesis in *Drosera spathulata*". *Acta Bot. Hung.*: 52:23-33.

[46] A. Chapman, S. Helleboid, A.S. Blerwacq, J. Vasseur and J.L. Hilbert (2000). "Removal of the fibrillar network surrounding *Cichorium* somatic embryos using cytoskeleton inhibitors: analysis of proteic compounds". *Plant Sci.*: 150: 103-114.

[47] T. Dubois, J. Dubois, M. Guedira, A. Diop and J. Vasseur (1992). "SEM characterization of an extracellular matrix around somatic proembryos in roots of *Cichorium*". *Ann. Bot.*: 70:119-124.

[48] M. Mazarei, H. Al-Ahmad, M.R. Rudis, B.L. Joyce and C.N. Stewart (2011). "Switchgrass (*Panicum virgatum* L.) cell suspension cultures: establishment, characterization, and application". *Plant Sci.*: 181:712-715.