Morpho- histological observations on somatic embryogenesis in mature embryo derived callus of *Oryza sativa* L. cv. Sakha 101

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Abstract

Cellular totipotency is one of the fundamental principles of plant biotechnology. The mature embryo is increasingly recorded as a valuable explant for somatic embryogenesis in rice biotechnology. In present study, rice cells dedifferentiation, proliferation and re-differentiation were investigated by culturing mature rice (*Oryza sativa* L. cv. Sakha 101) embryos in modified MS media fortified with different growth regulators alone and in combination. Mature embryo tissues competent for tissue culture and the chronological changes of cells morphology and histology were observed. The results showed that callus was induced only from mature rice seed (explant) cultured on MS media supplemented with 2, 4-D (2 or 2.5 mg/l) alone while the rest treatments showed negative response. Callus was initiated after 5 days of culture in MS media fortified with the lower 2, 4-D dose as clusters of undifferentiated cell masses while callus initiation was delayed 4 days more by increasing the applied dose. At morphological level, Pale yellowish and friable calli was noted in both tested doses. Calli texture exhibited different appearance, while was slightly nodular in the lower dose it was soft in the higher one. High callus induction frequency (70%) was estimated for 2mg/l 2, 4-D application while decreased frequency (40%) was concomitant with dose increment. Histological analysis for somatic embryogenesis revealed that within two weeks of culturing explants on callus induction medium (CIM), somatic embryos development began as clusters of embryonic cells at the peripheral parts of the proliferated calli while non-embryonic cells were observed at the inner regions of the induced callus. Embryogenic cells at the outer cell layer were observed as small and isodiametric with dense cytoplasm and clear nucleus located in the center of the cells, whereas the non embryogenic cells were large, vacuolated and had a very small nucleus located near the cell wall. Embryogenic cells undergo series of ordered divisions and protodermis observed surrounding globular embryo was recorded at the end of culturing in CIM fortified with lower 2, 4-D dose.

On the other hand, culture on MS fortified with higher dose delayed rice cells differentiation and globular stage was recorded two days after subculture embryonic callus into free hormone MS medium. After 2 days of subculture into free hormone MS medium, heart shaped embryo was observed in low dose of 2, 4-D.

This study assists to draw attention to the use of a histological approach as a helpful tool to follow the chronological series of embryo development *in vitro*.

Key wards: differentiation**,** histology**,** morphology**,** *Oryza sativa* L.**,** somatic embryogenesis and tissue culture.

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1. Introduction

Rice (*Oryza sativa* L.) is an annual grass belonging to tribe Oryzeae under subfamily Oryzoideae in family Poaceae (Jacquemin *et al.* 2013). It is the world's most important cereal crop with incomparable agricultural and economic importance as being a basic food for more than half of the world's population (Mohanty *et al*. 2010; Misratia *et al.* 2015). It represents nearly as 75% of the caloric intake of 2 billion humans living in Asia, and as much as 33% of caloric intake of approximately one billion humans in Africa and Latin America (Kinoshita and Mori 2001).

Rice is the second most widely cultivated cereal in the world, after wheat (Pazuki and Sohani 2013). In Egypt, rice plays a significant role as strategic crop for supporting the food self-sufficiency and raising the export (Anis *et al.* 2016). Frequently, humans eat rice in cooked form to obtain various nutrients, as well as to supplement their caloric intake (Kim *et al.* 2011). Most of the rice by-products, including rice husk and rice bran, are used as animal feeds. In latest years, rice by-products have received increased interest as functional foods because of their phenolic base compounds, in addition, it have high amounts of vitamins, minerals and fibers, which can help to decrease cholesterol and enact anti-atherogenic activity (Wilson *et al.* 2002).

In plants, there are two types of embryogenesis: zygotic and somatic embryogenesis. Zygotic embryogenesis is one of the most important steps in the life cycle of plants in which double fertilization occur leading to simultaneous formation of the endosperm and the embryo. Zygotic embryo undergoes cell division and cell differentiation to form a mature plant. Perhaps the main reason for the limited progress in understanding the developmental proceedings in plant embryos is that zygotic embryos of higher plants consist of several tiny cells that grow within motherly tissues (flowers or immature fruits) and it is quite difficult to collect sufficient embryos to study the biological events that occur early in the developmental process (Jimenez 2001). Whereas, Somatic embryogenesis is the process by which somatic cells, under induction conditions, generate embryogenic cells, which go through a series of morphological and biochemical changes (Quiroz-Figueroa *et al.* 2006), that result in the production of bipolar structure without vascular connection with the original tissue. The development of somatic embryos closely resembles the development of zygotic embryos by both morphologically and physiologically.

Somatic embryogenesis has been proved to be useful and efficient method for mass clonal propagation of selected material, production of mutants and artificial seeds, genetic engineering and germplasm cryopreservation (Gonzalez-Arnao *et al.* 2008; Hasbullah *et al.* 2013). In particular, when somatic embryogenesis is integrated with traditional breeding programs and molecular and cell biological techniques, it provides a valuable tool to enhance genetic improvement of crop species (Quiroz-Figueroa *et al*. 2006). It was found that, one of the most important uses of somatic embryogenesis its employment to study the initial events of zygotic embryogenesis in higher plants where somatic embryo is observable, its various culture conditions can be controlled, and a lack of nutrient is not a restrictive factor for experimentation. The previous characteristics have converted somatic embryogenesis into a model system for studying the morphological, physiological, molecular and biochemical events happening throughout the onset and development of embryogenesis in angiosperms (Quiroz-Figueroa *et al.* 2006).

The success of somatic embryogenesis depends on various factors including genotype, explant, plant growth regulator, basal salt, cultural conditions and developmental stages of the mother plants (Panjaitan *et al.* 2009; Rahman *et al.* 2015)

Several studies reported that plant growth regulator 2, 4-D was the most suitable auxin for callus induction in rice tissue culture (Chen *et al*. 1974; Maeda, 1980; Mondal *et al.* 2013) and however Schulze (2007) indicated that the combination of auxin with other plant growth regulators, such as cytokinins was more effective in regulation of morphogenesis.

Morphological and histological studies of callus and embryo induction of plant are important for enhancing the incidence of callus production and induction of embryogenic callus (Feng *et al.* 2007; Tan *et al.* 2009). In addition, the histological and morphological observation of callus can be used for characterizing the somatic embryogenesis process and evaluate the changes in the explant, the cell proliferation in the starting of the induction, and the cellular origin of calluses in embryogenesis (Soares *et al.* 2014). It is also an object of studies which leading to an understanding of the basis of the totipotency, differentiation, and changes in cell fate (Quiroz-Figueroa *et al.* 2006).

Therefore, the present study was aimed to evaluate the effect of different growth regulators (auxin and cytokinin), alone or in combination on rice somatic embryogenesis pathway. The functional link between morpho-histological changes during this developmental process was assessed.

2- Materials and Methods

2.1. Plant material and explant sterilization

Healthy and free disease mature rice grains (*Oryza sativa* L. cv. Sakha 101) were obtained from Field Crops Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Mature rice grains were de-husked manually, washed with tap water to remove dust and other particles, then washed with detergent (10%) and rinsed with running tap water for 5 min. Surface sterilization of mature seeds was carried out under laminar air flow cabinet. Seeds were surface sterilized for two minutes in 70% ethyl alcohol, followed by 50% Clorox (sodium hypochlorite 5.25%) supplemented with 1–2 drops of Tween-20 for 30 min, with continuous shaking and finally rinsed five times thoroughly with sterile distilled water according to Ibrahim and El Shihy (2012) with slight modification.

2.2. Culture media and culture condition

The medium Murashige and Skoog (1962) full strength composed of macro- and micro- elements, vitamins and glycine fortified with 25 g/l sucrose, 0.3 g/l casein hydrolysate, 5 g /l sorbitol, 0.5 g/l L-proline, 0.5 g/l L-glutamine, 0.45 g/l L-alanine and a solidifying agent 8 g/l agar (El-Shawaf *et al*. 2011) was used as a basal media (MS) in this study. To evaluate the effect of different plant hormones alone or in combination, MS medium supplemented with different concentrations of 2,4- dichlorophenoxy acetic acid (2,4-D) (0.5, 2 or 2.5 mg/l), kinetin (Kn) (0.5, 1, 1.5, 2 or 2.5 mg/l) was used. The pH of the medium was adjusted to 5.8 prior to the addition of agar and afterward the media was autoclaved at 121ºC, 1.1 kg cm^{-2} for 20 minutes. After autoclaving, media were allowed to cool to approximately 60ºC then about 30 ml were poured into each sterile Petri dish (100 x 15 mm) and sealed with parafilm and

stored at room temperature for least 3 days before use under completely darkness to examine contamination.

2.3. Response of mature rice seeds to different hormones

Ten surface sterilized seeds (explants) were inoculated under the laminar airflow cabinet on free-hormone MS medium and on MS media supplemented with different plant hormones alone or in combination to assess rice cell response. Plant hormone treatments and their concentration were symboled in Table 1. Within 2 weeks of inoculation, responses of the mature seeds to all treatments were recorded in Table 1 and Figure1.

2.4. Callus, somatic embryo induction and development through morphological study

After one week of culture, induced calli were sub cultured for another week on same fresh callus induction media (CIM). All cultures were incubated under controlled condition at 30 ± 1^0C at complete darkness. Within 2 weeks of inoculation, callus initiation day, frequency of callus induction as percentage of seeds which produced a callus (Lee *et al.* 2009), morphological characters of induced calli (type, color & texture) and days after which somatic embryos initiated was recorded in Table 2 and Figures 2 & 3.

For rice somatic embryos development, embryonic calli were sub cultured after 2 weeks from inoculation on free-hormone medium for more one week at 30°C in the dark. Rice sample tissues were harvested after 16 days after culture (DAC) for histological analysis.

2.5. Histological analysis

Rice tissue samples were harvested at 5^{th}, 7^{th}, 9^{th}, 11^{th}, 14, and 16^{th} days after culture (DAC) and were fixed immediately in 10% formaldehyde, 5% glacial acetic acid, 50% of 95% ethyl alcohol and 35% d-H_2O (FAA solution) for 24 h then preserved in 70% ethyl alcohol for histological evaluation. The fixed materials were dehydrated through a graded series of ethanol (80% for 48 hrs, 95 % and 100% for 2 hrs each). Tissues were then infiltrated in absolute ethyl alcohol: chloroform graded series (3:1, 2:1, 1:1, 1:2, 1:3) for 2 hrs each and finally absolute chloroform for 12 hrs before they were embedded in paraffin wax. Serial sections were prepared at 9 μm thickness using a rotatory microtome, mounted on glass slides and double stained with safranin (2% dissolved in 50% ethyl alcohol) for 15 minutes and in light green (1% dissolved in absolute alcohol) for 30 sec. It was then rinsed in tap water twice following Johansen (1940) protocol. Samples were examined and photographed with the use of Olympus CX21FS1 microscope (Olympus, Japan) equipped with digital microscope eye piece (HiROCAM type MA 88-500, 5 Mega pixels) (Figures 4-6).

3. Results and discussion

3.1. Response of mature rice seeds to different hormones

Response of mature seeds was varied according to the type and concentration of growth regulators employed either alone or in combination (Table1). Present results indicated that growth regulator (2, 4-D) alone played an essential role in callus induction. De-husked rice seeds cultured in the free-hormone MS medium was germinated (Figure 1A) and the absence of calli revealed that 2, 4-D is a key factor to stimulate its formation. George *et al.* (2001) reported that the presence of auxins increased the DNA methylation than usual and

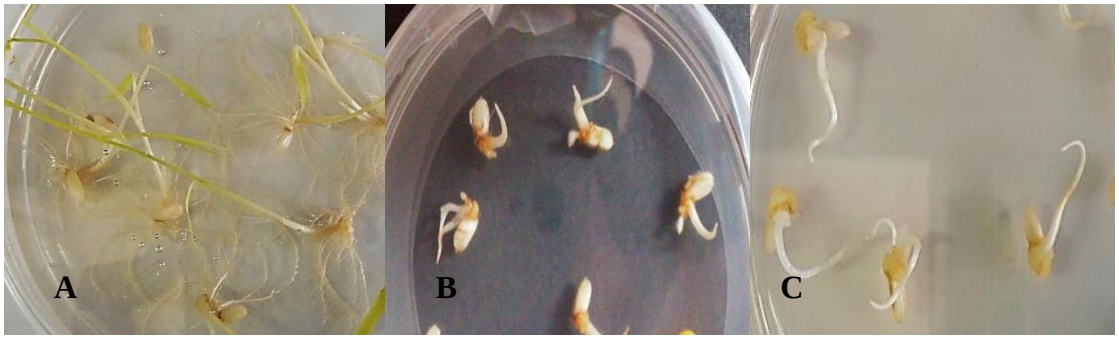
this might be required for programming of differentiated cells and made them able to begin division.

Similarly**,** in MS media fortified with kinetin separately, callus was failed to be formed and rice seeds were germinated. Our result was confirmed by Mondal *et al.* (2013). In T1 treatment (MS + 0.5 mg/l 2, 4-D) rice seeds were germinated (tiny root and shoot) instead of callus formation as shown in Figure 1B and this result is on line with Abiramasundari *et al.* (2014) in which they indicated that the lower level of 2, 4 D concentration (below 1 mg/l) was increased the shoot and root growth of the embryo in place of callus induction. While T2 (MS+2mg/l 2, 4-D) and T3 (MS+2.5 mg/l 2, 4-D) treatments tested alone have the ability to induce callus (Figure 1C). In MS media fortified with kinetin in combination with each concentration of 2, 4-D tested, callus was failed to be formed and this result is contrary to the work of Mondal *et al.* (2013) in which they reported that the use of 2, 4-D along with low concentration of Kn (0.5 mg/l) could be helpful for callus production and 2, 4-D 2mg/l alone was the best dose for callus induction. The failure of callus induction using combination of 2,4-D and kn in current study may be due to auxin (2, 4-D)/ cytokinin (Kn) ratio used and it was found that high auxin/ cytokinin ratio is necessary for starting embryogenic callus formation compared to a low ratio for the regeneration of plantlets (Ge *et al.* 2006). As the results obtained previously, the present study was carried out on two concentrations of 2, 4-D (2 or 2.5 mg/l).

Table (1): Effect of different concentration of 2, 4-D and Kn alone or in combinations on callus induction derived from rice (*Oryza sativa* L. cv. Sakha101).within two weeks from inoculation.

Treatment number	Media tested	Response of explant
T0	free- hormone MS medium	Germination
T1	MS + 0.5 mg/l 2,4-D	Germination
T2	**MS + 2 mg/L 2,4 D**	**callus**
T3	**MS + 2.5 mg/L 2,4 D**	**callus**
T4	MS + 0,5 mg/L kn	Germination
T5	MS + 1 mg/L Kn	
T6	MS + 1.5 mg/L Kn	
T7	MS + 2 mg/L Kn	
T8	MS + 2.5 mg/L Kn	
T9	MS + 2 mg/L 2,4 D + 2 mg/L Kn	
T10	MS + 2 mg/L 2,4 D + 2.5 mg/L Kn	
T1	MS + 2.5 mg/L 2,4 D + 2 mg/L Kn	
T12	MS + 2.5 mg/L 2,4 D + 2.5 mg/L Kn	

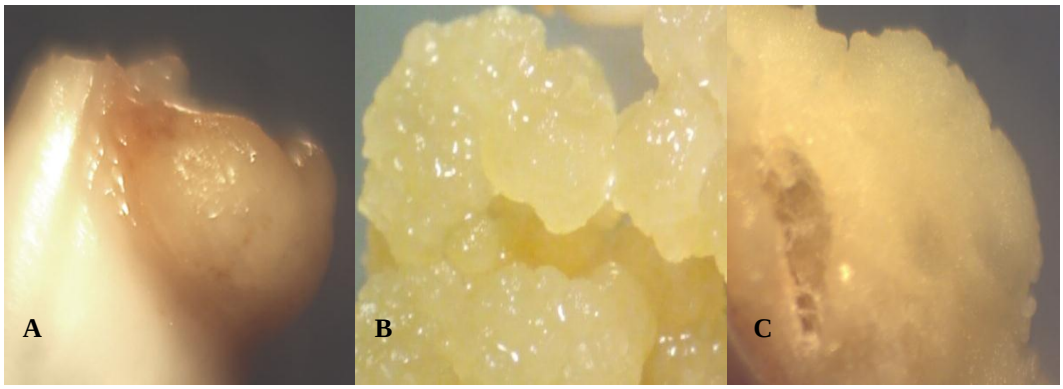
Fig.1 (A-C): Response of plant hormones on mature rice seeds. (A) Germination, (B) Germination with tiny root and shoot in T1 (MS + 0.5mg/l 2, 4-D), (C) Induced callus in T2 (MS + 2mg/l 2, 4-D)



3.2. Callus, somatic embryo induction and development through morphological study

The scutellum was appeared to be swollen after 2 days of culture (Figure 2A) then callus was initiated as unorganized cells masses in MS medium supplemented by 2mg/l 2, 4-D (T2) after 5 days of culture whereas callus initiation was delayed 4 days more after culture in MS medium fortified with 2.5mg/l 2, 4-D (T3). This result confirmed by Benlioğlu *et al.* (2015) who reported that callus formation was started from mature rice embryos after 4-5 days of culture. In the current study, Friable, pale yellowish and slightly nodular calli were produced after 2 weeks from mature zygotic embryos of rice (*Oryza sativa* L. cv. Sakha 101) when cultured in T2 (low dose level) treatment while friable, soft and pale yellowish calli was produced when cultured in T3 treatment as shown in Figure 2B and 2C respectively. Maximum callus induction frequency was recorded in T2 treatment (70%) while the response to callusing reduced (40%) as the level of 2, 4-D increased in the medium T3 (MS+2.5 mg/l 2, 4-D) as shown in Table 2. The results of the current study are on line with those of Pandey *et al.* (1994), Abeyaratne *et al.* (2004), Afrasiab and Jafar, (2011) and Upadhyaya *et al.* (2015) which reported that concentration of 2 mg/1 2, 4-D gave the highest response for callus formation in rice while Libin *et al.* (2012) observed that the frequency of callus decreased when the concentration of 2, 4-D exceeded 2.0 mg/l.

Fig.2 (A-C): Morphological characters of induced calli derived from mature rice seed. (A) Swollen scutellum with non-embryonic callus; (B)Embryonic calli (yellowish, friable and slightly nodular) derived on T2 treatment (2mg/l 2,4-D); (C) Embryonic calli (yellowish, friable and soft) derived on T3 treatment (2.5mg/l 2,4-D).



Afterward, globular embryos were observed on the periphery of the proliferated embryogenic calli as initial sign of re-differentiation after 14 days of inoculation on induction and somatic embryo initiation media in T2 treatment (Figure 3A) and after 2 days of culture on free-hormone media, the upper part of the globular embryos enlarged and became bulb-like structure and finally assumed the heart shape through further differentiation accompanied with globular embryo (Figure 3A1) while re-differentiation of somatic embryogenesis (globular embryo) was delayed till sixteenth day from inoculation time in high dose level of 2,4-D (Figure 3B). This result is in line of study of Deepti *et al.* (2002) in which they described the morphological differentiation of globular embryo toward heart shaped embryo.

Table 2: Effect of different concentration of 2, 4-D on callus induction and initiation somatic embryogenesis derived from mature embryo of rice (*Oryza sativa* cv. Sakha101).

Treatment number	Callus initiation days	Callus morphology			Callus Induction frequency %	Somatic embryos initiation days
		color	type	texture		
T2	**5th**	Pale Yellowish	Slightly nodular	Friable	70	14th
T3	**9th**	Pale yellowish	Soft	Friable	40	16th

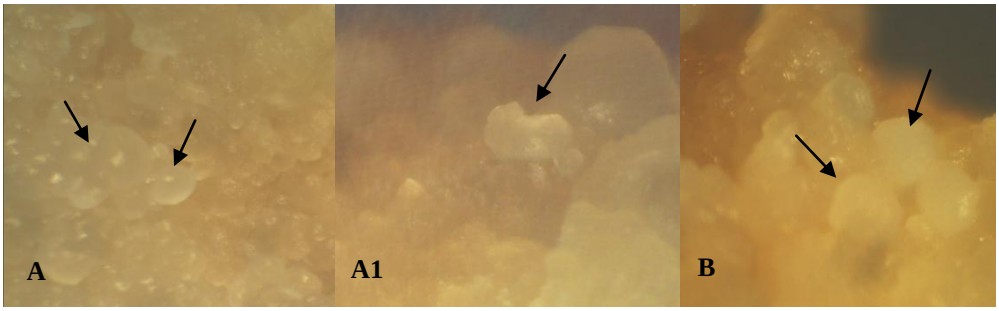


Fig.3 (A, A1 and B): Morphological characters of induced somatic embryos from mature *Oryza sativa* cv. Sakha 101 seed explant :(A) Globular somatic embryos produced in T2 (2 mg/l 2, 4-D) on somatic embryo induction media at 14th days after culture, (A1) Heart- shaped somatic embryo on development media at 16th days after culture and (B) Embryonic calli show globular somatic embryos cultured on T3 (2.5mg/l 2,4-D) at 16th days after culture.

3.3. Histological analysis

In present study, microscopic observation in cross section of scutellum taken before callus initiation revealed that the epithelial cells of scutellum are columnar cells compactly arranged with dense cytoplasm and a prominent nucleus and nucleolus while the ground tissue of scutellum consists of relatively large, compact parenchyma cells (Figure 4A). Callus initiation was detected from the more mitotically active epithelial cells of rice scutellum in both 2, 4-D treatments (T2 and T3) as shown in Figure 4B. Our observation confirmed with previous observations in immature and mature zygotic embryos of *Pannicum maximum* (Lu & Vasil 1985) and in rice (Hartke & Lorz 1989, Vega *et al*. 2009, Mahmudul Islam *et al.* 2013). Also callus initiated from meristematic patches proliferated from outermost layer of central mesocotyle bundle in both 2, 4-D treatments (T2 and T3) as shown in Figure 4C and from perivascular parenchyma cells of scutellum in T2. The present result was in agreement with those of Nishimura & Maeda (1977) and Maeda (1980) whom reported that mesocotyl and scutellum were the precursors of callus formation of rice.

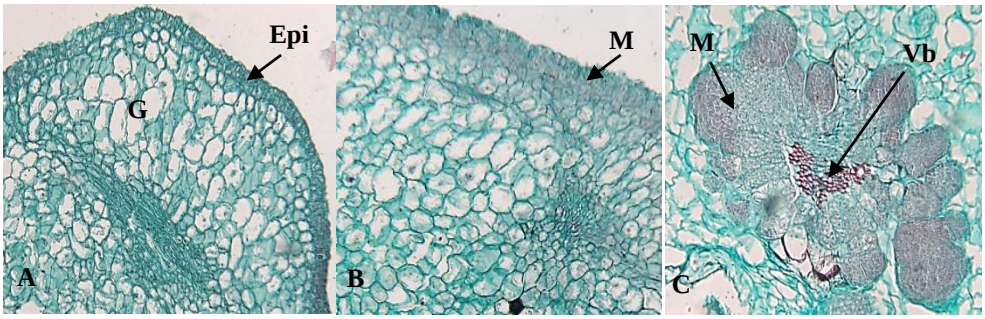
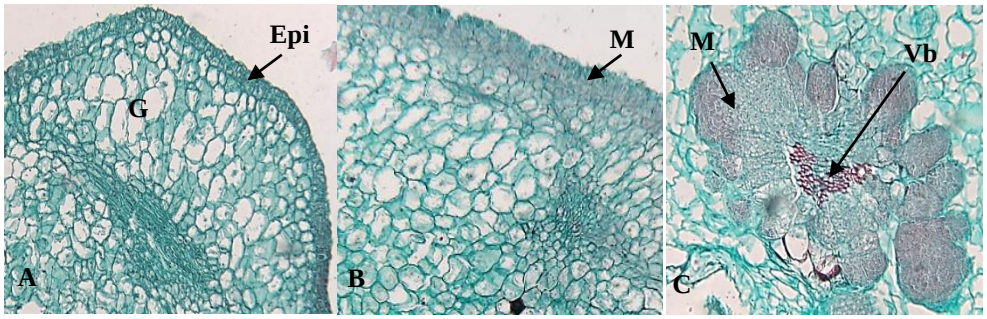
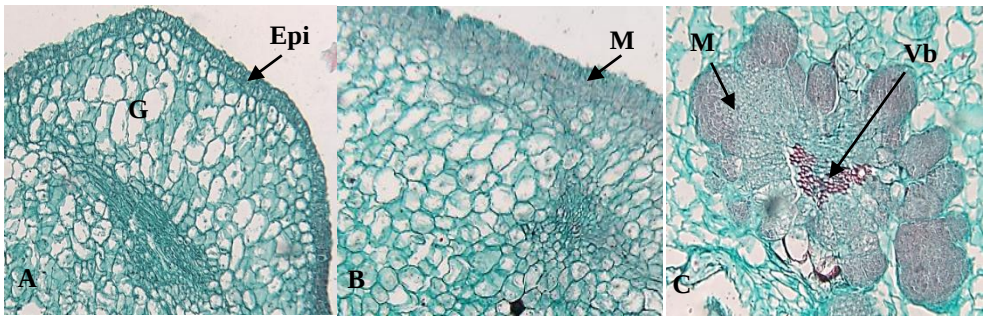
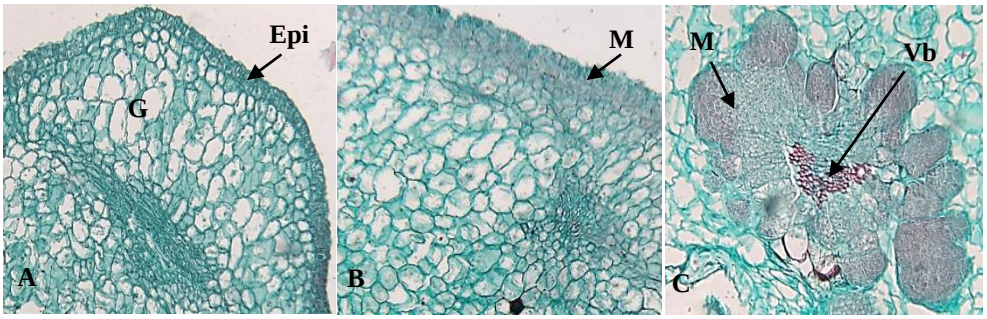


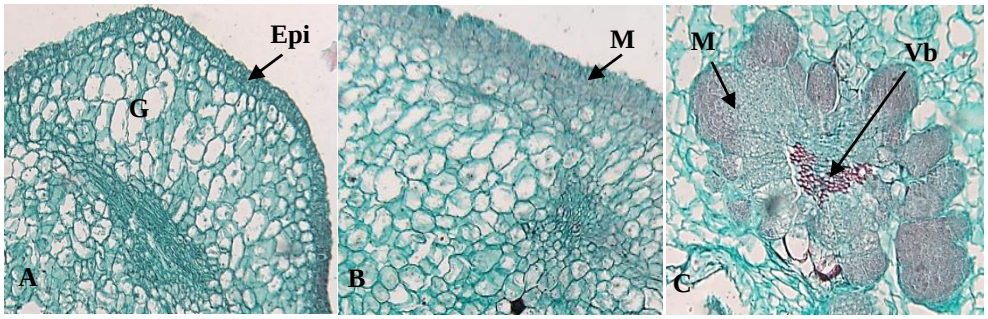
Fig. 4 (A-C): Histological sections showing the position from which derived-callus initiated from mature seed of rice. (A) Internal characters of scutellum (B) Embryonic cells (meristematic cells) induced from the epithelial cells of scutellum; (C) Embryonic cells (meristematic cells) induced from the outermost layer of mesocotyle bundle, where M meristematic cells, Vb vascular bundle, Epi c epithelial cells and G ground tissue of scutellum.

Histological observation also revealed two types of cells constitute embryogenic calli (Figure 5A); the external cell layers resembled meristematic cells (embryonic cells) consisted of small isodiametric compactly arranged cells in the company of dense cytoplasm and a prominent nucleus and nucleolus (Figure 5B). On the contrary, the internal cell layers (non-embryonic cells), consisted of large cells with abundant intercellular spaces. Most of non-embryonic cells had no nucleus but some had very small nucleus located near the cell wall and large vacuole were observed (Figure 5C). Results of the present study were in agreement with those of Alfonso-Rubi *et al.* (1999), Vega *et al.* (2009) and Narciso & Hattori (2010) which showed the presence of parenchymatic cells in the interior part and meristematic cells in the peripheral part of rice callus.









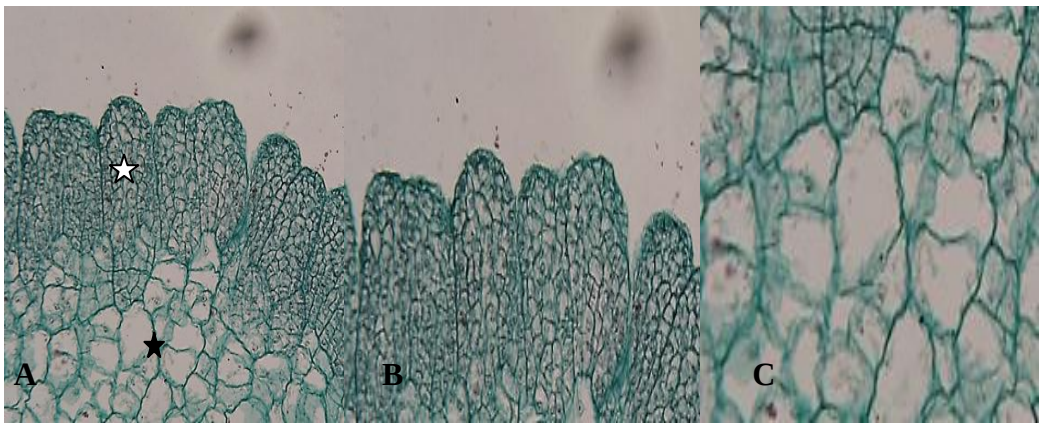


Fig. 5 (A-C): Histological analysis of somatic embryogenesis of *Oryza sativa* L. cv. Sakha101. (A) embryonic cells (☆) and non-embryogenic cells (★) (B) Embryonic cells showing isodiametric cells (C) Non-embryogenic cells with large vacuole and small nucleus located near the cell wall.

Histological changes of the tissues during culture period were detected as follows: After five days of culture, callus initiated from the differentiated epithelial cells of scutellum in T2 treatment (MS+ 2 mg/l 2, 4-D) (Figure 6A) as the first signs of an active cellular division and by increasing days of culture (after 7 days of culture) the cellular division was increased and callus proliferation increased (Figure 6B). This result in agreement with Revathi and Arumugam Pillai (2011) who reported that 2, 4-D role in cell division was to

increase the rate of cell division which leads to increased amount of callus. Also, shoot bud primordium was initiated as a meristematic dome from the outermost layer of mesocotyle bundle and developed to an adventitious shoot apical meristem surrounded by a small leaf primordium by increasing days of culture (Figure 6B1). The current result is in contrast with the result of Nishimura and Maeda, (1977) in which they reported that the outermost layer of the central cylinder in mesocotyl formed stunted primordium of lateral roots by abnormal proliferation of the cells which should have formed lateral roots when 2, 4-D concentration decreased to 3 x 10^{-5} M. Our result may be happened due to the difference in genotype or in the difference of endogenous hormones ratios. Proembryos were observed in the peripheral layer of embryonic calli as early as 9 days after culture in low dose of 2,4-D and were attached to it with a well-known multicellular suspensor (Figure 6C). The present result confirmed by Miroshnichenko *et al.* (2017) who reported that during the transition form from proembryonic structure to globular embryo, a multicellular suspensor-like structure developed, connecting the globular embryos to the callus. After 11^{th} days of culture (DAC), the number of proembryos was increased. Also, redifferentiation of adventitious shoot was increased (shoot organogenesis). While in T3 treatment (MS+ 2.5mg/l 2, 4-D) callus initiation was delayed till 9^{th} day of culture (Figure 6D) and adventitious shoot apical meristem surrounded by leaf primordial was observed. Proliferation of embryonic callus was increased by increasing days of culture. After 14 days on the callus and somatic embryo initiation media, globular somatic embryos were observed as a sign of rice cells re-differentiation in T2 treatment. Globular somatic embryos were covered with protodermis and consisted of zones of dividing cells with dense cytoplasm and small vacuoles which distinguished by the absence of vascular connection with the original tissue at basal side (Figure 6E) and this result was confirmed by Miroshnichenko *et al.* (2017) while in T3 treatment, proembryos were initiated from peripheral layer of embryonic callus and this initiation were delayed as compared with low dose of 2, 4-D (Figure 6F). Finally, After 2 days from subculturing on embryo development media (free-hormone media), globular somatic embryo accompanied with heart-shaped somatic embryos were observed in T2 (2mg/l 2, 4-D). Also root primordia (rhizogenesis) were noticed from preivascular parenchyma cells of scutellum in the same treatment. All somatic embryos covered with protodermis as shown in Figure 6G. Also, adventitious shoot apical meristem and leaf primordia was well developed forming leaf like structure (Figure 6G1). In T3 (2.5 mg/l 2, 4-D) globular somatic embryos were initiated (Figure 6H). Globular somatic embryos were covered with protodermis and attached to the embryonic callus with prominent multicellular suspensor like structure (Figure 6H). Also, adventitious shoot apical meristem surrounded by leaf primordia was observed and distinguished by presence of vascular connection with the maternal tissue (Figure 6H1) as shoot organogenesis. The results of the current study are on line with those of Angelo *et al.* (2009), Demeter *et al.* (2010), Elviana *et al.* (2011) and Steinmacher *et al.* (2011) who reported that protodermis was a unique character and the first tissue identified somatic embryogenesis development .

In our study, somatic and organogenesis morphogenic pathways was reported to occur simultaneously in T2, T3. The result of the current study is on line with those of Boissot *et al.* (1990) and Gairi & Rashid (2004) whom stated that organogenesis and embryogenesis may occur simultaneously, as regeneration pathway.

Conclusion:

The current study shed light on the importance of histological approach as a helpful tool to follow the chronological series of embryo development *in vitro* and indicated that growth regulator (2, 4-D) alone played an essential role in callus induction from matured seeds of *Oryza sativa* L. cv. Sakha101.

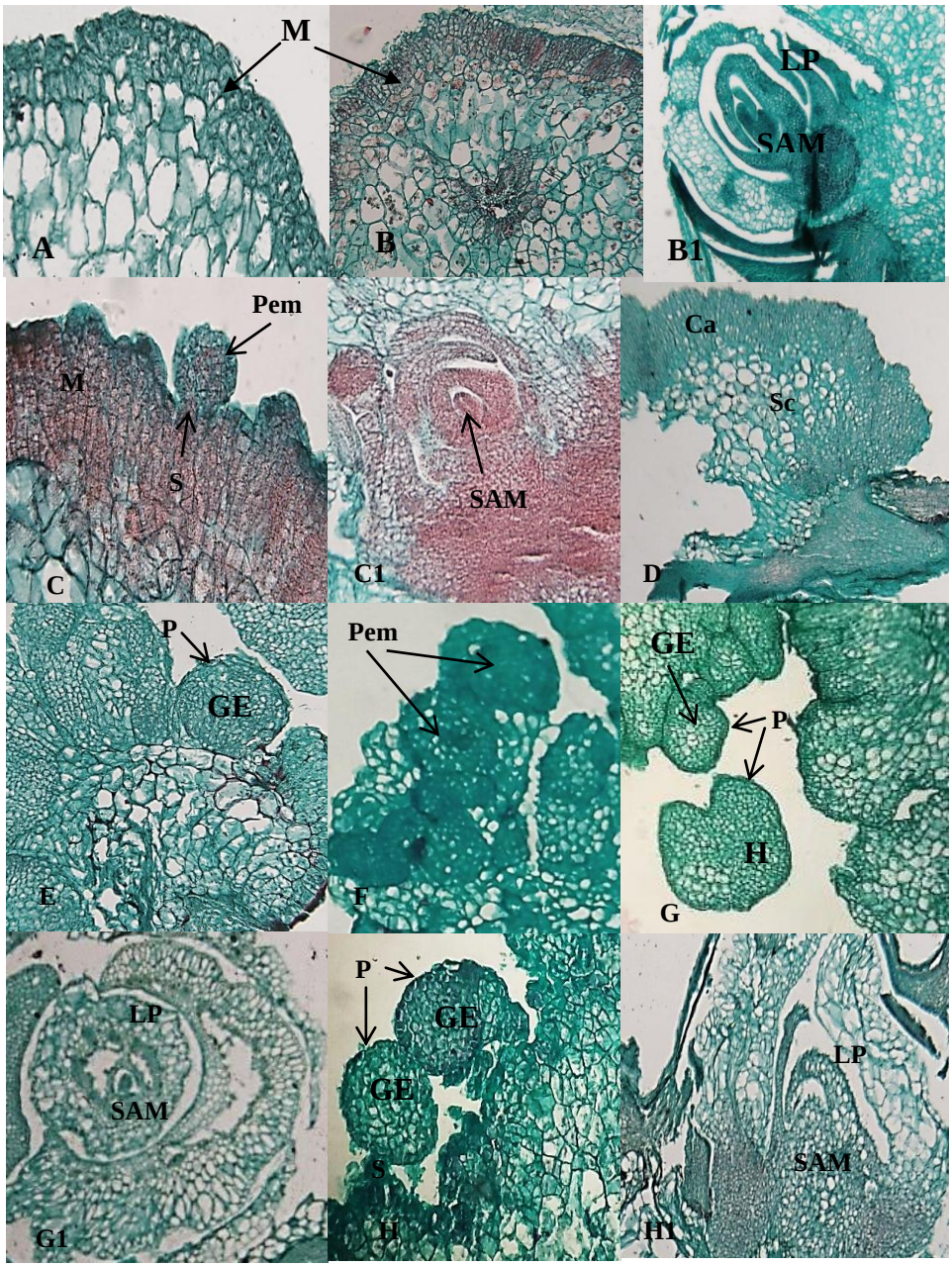


Fig.6 (A-H2): Histological characters of somatic embryogenesis of callus derived from mature rice seeds (A): callus initiation from the epithelial cells of scutellum at 5th DAC in T2 (2 mg/l 2,4 –D); (B): increasing in cellular division at 7th DAC in T2; (B1): shoot apical meristem (SAM) with small leaf primordia (LP) in T2; (C, C1): proembryos (pem) and shoot organogenesis (SAM) at 9th day after culture; (D): callus induction (Ca) at 9th day after culture in T3 (2.5 mg/l 2,4 –D); (E): globular embryo (GE) at 14th day after culture in T2; (F): Proembryo (Pem) at 14th day after culture in T3; (G): globular embryo (GE) accompanied with heart shape somatic embryo (H) in T2; (G1): well differentiated shoot apical meristem (SAM) with leaf primordia (LP) at

16th day after culture in T2; (H): globular embryo and differentiated shoot apical meristem with leaf primordia (H1) at 16th day after culture in T3. Where, Sc scutellum, S suspensor and P protodermis.

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الملخص باللغة العربية

رصد التغيرات الشكل- تشريحية خلال مسار انتاج الاجنة الجسدية المستمدة من الجنين الناضج لنبات الارز صنف سخا ١٠١

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تعتبر القدرة الكامنة للخلية النباتية للتحول الى نبات كامل احد المبادئ الاساسية للتكنولوجيا الحيوية النباتية. يتم تسجيل الجنين الناضج على نحو متزايد باعتباره جزءا نباتيا قيما لتكوين الجنين الجسدي في التكنولوجيا الحيوية للأرز

فى الدراسة الحالية، تم تدارس فقد تمايز خلايا الارز وزيادة عددها واعادة تمايزها باستزراع الاجنة الناضجة فى بيئات الاستزراع موراشيج وسكوج المعدلة والمدعمة بمنظمات نمو مختلفة بمفردها وبالدمج بينهما. تمت ملاحظة كفاءة انسجة الاجنة الناضجة لزراعة الانسجة والتغيرات الزمنية فى مورفولوجيا الخلايا والانسجة .

اظهرت النتائج ان الكالس كان مستحثًا فقط من بذور الأرز الناضجة الذى تمت زراعته على بيئة موراشيج وسكوج المحسنة ب ٤،٢- ثنائى كلوروفينوكسى حمض الخليك (٢ أو ٢,٥ ملغم/ لتر) منفردا بينما اظهرت المعاملات الباقية نتيجة سلبية. وقد بدأ الكالس بعد ٥ أيام من الزراعة على بيئة موراشيج وسكوج المحسنة بالتركيز الاقل من ٢ ، ٤ - D كمجموعات من كتل خلايا غير متمايزة في حين تأخر ظهور الكالس ٤ أيام أكثر بزيادة الجرعة المستخدمة. على المستوى المورفولوجى ، لوحظ الكالس ذو اللون الاصفر الباهت والهش فى كلا الجرعتين المختبرتين واظهر الكالس مظهرا مختلفا فى حين كان قليل العقد فى الجرعة الاقل كان املسا فى الجرعة الاعلى. وقد تم تقدير معدل استحثاس عالى للكالس (٧٠%) مع ٢ ملغم/لتر من ٢و٤- ثنائى كلوروفينوكسى حمض الخليك فى حين ان المعدل المنخفض لاستحثاس الكالس (٤٠%) كان مصاحب للزيادة فى التركيز.

كشف الفحص والتحليل التشريحى لتكوين الجنين الجسدي أنه في غضون أسبوعين من الزراعة على بيئة تحريض الكالس (CIM) ، بدأ تطور الأجنة الجسدية كمجموعات من الخلايا الجنينية في الأجزاء الطرفية من الكالس المتكاثر بينما لوحظت خلايا غير جنينية في المناطق الداخلية للكالس المستحث. ولوحظ ان الخلايا الجنينية في الطبقة الخارجية كانت صغيرة الحجم ومتساوية الاقطار وذات سيتوبلازم كثيف ونواة واضحة موجودة في مركز الخلايا ، في حين كانت الخلايا غير الجنينية كبيرة الحجم ،مفرغة وبها نواة صغيرة تقع بالقرب من جدار الخلية. خضعت الخلايا الجنينية لسلسلة من الانقسامات المنظمة ولوحظت طبقة من البروتودرمس (منشأ البشرة) تحيط بالجنين الكروي في نهاية الانبات على بيئة CIM المحسنة بالجرعة الاقل من ٢ و ٤-D ومن ناحية أخرى ، تأخر تمايز خلايا الأرز التى تم انباتها على بيئة MS المدعمة بالجرعة الاعلى من ٢ و ٤-D حيث لوحظ ظهورالجنين الكروي بعد يومين من نقل الكالس الجنينى لبيئة خالية من الهرمون كما لوحظ ظهور الجنين قلبى الشكل بعد يومين من النقل لبيئة خالية من الهرمون في الجرعة المنخفضة من ٢،٤-D.

هذه الدراسة تلفت الانتباه إلى استخدام النهج التشريحى كأداة مفيدة لمتابعة سلسلة التسلسل الزمني لتطورالجنين في المختبر.