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平贝母不同发育期体细胞胚的核酸与蛋白质合成的研究

SYNTHESES OF NUCLEIC ACID AND PROTEIN IN SOMATIC EMBRYOS OF *Fritillaria ussuriensis* MAXIM IN DIFFERENT DEVELOPMENTAL STAGES

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平贝母不同发育期体细胞胚的核酸与蛋白质合成的研究*

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摘　要

在获得比较理想的平贝母体细胞胚胎发生体系的基础上，应用放射自显影和液闪计数方法研究了平贝母体细胞胚胎发生过程中球形胚、心形胚、鱼雷胚、子叶胚和成熟胚等时期的DNA，RNA和蛋白质的合成动态。研究表明，从球形胚到子叶胚核酸与蛋白质的合成速度递增，在子叶胚前期RNA合成、在子叶胚期蛋白质合成和子叶胚后期DNA合成均达到高峰，即在子叶胚期核酸与蛋白质的合成比其他期更多。核酸与蛋白质合成速度的变化与胚体细胞增殖及器官分化相关。

* 由中国原子能农学会供稿。

SYNTHESES OF NUCLEIC ACID AND PROTEIN IN SOMATIC EMBRYOS OF *Fritillaria ussuriensis* MAXIM IN DIFFERENT DEVELOPMENTAL STAGES*

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ABSTRACT

After developing a procedure for somatic embryogenesis in *Fritillaria ussuriensis*, dynamics on the syntheses of DNA, RNA, and protein during globular, heart-shaped, torpedo-shaped, cotyledonary, and mature somatic embryo stages was demonstrated by both autoradiography and scintillation counting. The rates of syntheses of DNA, RNA, and protein gradually increase between the globular and cotyledonary somatic embryos stages. DNA, RNA, and protein synthesis rates are in peak at the cotyledonary later stage, precotyledonary stage, and cotyledonary stage, respectively. It appears that more DNA, RNA, and protein are synthesized in the cotyledonary somatic embryo stage than in other stages. All these results indicate that an increased syntheses of DNA, RNA, and protein is associated with the differentiation of embryogenic cells and organogenesis in somatic embryos.

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INTRODUCTION

Somatic embryogenesis is closely related with the cell totipotency and zygotic embryogenesis. Some angiospermous embryos are wrapped in the ovule, making it very difficult to measure rates of syntheses of DNA, RNA, and protein during embryogenesis and development. Somatic and zyyotic embryogenesis are similar. Somatic embryogenesis can be used to study the dynamics of syntheses of DNA, RNA, and protein, and to probe the mechanisms of cell differentiation. Fujimura et al[1]. found that carrot embryogenic cells growing without auxin have a higher DNA content at 3～4 days after culturing compared to nonembryogenic cells growing in auxin-enriched media. Sengupta et al[2]. noted that RNA and protein content in carrot embryogenic cells slightly increase after transferring it to new media. On the other hand, embryogenic cells synthesize poly (A) containing RNA (poly A+ RNA) at a higher rate than nonembryogenic cells[3]. From the work of Vergara et al.[4], it appears that the reversible variation in theomethylation pattern of DNA is associated with the development of carrot somatic embryos. At present, no reports are found on the physiology and biochemistry on somatic embryogenesis in *Fritillaria*. For these reasons, the objectives of this work were to determine the dynamics on the syntheses of DNA, RNA, and protein during various developmental stages in somatic embryos of *Fritillaria ussuriensis*.

1 MATERIALS AND METHODS

1.1 Test material

F. ussuriensis Maxim.

1.2 Labelled compound

^{3}H-Thymidine; ^{3}H-Uridine; DL-(4,5-^{3}H)-Leucine.

1.3 Instrument

LS-5801 Liquid Scintillation Counter.

1.4 Somatic embryogenesis system

Bulb segments of regenerated plants (0.1×0.2×0.3 cm^3) were inoculated on a filter paper bridge liquid media containing MN+2.5 ppm 2, 4-D+1.0 ppm KT +5.0 ppm A+500 ppm CH(MN: MS macroelements+N$_6$ microelements, EDTA-Fe, organic substances). After 28 days, light yellowish calli appeared on the media.

The calli were transferred to solid media containing N_6+1.0 ppm KT+5.0 ppm A +500.0 ppm CH, globular somatic embryos were formed after 12 days and mature somatic embryos with shoots and roots formed after 25 days. Sucrose concentration in the media was 30 g/L. All cultures were maintained at 23±2℃ under cool white fluorescent light of 1500 lux with a photoperiod of 12 hours.

1.5 ^{3}H-labelling somatic embryos

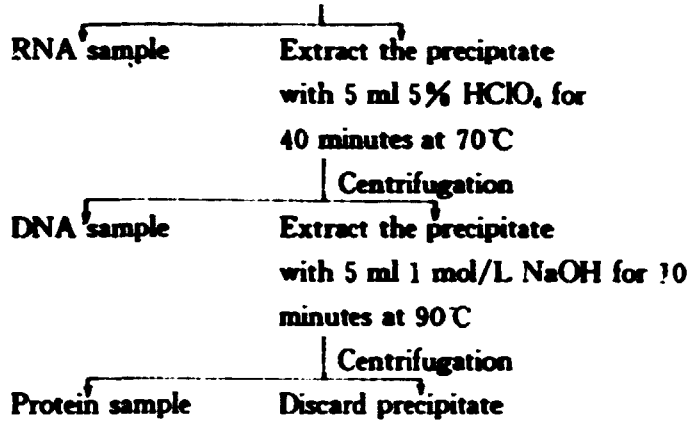
12, 15, 18, 20, 21, 23, 25, and 28 days after initiating embryogenic culture, 10 somatic embryos of the same size and shape were respectively placed in labelling solutions containing ^{3}H-Thymidine (3.7×10^5 Bq/ml, 1.486×10^{12} Bq/mmol), ^{3}H-Uridine (3.7×10^5 Bq/ml, 8.362×10^{11} Bq/mmol), and DL-(4,5-^{3}H)-Leucine (3.7×10^5 Bq/ml, 2.03×10^{12} Bq/mmol). The embryos were incubated for 4 hours at 25±1℃. At the same time, the structures of somatic embryos of different development stages were observed by a histocytological method.

1.6 Separation of samples

DNA, RAN, and protein samples were extracted and purified by a modified procedure from Cherry et al[5]. All centrifugations were maintained for 10 minutes at 5000 g and 4℃.

Labelled somatic embryos were homogenized in a grinder with 2 ml chilled CH_3OH, and the grinder was washed twice with 2 ml chilled CH_3OH washed solution with homogenate was blent.

Homogenate
↓ Centrifugation
Discard supernatant ← Wash the precipitate twice with 4 ml 0.2 mol/L $HClO_4$
↓ Centrifugation
Discard supernatant ← Wash the precipitate twice with 4 ml C_2H_5OH
↓ Centrifugation
Discard supernatant ← Extract the precipitate with 5 ml C_2H_5OH : $(CH_3CH_2)_2O$(2 : 1) for 30 minutes at 50℃
↓ Centrifugation
Discard supernatant ← Extract the precipitate with 5 ml 0.5 mol/L KOH for 16 hours at 37℃
↓ Centrifugation



1.7 Determination of ^3H-Labelled compounds

Labelled DNA, RNA, and protein samples were placed individually into scintillation vials containing 5 ml scintillation fluid (100 g naphthalene + 5 g 2,5-diphenyloxazole + 1000 ml dioxane), and disintegrations per minute were determined with an LS-5801 Liquid Scintillation Counter.

2 EXPERIMENTAL RESULTS

2.1 The histocytological observation

The bulb segments of regenerated *F. ussuriensis*. Maxim plants were inoculated on MN medium. After 25 days, many light yellowish calli had formed (Fig. 1.1). On the 12th day, after callus was transferred to N_6 medium, globular somatic embryos had formed. Histocytological observations of the globular somatic embryos revealed a densely packed cells, large nuclei, dense cytoplasm, and active division (Fig. 1.2). On the 15th day, heart-shaped somatic embryos had formed (Fig. 1.3). On the 18th day, torpedo-shaped somatic embryos had formed, cell division had become directional, and primordial cotyledons had begun to form (Fig. 1.4). On the 21th day, cotyledonary somatic embryos had formed with cotyledons positioned on top of the embryo, and primordial shoots and roots had begun to form (Fig. 1.5). On the 25th day, mature somatic embryos with shoots and roots were observed (Fig. 1.6). After subculturing, mature somatic embryos had developed into regenerated plants with roots, stems, and leaves (Fig. 1.7). The process of somatic embryogenesis, from globular somatic embryos to regenerated plants, is illustrated in Fig. 1.8.

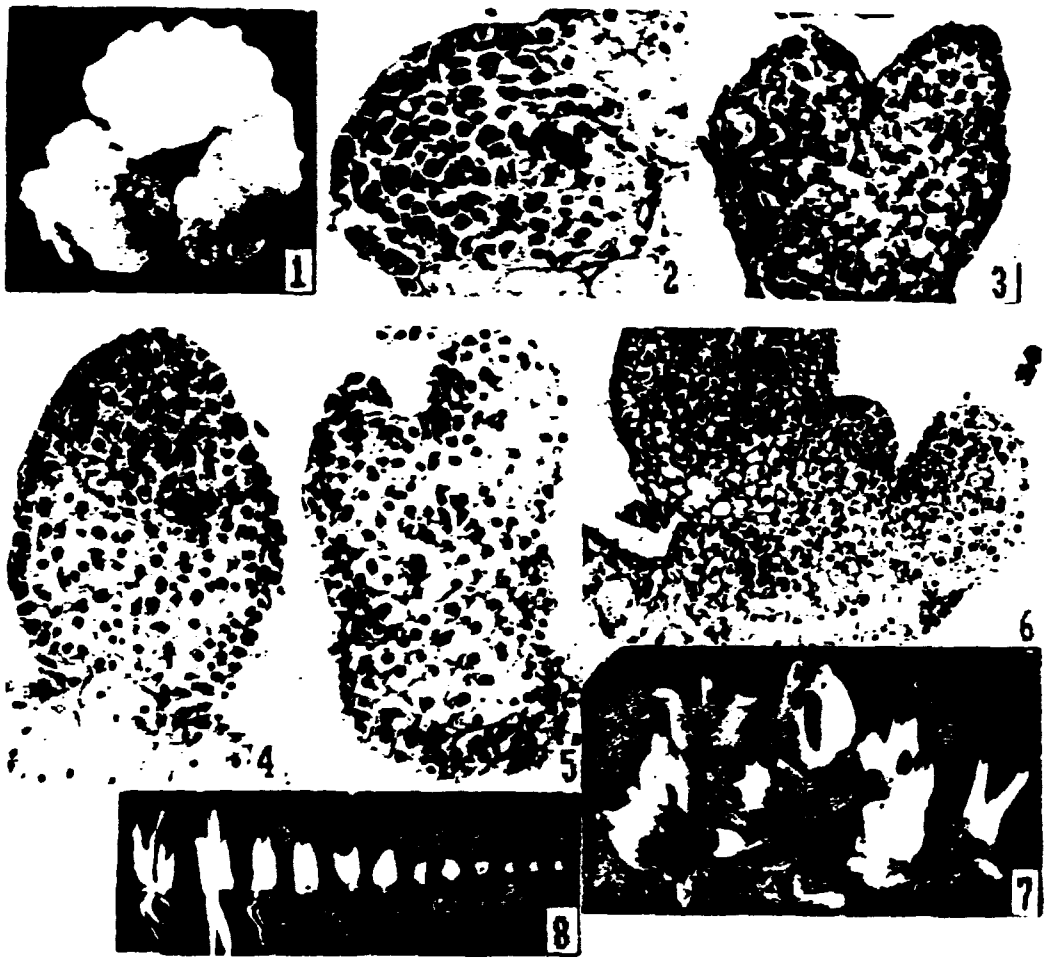


Fig. 1 Explanation of Plate

Fig. 1. 1 Callus (×1. 5)

Fig. 1. 2 Globular somatic embryo (×165)

Fig. 1. 3 Heart-shaped somatic embryo (×125)

Fig. 1. 4 Torpedo-shaped somatic embryo (×99)

Fig. 1. 5 Cotyledonary somatic embryo (×99)

Fig. 1. 6 Mature somatic embryo (×49)

Fig. 1. 7 Regenerated plants (×1. 5)

Fig. 1. 8 Developmental sequence from globular somatic embryo to regenerated plants (×2. 5)

2.2 Dynamic Characteristics of DNA, RNA, and Protein Syntheses

2.2.1 Changes in the rate of incorporation of ^{3}H-Thymidine

Changes in the rate of incorporation of ^{3}H-Thymidine into somatic embryos of *F. ussuriensis* showed in Fig. 2 (dpm = decay/min) by both autoradiography and scintillation counting. The DNA synthesis rate in the somatic embryos gradually increased between 12 and 23 days after initiation of embryogenic culture. The DNA synthesis rate in cotyledonary somatic embryos was in peak at 23 days after culture initiation. ^{3}H-Thymidine incorporation data showed that active synthesis of DNA in the cotyledonary and later stages of somatic embryos is a biochemical transition point during the primordial differentiation of shoot and root. The rate of DNA synthesis was maintained at higher levels in the mature somatic embryo stage.

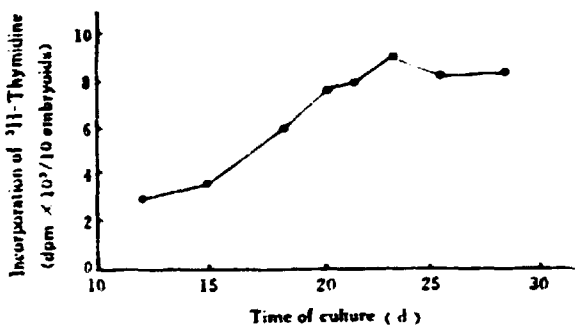


Fig. 2 ^{3}H-Thymidine incorporation into somatic embryos of *F. ussuriensis*

2.2.2 Changes in the Rate of Incorporation of ^{3}H-Uridine

The pulse-labelling experiments using ^{3}H-Uridine as a precursor of RNA synthesis demonstrated that the rate of RNA synthesis in somatic embryos rapidly increased between 12 and 20 days after embryogenic culture (Fig. 3). A peak value in RNA synthetic activity of the somatic embryos occured 21 days after embryogenic culture initiation. This result suggests that active gene expression may be occurring in cells of these pre-cotyledonary somatic embryos. The rate of RNA synthesis decreased at later somatic embryo stage.

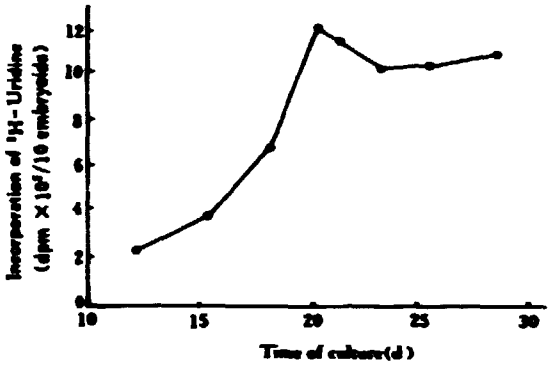


Fig. 3 ^{3}H-Uridine incorporation into somatic embryos of *F. ussuriensis*

2.2.3 Changes in the Rate of Incorporation of DL-(4,5-^{3}H)-Leucine

As DL-(4,5-^{3}H)-Leucine incorporation showed in Fig. 4, protein synthesis rate was in peak in 21-day-old cotyledonary somatic embryos. DNA, RNA, and protein syntheses rates were in peak at the cotyledonary later stages, pre-cotyledonary stage, and cotyledonary stage, respectively. These results suggest that changes in the rate of macromolecule synthesis in somatic embryos be correspond with the primordial differentiation of shoots and roots. Protein is a main structural macromolecule of the cell. The rate of protein synthesis in somatic embryos is higher than that of DNA and RNA.

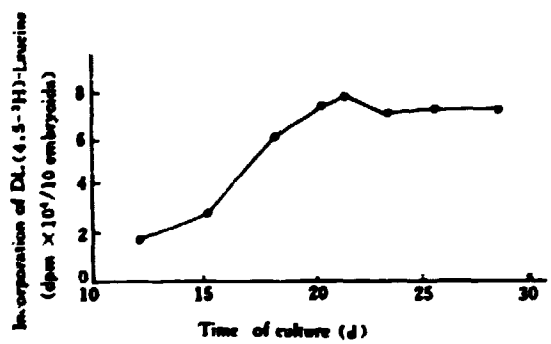


Fig. 4 DL-(4,5-^{3}H)-Leucine incorporation into somatic embryos of *F. ussuriensis*

3 DISCUSSION

The process of somatic embryogenesis at high frequency was developed in *F. ussuriensis*. The structures of the somatic embryos of different development stages were histocytologically observed. The development of somatic embryos is closely parallel to embryogenesis of a fertilized egg cell in *F. ussuriensis*.

Somatic embryogenesis was first described clearly in carrot, and to date the carrot system is the most comprehensively studied with respect to culture conditions and developmental physiology and biochemistry of somatic embryogenesis. Though biochemistry on somatic embryogenesis have mainly been done with carrot, similar research have been accomplished in other crop plants using simple manipulative techniques. From the work of Shoemaker et al.[6], changes in the levels of 60 kd and 70 kd proteins of cotton somatic embryos may serve as a biochemical indicator of different stages of development. Coppens et al.[7], found changes of certain isoenzymes during somatic embryogenesis in barley. Recently, Raghavnan et al.[8] studied the dynamics on nucleic acid and protein syntheses during normal pollen development and pollen embryogenesis in *Hyoscyamus niger*. We found that the rates of DNA, RNA and protein syntheses during the early stages of development of *F. ussuriensis* somatic embryos rapidly increased. In different stages of cotyledonary somatic embryos, peak value in the DNA, RNA and protein syntheses rates were observed. Primordial differentiation of shoot and root in somatic embryos depends on increase of syntheses of DNA, RNA, and protein in *F. ussuriensis*. The dynamics on macromolecule synthesis in somatic embryos as shown in this investigation suggest the need for further research in the synchrony of events in somatic embryogenesis.

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