INDUCTION OF CALLUS TISSUES FROM DIFFERENT SOMATIC ORGANS OF RICE PLANT BY VARIOUS CONCENTRATIONS OF 2,4-Dichlorophenoxy acetic acid[1]

L. Wu and H. W. Li[2]

Abstract

Callus tissues were induced from different somatic organs of *O. sativa* variety Taichung No. 65. Specific 2,4-dichlorophenoxy acetic acid (2,4-D) concentration in a medium is necessary for the induction of callus from different organs. The optimum 2,4-D concentrations for callus induction from different organs are: 0.5 ppm for roots, 2 ppm for scutellum and cotyledonous node, 6 ppm for coleoptile and 8 ppm for stem nodes and leaf-sheath. All cultures were incubated in darkness at 28°C.

Anatomical studies showed that callus tissues from leafsheath and coleoptile are originated from the meristematic cells located very closely by the immature vascular bundles. The initiation of callus in nodes seems to be similar to those of adventitious roots which are initiated near the differentiating vascular tissue or directly from the procambium.

Introduction

Somatic tissues from a wide variety of dicotyledons have been grown *in vitro* as callus tissues which were induced from different organs. Most of these attempts to culture comparable tissues from monocotyledons have been successful in some species in the past but not with rice plant.

Carter and Schwarting (1958) attempted to obtain callus from nodes, endosperm, and young embryos of rye but they were successful only with the embryos. Nickle (1964) cultured internodal parenchyma tissues from the stem of several sugarcanes. Mascardnhas, Sayagaver and Jagannatha (1965) obtained callus from maize roots. This callus had an absolute requirement for diphenylurea. Tamaoki and Ullstrup (1958) using small explant of intercalary meristem of corn stem obtained callus, but their subcultures did not grow.

(1) This study was partly supported by the National on Science Development of Republic of China.

(2) Assistant Research Fellow and Research Fellow of the Institute of Botany, Academia Sinica.

(3) Journal Series No. 90 Institute of Botany, Academia Sinica.

Webster (1966) established callus from germinating oats seeds cultured the Chysanthemum nematode upon the callus. Carter, Yamada and Takashi (1967) found that oat-callus induction and growth were very sensitive to auxin but relatively unaffected by Kinetin. Trione Jones and Metzger obtained callus tissue from the cotyledonary node of wheat and grew well thereafter (1968). The tissue culture of rice has been studied in recent years. However the callus can be induced only from the embryo (Yamada, Taneka and Takahashi 1967).

Corn endosperm was first cultured by La Rue (1949) and subsequently by many others (Piezur, 1952; Sternkeimer, 1954; Strans and La Rue, 1954; Tamaoki and Ullstmp, 1958). Norstog (1965) cultured the endosperm of rye grass. These endosperm on cultures are triploid tissues which do not differentiate to form recognizable organs in culture. All in all so far there have been a few reports of successful culture of cells from monocoty-ledonous plants.

This paper gives the result of our study on tissue culture of rice plant *O. sativa* as the methods for inducing the formation of callus from different somatic organs using different concentrations of 2,4-D as well as the anatomical studies of the origin of these cells from which callus tissues are originated.

Materials and Methods

Various organs of seedlings and older rice plants of *O. sativa* var. Taichung No. 65 were used in this experiments. Dehusked seeds and older plant organs were washed and surface sterilized in 1 percent sodium hypocholoride for 15 min and rinsed four times with sterile distilled water. The sterilized seeds were soaked in sterile distilled water for 12 hours, then they were implanted on the selected medium. The modified medium contained (mg/1); NH_4NO_3, 1000; $Ca(NO_3)_2 \cdot 4H_2O$, 1000; KNO_3, 800; KCl, 65; $MgSO_4 \cdot 7H_2O$, 370; NaH_2PO_3, 16.5; $Fe_2(SO_4)$, 2.5; Na_2-EDTA, 13.5; $ZnSO_4 \cdot 7H_2O$, 3.8; H_3BO_3, 4.5; KI, 0.75; glycine, 3; thiamine, 0.1; Pyriodoxine, 0.1; nicotinic acid 0.5; sucrose, 20,000; yeast extract, 5000; agar, 8,000. Then the growth regulators such as kinetin, 2.4-D and 3-indolyl-acetic acid (IAA) were added for specific purpose. With excised nodes and young leaf sheath of older plants they were placed on the surface of agar medium containing 8 ppm 2,4-D in addition for callus induction. Whereas with soaked seeds they were on the medium containing 0.5 ppm 2,4-D for the callus induction from roots. When the medium containing 6 ppm 2,4-D callus induction could be obtained from scutellum and cotyledonous nodes. On the medium containing 2 ppm 2,4-D the coleoptile could be grown to the length of 2 to 4 centimeters. The excised coleoptile was then transfered to the medium containing 6 ppm 2,4-D for callus induction. All the cultures were incubated in dark room at 28°C.

For anatomical observation of the initiation of callus tissues, paraffin

sections were obtained and stained with Hematoxylin as well as Fast green.

Results

Excised young leaf-sheath and nodes were incubated three weeks (in darkness at 28°C) on the medium containing 8 ppm 2,4-D. The outer epidermis of the excised leaf-sheath and nodes were proliferated to form callus tissues (Plate 1 A, B). However, the inner epidermis of leaf-sheath sometimes were found to form callus. The callus formation usually limitted in the node region. Although we tried to induce callus from internode, it had never been succeeded. A cross section of the callus induced leaf-sheath shows many groups of callus which proliferate from the external region of immature vascular bundles (Plate 1 F). An enlarged photograph of transverse section of the vascular bundle of the callus induced leaf-sheath (Plate 1 G) shows that the callus are initiated from the external meristematic cells located very closely to the immature vascular tissues of the differentiating vascular bundle. A cross section of callus induced node shows that masses of callus tissues arise from the meristematic cells of procambium (Plate 1 H).

The excised coleoptile were implanted in the medium containing 6 ppm 2,4-D and incubated in darkness at 28°C for three weeks. The surface of the coleoptile were proliferated to form callus (Plate 1 C). Similarly callus were formed from cotyledonous node and scutellum of germinated seeds which were incubated in the medium containing 2 ppm 2,4-D (Plate 1 D). Further investigations show that the callus tissues were initiated from the meristematic cells near the differentiating immature vascular bundle of the coleoptile (Plate 2 A). In cotyledonous node the callus tissues initiated from the meristematic cells of procambium sometimes initiated from the peripherial meristematic tissues of the external phloem region (Plate 2 B). However, the callus tissues were formed by the vigrous cells division of the epidermis cells of the scutellum (Plate 2 C, D).

The meristematic portion of the root tips of seedlings were swollen to form callus (Plate 1 E) after being planted on the medium containing 0.5 ppm 2,4-D and incubated in darkness at 28°C.

All callus tissues induced from different somatic organs had been subcultured successful on the basic medium which supplement with Kinetin (2 ppm) and IAA (2 ppm) and 2,4-D (4 ppm).

Discussion

In the case of seeds, they must be presoaked for 12 hours in order to induce callus formation. When the seeds are not presoaked a mass of callus

tissue will be proliferated from embryo, having the rest of the organs to be inhibited from further development such as coleoptile, hypocotyle and roots. Since only the mass of callus tissues are formed, the origin of the tissue from which the callus is originted remainds unknow.

For callus differentiation Kinetin and IAA seem to be not essential in the medium. However 2,4-D is necessary hormone required.

The variation of the concentration of 2,4-D in the medium is sensitive for the callus induction from different organs. When the concentration is low (0.5 ppm) only the root tip cells will form callus the other organs would differentiated and grow as usual. When the concentration of 2,4-D was increased to 2 ppm root differentiation stopped, coleoptile and hypocotyle seem not to be effected and they continue to differentiate. Calluse tissues can be frequently initiated from scutellum and cotyledonous nodes. Coleoptile may increase up to 4 centimeter in length. The lengthened coleoptile can be excised and planted on the medium containing 6 ppm 2.4-D; callus tissues would then be initiated.

Anatomical studies showed that the callus tissues from leaf-sheath and coleoptile are originated from the meristematic cells located around the immature vascular bundles. From that, the callus induction is easier to be obtained from leafsheath of young seedlings. On the contrary it is harder to obtain a callus from young leaf-sheath of an older plant. It was tried to callus from the old leaf-sheath but there was no success.

Callus tissue can be induced from any node of rice stem even from nodes of panicles. The initiation of callus in nodes seems to be similar to the

Explanation of Plates

Plate 1

1A A mass of callus formed from the leaf-sheath which was excised from the older rice plant and incubated on the agar medium containing 8 ppm 2,4-D ×6

2B Callus tissue proliferated from the excised node of older rice plant. The induction medium containing 8 ppm 2,4-D. ×2

1C Masses of callus tissues burst out from the epidermis of the excised coleoptile using medium containing 6 ppm 2,4-D. ×4

1D Two groups of callus are differentiated from the scutellum and cotyledonous node using medium containing 2 ppm 2,4-D. ×4.

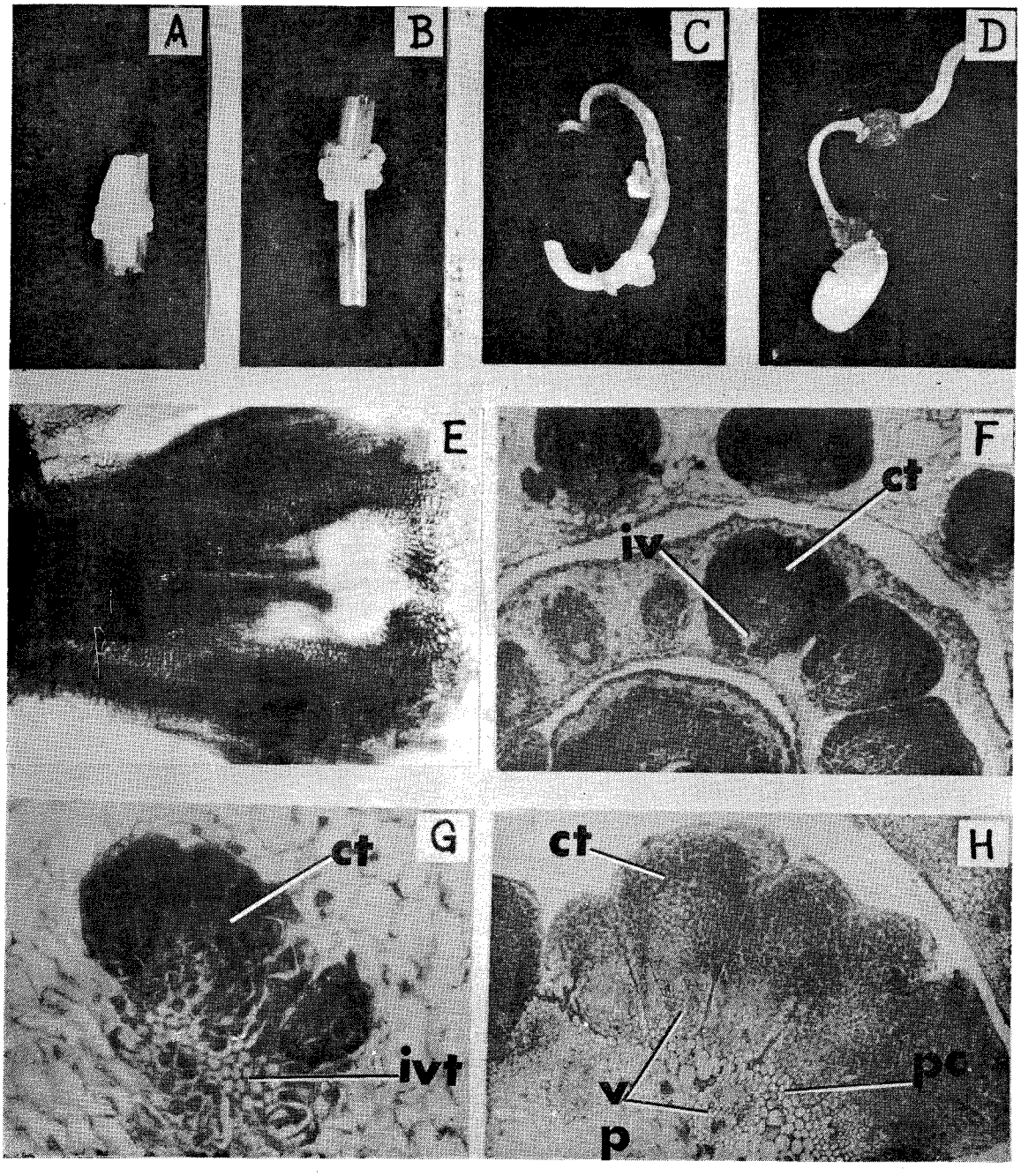
1E A root tip is swollen and collasped to form callus using medium containing 0.5 ppm 2,4-D. ×100

1F Transverse section of callus induced young leaf-sheath of rice showing masses of callus tissue grow from the external region of immature vascular bundles. ×200

1G An enlarged photomicrograph of differentiating vascular bundle of callus induced leaf-sheath showing a group of meristematic cells initiated from the meristematic cells near the immature vascular tissues. ×400

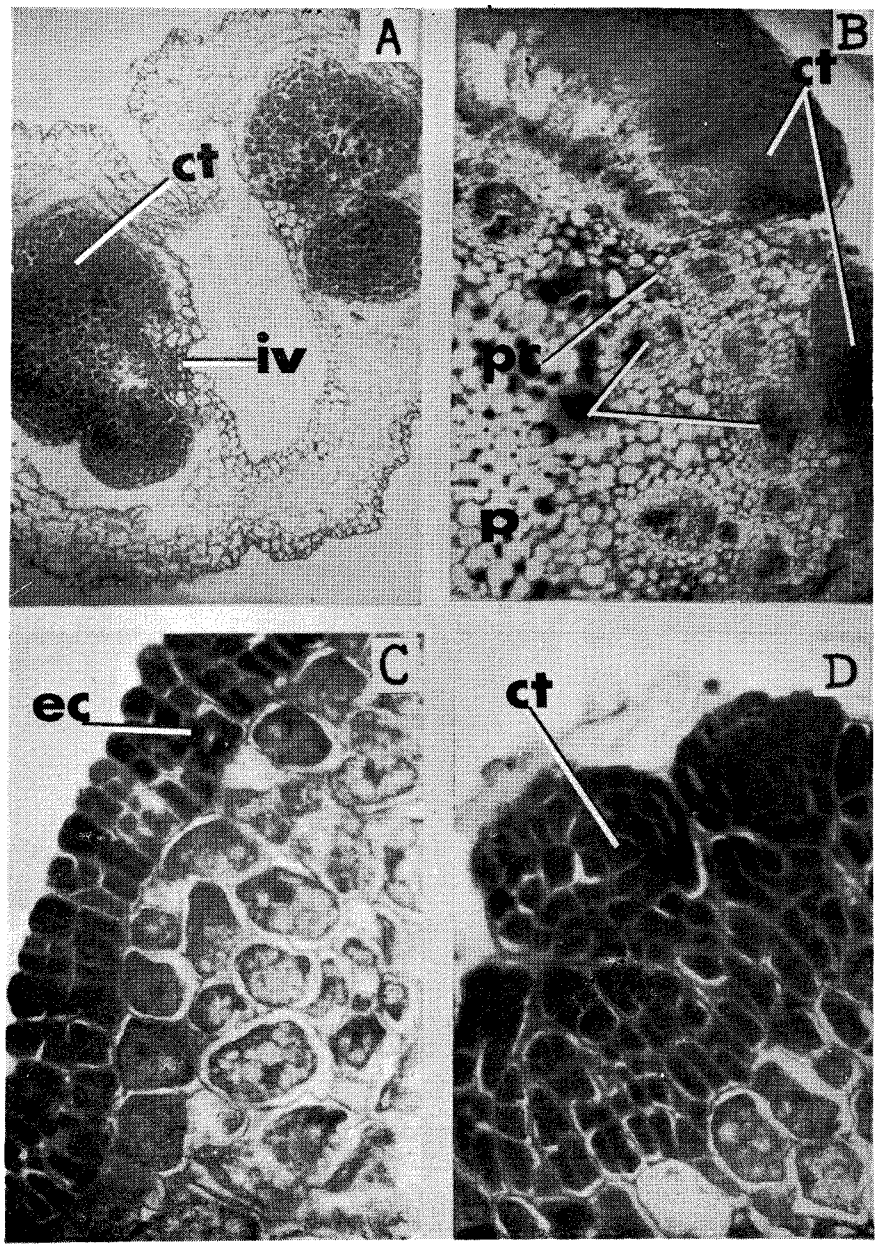
1H A transverse section of callus induced node of rice showing callus tissues formed from the meristematic cells of procambium. ×200

Plate 1



iv =immature vascular bundle
pc =procambium
ivt=immature vascular tissue
ct=callus tissue
v =vascular bundle
p =pith

Plate 2



ec=activated scutellum epidermis cells

initiation of the adventitious roots which are initiated near the differentiating vascular tissue or arise directly from procambium (Katayama).

以不同濃度 2,4-Dichlorophenoxy acetic acid (2,4-D) 自水稻不同器官誘發腫瘤組織 (Callus) 之研究

吳　麟　李先聞

以不同濃度之 2,4-D 加入培養基中可自水稻之不同器官誘發出可供組織培養研究使用的腫瘤組織（Callus）經解剖學上的觀察，發現其腫瘤組織之發生在水稻組織內起自特定的部位，如節部的原始形成層（Procambium），根之尖端細胞，葉鞘與子葉鞘未成熟輸導組織週圍之分裂細胞與胚盤之表皮細胞等爲腫瘤組織的發源處。

Literature Cited

CARTER, O., Y. YAMADA, and E. TAKAHASHI, 1967. Tissue culture of oats. Nature **214**: 1029-1030.

CAREW, D. P., and A. E. SCHWARTING, 1958. Production of rye embryo callus. Bot. Gaz. **119**: 237-239.

TRIONE, E. J., L. E. JONES, and R. J. METZGER, 1968. In vitro culture of somatic wheat callus tissue. Amer. J. Bot. **55**(5): 529-531.

LA RUE C. D. 1949. Culture of the endosperm of maize. Amer. J. Bot. **34**: 585-586.

MASCARENHAS, A. F., B. M. SAYAGAVER, and V. JAGANNATHAN, 1965. Studies on the growth of callus cultures of zea mays. Tissue culture seminar Pro., Univ. Baroada, India.

NICKELL, L. G. 1964. Tissue and cell culture of sugar can-another research tool. Hawaiian Planters Rec. **57**: 223.

NOSTOG, K. J. 1956. Growth of rye-grass endosperm in vitro. Bot. Gax. **117**: 253-259.

PIECZUR, E. A. 1952. Effect of tissue culture of maize endesperm on the growth of excised maize embryos. Nature **170**: 241-242.

STRAUS, J. 1954. Maize endosperm growth in vitro. II. Morphology and Cytology. Amer. J. Bot. **41**: 833-839.

Plate 2

2A A transverse section of coleoptile of rice showing two groups of callus tissues was formed from the meristematic cells of the external differentiating vascular bundle. ×200

2B A transverse section of callus induced cotyledonous node of rice showing callus tissues grow from the procambium and the external phloem region. ×200

2C A section of the scutellum of rice embryo cultured on the medium containing 6 ppm 2,4-D showing the epidermis cells being activated into meristematic cells. ×400

2D A section of callus tissues are initiated from the activated epidermis cells of scutellum. ×400

STERNHEIMER, E. P. 1954. Method of culture and growth of maize endosperm in vitro. Bull. Torry Bot. Ciub **81**: 111.

TADDA C, KATAYAMA 1966. Anatomy of the root of *Oryza sativa* with emphasis on developmental processes. Seiken Ziho **18**: 6-22.

TAMAOKI, T., and A. J. ULLSTRUP 1958. Cultivation in vitro of excised endosperm and meristem tissues of corn. Bull. Torry Bot. Club **85**: 260-272.

WEBSTER, J. M. 1966. Production of oat callus and its susceptibility to a plant parasitic nematod. Nature **212**: 1472.