ABSTRACT
Sites of callus origin have been histologically traced in the 40-day-old sugarcane cv. F168's young leaf and stem tip explants cultured on a modified Murashige and Skoog medium containing 3 mg/l 2,4-D. Panoratically, calli are formed on those regions of the explants which have cut wounds and are exposed to the medium. Microscopically, callus cells arose from the cambium-like cells, phloem parenchyma and the primary xylem in the vascular bundle of the leaf and stem tip explants. The newly formed callus cells are large and vacuolated in appearance but some of them soon grow into a meristemoid. Pressure from the centrifugal growth of the meristemoids results in the appearance of many nodule-like structures along the periphery regions of the explants.

INTRODUCTION
A number of researchers have investigated the original site of callus formation in various explants of different plant species. Information on this subject would facilitate the culturists to know the potential of different tissues to proliferate in vitro and hence a more efficient working scheme can be undertaken. Gautheret6 has given a diagram showing callus initiation from a variety of sites including cambium in a cultured carrot root, but his text presented no convincing histological sections. White13 has examined the callus origins in a mature spruce tree explant and concluded that callus formed first from the cambium, then from phloem parenchyma and finally from the lining of xylem resin ducts. Lai8 has found that the rice's callus arose from the pericycle and endodermis of roots. Gupta7 traced the hypocotyl callus of fenugreek by paraffin section method and revealed that the callus stemmed from the pericycle region. Dunstan et al4 examined histologically a sorghum explant and found that cell divisions occurred internally and on the periphery of the seed scutellum. Liu and Chen9 have made a morphogenetic study on the hormonal effects on the capability of organogenesis in sugarcane (Saccharum species hybrid) callus but presented no data to show the exact sites of callus initiation. The present investigation is intended to fill this gap and to provide sufficient histological evidences to verify the origin of the callus cells in the explants.

MATERIALS AND METHODS
Sugarcane cv. F168's stem tip tissue including 1st to 9th internodes (node
FIGURE 1. Top part of sugarcane plant with leaf sheaths cut off and stem tip split open so as to expose the growing point and the leaf portion proposed to be excised for explantation. Nodes 14, 13 and 12 have leaf sheaths already mature, d indicates the position of the termination of the leaf sheath of node 11, e of node 10 and f of node 9.

FIGURE 2. A portion of a leaf explant exhibiting the primary interior structures of a leaf blade, a = Protoxylem, b = Chlorophyll-bearing bundle sheath, e = Protophlem, u,e = Upper epidermis, i.e. = Lower epidermis. (x 125)

designation is according to Artschwager¹) and the 2 to 3 cm portion of the second innermost rolled young leaves right above the tip (Fig. 1) were aseptically excised and immediately inoculated on a modified Murashige and Skoog medium¹ containing 3 mg/l 2,4-dichlorophenoxyacetic acid (2, 4-D) which is designated as non-organ-forming (NOF) medium thereafter (Liu et al¹⁰). About 5 to 7 days, callus cells started to initiate from the cut wounds of the explants. Six weeks later the explants with massive calli were cut into an appropriate size and fixed in formalin-propionic-alcohol (FPA) for 24 hrs., dehydrated with a series of tertiary butyl alcohol before being embedded in paraffin. Serial sections cut at thickness of 10 μm were stained with safranin-fast green or safranin, tannic acid and orange G (Sharman¹¹).
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FIGURE 3. Whole view of a leaf explant with massive callus. Note the region where the callus is proliferating, at the lower epidermis (rectangular areas are enlarged in Figs. 4 and 5). (X 16).

RESULTS AND DISCUSSION

Site of young leaf callus

The young leaf when excised was not developed into clear-cut distinction between sheath and blade (Artschwager¹). It is composed of cells in meristematic state which are similar in size (Cook³). Five to seven days after explanation, the
The former is surrounded by the latter.

After 40 days in culture, it can be seen that numerous vascular bundles in different sizes have emerged along the lower epidermis. The small vascular bundles are situated near to the lower epidermis than the larger ones (Figs. 2 and 3).

Panoramically, callus is usually formed at the cut wounds of the explant.
Figure 6. Whole view of a stem-tip explant with massive callus which is growing on the periphery region (arrowed areas are enlarged in Fig. 7,8,9 and 10). X-75

Histological examination shows that callus is obviously proliferated from the cells near or in the vascular bundle because only those regions of the leaf which house the vascular bundles can produce callus (Fig. 3). This observation is of interest since Artschwager\(^1\) has stated that in young developing leaves, cambium-like cells occur between xylem and phloem. They constitute an actively dividing tissue which some-
times takes on the appearance of a typical cambium. Other evidence from the microscopical slides (Figs. 3, 4 and 5) is that the primary phloem cells possess dense cytoplasm. Thus, the cambium-like cells, the phloem parenchyma and the primary xylem are more easily amenable to proliferation than other parenchymatous cells.

By enlargement of the two portions (labelled by rectangles) on Fig. 3, some callus growth patterns can be observed. Fig. 4 shows that the newly formed cells...
FIGURE 8. Similar to Fig. 7 except that the enlarged area is at b (arrowed) in Fig. 6 indicating the callus mass is intruding the epidermis. (X 80).

from the primary vascular bundles are larger in size than their mother cells, but some of them soon develop into a nodule-like structure which could be appropriately referred to as "meristemoid" (Bunning). The meristemoid in its early stage is composed of cells in small size but with dense cytoplasm and striking nuclei. The heterogeneous phenomenon exhibited by the sugarcane callus in its initial stage was also observed in carrot callus cultures as reported by Gautheret and his co-workers. They found that the callus cultures were actively growing and usually contained a high proportion of vacuolated parenchymatous cells together with
FIGURE 9. Similar to Fig. 7 except that the enlarged area is at the lower part of the explant (z, arrowed) showing the intruding growth of a meristemoid toward the periphery region (X 80).

more localized groups of smaller and obviously meristematic cells.

Site of stem tip callus

Fig. 6 is a radial section through a 40-day-old explant with massive callus. The stem tip virtually contains the top 1-9 internodes in height of 6-10 mm. The
FIGURE 10. Enlarged view of a well-developed vascular bundle (d, arrowed) 6 mm away from the tip, deep embedded in the ground tissue, demonstrating no callus cell arises at all. (X 125).
apical portion is made of thin-walled meristematic cells which are in a state of active division. Some of them gradually develop into the primordia of vascular bundles and become fullpledged later (Artschwager1). By enlarging the callus-forming site, it is apparent that the callus is incepted form the extensive proliferations of ground vascular bundle in which the protoxylem cells play a very important role. This is based on the observation that some cells with secondary wall thickening are clearly connected with the cells of a meristemoid as seen in Fig. 7 and 9. Massive callus arising from spruce tree’s cambium and young phloem region has also been reported by White13. The development of a meristemoid from the newly born cells is similar to the case occurring in leaf explant. The meristemoid is virtually a pro-embryoid which will develop into a shoot when transferring it onto an organ-forming medium (Liu and Chen9). Phloem parenchyma may also give rise to callus but this is not clearly shown in these slides. Pressure from the centrifugal growth of the meristemoids has pushed off the epidermis as shown in Fig. 8.

Those vascular bundles which are deeply embedded in the parenchymatous ground tissue of the stem tip explant will not be induced to form callus mass (Fig. 10) whereas those situated near the periphery region can do so (Figs. 3 and 6).

From this studies it can be concluded that callus cells originate from the primary vascular bundles in both the leaf and stem tip explants. Microscopical tracing of callus origin shows that the cambium-like cells, phloem parenchyma and the primary xylem in the vascular bundles are responsible for the callus production. However, any cell which is in meristematic state can, under suitable conditions, be brought to grow and to provide callus.

REFERENCES


SITIOS DE FORMACION CALLOS EN HOJA JOVENES TROZOS DE TALLOS SECCIONADOS DE CAÑA DE AZUCAR.

M.C. Liu, W.H. Chen and S. C. Shih

RESUMEN

Lugares de origen del callo han sido histologicamente reconstruidos en una planta de caña de azucar de 40 días de edad, clon varietal F 168. Se trabajó con hojas jóvenes y puntas de los tallos de la ex-planta, en el medio de cultivo Murashige y Skoog modificado, conteniendo 3 mg/l de 2,4 D.

Panoramicamente, el callo estaba formado en aquellas regiones de la ex-planta que fueron cortadas y ese corte expuesto al medio citado. Microscópicamente las células del callo fueron apareciendo de las células parecidas al cambium, floema, parenquima y del xilema primario en los vasos vasculares de los órganos mencionados al principio. La nueva formación de células en los callos fueron alargadas y vacuoladas en apariencia pero algunas de ellas crecieron rápidamente a meristemoides.

La presión del crecimiento centrifugo de los meristemoides, resultaron en la aparición de muchas estructuras parecidas a nodulos, a lo largo de la región periferica de la ex-planta.