ANATOMY OF SHOOT PRODUCTION IN VITRO FROM EXPLANTS OF CAULIFLOWER CURD (BRASSICA OLERACEA L. VAR. BOTRYTIS SUBVAR. CAULIFLORA DC.)[1]

ANTONIO CARLOS TORRES[2], THEREZINHA ISAIA PAVIANI[3], LINDA STYER CALDAS[4] e PAULO TARCISIO DELLA VECCHIA[5]

ABSTRACT - The cauliflower curd consists of a main shoot from which many first-order branches develop in acropetal succession. Successive branching orders develop from the apices which exist on each branch and which form the surface of the marketable curd. The branches have the same anatomical structure as the majority of herbaceous dicotyledons. Explants taken from the surface of the marketable curd of cauliflower were cultured, in nutrient medium. Starting on the fifth day after inoculation of the explant in the nutrient medium, the anatomical modifications in the explant were observed. The parenchymatic cells adjacent to the vascular bundles of the peduncle divide periclinally and increase in volume. Apparently this phenomenon is related to the differentiation of vascular elements which occur in a disorganized fashion. Concomitantly there is a progressive and gradual differentiation of the apical regions from undifferentiated meristems to organized shoot meristems, producing leaf primordia in a regular fashion. In approximately 10% of the cultures, bracts which were present on the explant developed greatly when in contact with medium, and shoots were formed from their blades.

Index terms: cauliflower, plant anatomy, tissue culture, organogenesis.

ANATOMIA DA CABEÇA E DO DESENVOLVIMENTO DE PARTE AÉREA EM EXPLANTES DE COUVE-FLOR (BRASSICA OLERACEA L. VAR. BOTRYTIS SUBVAR. CAULIFLORA DC.) CULTIVADOS "IN VITRO"

RESUMO - A cabeça da couve-flor consiste de um eixo caulinar do qual se desenvolvem vários eixos pedunculares primários, em sucessão acrópeta. Os eixos pedunculares secundários se iniciam a partir dos ápices dos eixos primários e assim sucessivamente, até a formação da cabeça. Os eixos apresentam anatomia semelhante à de caules de dicotiledôneas herbáceas. Os eixos pedunculares superiores são recobertos por ápices meristemáticos. Explantes tomados da superfície da cabeça de couve-flor em estádio de colheita foram cultivados em meio nutritivo. A partir do quinto dia após introduzido no meio do cultivo, observaram-se modificações no material. As células parenquimatosas que cercam os feixes vasculares do pedúnculo do explante dividem-se periclinalmente e aumentam em volume. Este fato parece estar relacionado com a diferenciação de elementos vasculares que ocupam posições e arranjos desordenados. Concomitantemente, os ápices meristemáticos encontram-se em estado de ativa mudança ontogênica, resultando desta atividade a diferenciação gradual e progressiva destas células, que passam de meristemática a elementos diferenciados e, até, organizando-se em caules e folhas. Constatou-se, em 10% do material em cultura, que algumas brácteas presentes no explante se desenvolvem bastante em contato com o meio de cultivo, e de seu limbo foliar pode ocorrer a diferenciação e desenvolvimento de parte aérea.

Termos para indexação: couve-flor, anatomia vegetal, cultura de tecido, organogênese.

INTRODUCTION

The importance of vegetative propagation in cauliflower has been discussed in several papers: some authors used the technique of *in vitro* culture of cauliflower to propagate and maintain clones of parent lines (Crisp & Walkey 1974, Grout & Crisp 1977, Pow 1969, Trimboli et al. 1977), whereas others have employed it in breeding of summer cultivars (Torres et al. 1978) or simply in producing virus-free clones (Grout & Crisp 1977, Walkey et al. 1974). Reports of *in vitro* propagation of cauliflower described the formation of callus before shoot differentiation on the explants. This method of propagation offers great potential for the production of large numbers of plants in each sub-culture (Vazquez et al. 1977). On the other hand, callus cultures lead to patterns of differentiation which are not under the normal physiologi-

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[2] Engº Agrº, M.Sc., Unidade de Execução de Pesquisa de Âmbito Estadual (UEPAE) - EMBRAPA, km 9 da Rodovia Brasília-Anápolis, Caixa Postal 11-1316, CEP 70.000 - Brasília, DF, Brasil.

[3] Botânica Livre Docente da Universidade de Brasília (UnB) - Departamento de Biologia Vegetal, CEP 70.910 - Brasília, DF, Brasil.

[4] Biól., Ph.D., Universidade de Brasília (UnB) - Departamento de Biologia Vegetal.

[5] Engº Agrº, UEPAE/EMBRAPA, Brasília, DF, Brasil.

cal-genetic control of the meristems (Lavee & Galston 1968), permitting the appearance of genetic variations (Crisp & Walkey 1974, D'amato 1977, Lavee & Galston 1968, Vazquez et al. 1977). These variations presumably can be avoided with meristem or shoot tip cultures which have been successfully used in clonal propagation *in vitro* with different species of plants (Kartha et al. 1974a, Kartha et al. 1974b, Pow 1969). Considering the importance of the origin of the new plants formed *in vitro*, the present anatomical study was undertaken to describe the anatomy of the curd of cauliflower and the organogenesis of explants cultivated according to the technique previously described by Torres et al. (1978).

MATERIAL AND METHODS

Anatomy of the cauliflower curd

Curds of market-ready cauliflower, cultivar "Teresópolis Precoce", were divided into main shoot, first-order branches, second-order branches and so on to tenth-order branches. Segments of the proximal and distal ends of each branching order were fixed in FAA (Formol: acetic acid: 50% ethyl alcohol, 1:1:10 v/v/v), included in paraffin and sectioned (10-15μ). The sections were stained with safranine and fast-green.

Differentiation of the explants

Explants of about 3-4 mm in diameter taken from the surface of the market-ready cauliflower curd were sterilized in 2% calcium hypochlorite, washed in sterile water, and placed in nutrient medium containing macro and micronutrients and vitamins of Murashige & Skoog (1962) with the addition of 4 mg/l of kinetin, 5 mg/l of indoleacetic acid, 27 mg/l of adenine, 100 mg/l of myo-inositol, 170 mg/l of NaH_2PO_4, 6,5 g l of agar, 30 g/l of sucrose with the pH adjusted to 5.7 + 0.1. The recipients containing the medium inoculated with the explants were submitted to a light intensity of 4.000 lux, photoperiod of 16 hours and day/night temperature regime of 24/16°C. The histological examination of the explants was initiated on the fifth day of culture and was repeated at 5-day intervals during a period of 30 days. Both transverse and longitudinal sections of the explants were made as described above. After the differentiation and development of the shoot, apical segments from 2 to 3 cm shoots were transferred to the nutrient medium and culture conditions described by Torres et al. (1978), in order to obtain root differentiation.

RESULTS AND DISCUSSION

Anatomy of the cauliflower curd

The cauliflower curd consists of a main shoot from which many first-order branches develop in acropetal succession. The second-order branches are initiated from the apices of the first-order branches. Each successive branching order develops similarly until the market-ready curd is formed. These observations are similar to the findings of Sadik (1962). The higher order branches are covered with meristematic apices (Fig. 1), which, in a normal course of development, will give rise to the floral primordia. On the other hand, if the curd is exposed to suitably high temperatures, the meristematic apices will be devernalized and form new vegetative organs (Crisp & Walkey 1974). Sadik (1962) mentioned that in the varieties which require a cold treatment for curd formation, this structure behaves as an integral part of the inflorescence.

The first-order branches, as well as those of the higher orders, present a bract at the base of the branch and have the same anatomical structure as the majority of herbaceous dicotyledons (Fig. 2). The lower-order branches (first-order) have an epidermis, formed of a homogeneous layer of cells, which are continuous, tabular and covered by a thin cuticle. The stomates are slightly elevated with respect to the level of the other epidermal cells. The cortex is constituted of parenchyma cells of diverse sizes and shapes (Fig. 2). The phloem is very distinct (Fig. 3). The external phloem elements have thicker walls. These elements could develop into fibers, marking the pericycle region. The sieve tube elements are distinguished by their larger diameter and in some cases

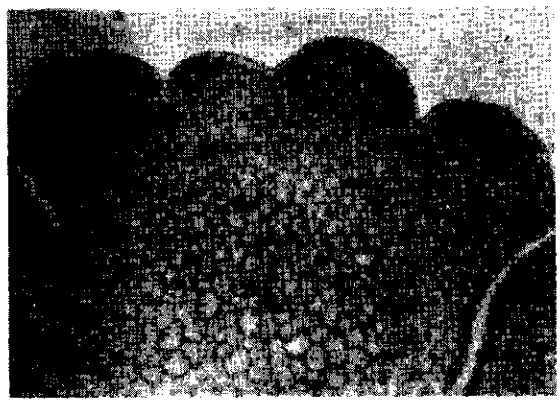

FIG. 1. Longitudinal section of the surface of the cauliflower curd, showing several apical meristems.

simple sieve plates are visible. The companion cells are smaller, with dense cytoplasm and, in some cases, a variable number of companion cells is associated with each sieve tube element. In this stage of development, the vascular cambium is limited to the region of the vascular bundles, constituting the fascicular cambium. The interfascicular areas are made up of parenchyma. The phloem and the xylem are mostly primary and both are present in the form of a narrow, elongated strip when viewed in a transverse section (Fig. 3 and 4). In the smaller vascular bundles which are still undergoing differentiation, phloem bundles were noted without corresponding xylem bundles differentiated as yet. The subsequent branching orders present a similar aspect to that described above. The differences are only in the level of development. As one approaches the higher level branches, the epidermal cells become progressively less homogeneous, the area of the cortex decreases, the phloem and the xylem gradually lose their elongated form (Fig. 2 and 5). The occurrence of phloem without corresponding xylem is more common, since these are less differentiated areas closer to the meristem. The medulla occupies a smaller area.

Organogenesis of explants

The histological observations, during morphogenesis, were made at different stages of development. Starting on the fifth day after inoculation of the explant in the nutrient medium, the modifica-

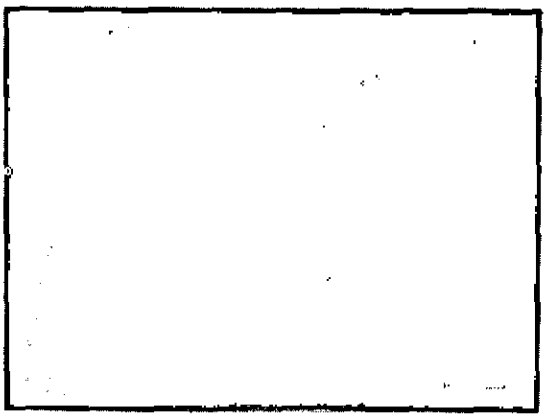

FIG. 2. Transverse section of an eighth-order branch of a cauliflower curd, showing the epidermis, cortex, vascular bundles and medulla.

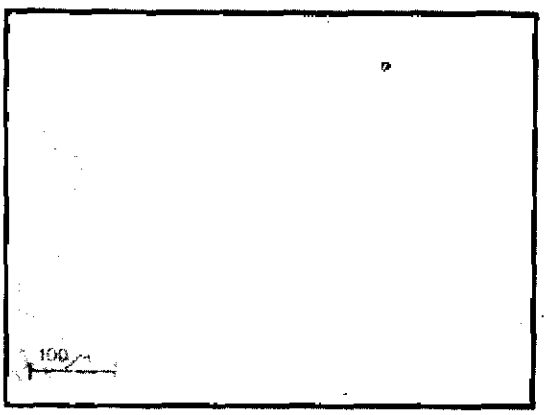

FIG. 3. Aspect of a transverse section of a third-order branch of a cauliflower curd in which the phloem and vascular cambium can be observed.

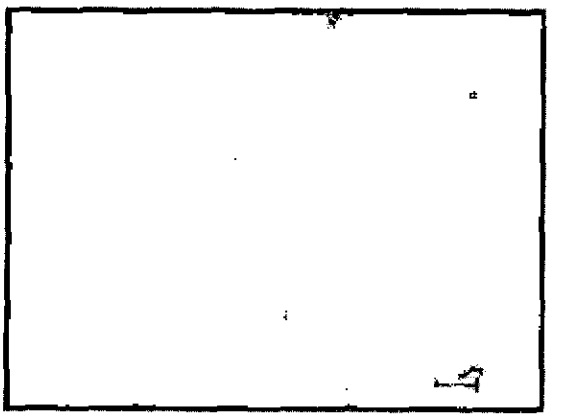

FIG. 4. As Fig. 3, showing xylem.

FIG. 5. Transverse section of an eighth-order branch of a cauliflower curd, in which the vascular tissue is no longer seen in elongated strips but rather is present in a circular pattern.

tions in the explants were observed (Fig. 6). Macroscopically, the cauliflower explant was seen to increase in volume and begin development of shoots on its distal surface (Fig. 6b). Anatomical examination at this stage showed that the parenchymatic cells adjacent to the vascular bundles of the peduncle of the explant divide periclinally and increase in volume. Apparently this phenomenon is related to the differentiation of vascular elements which occur in a disorganized fashion (Fig. 7). These vascular elements are more numerous than those in the original material and the disorganization is more pronounced in the basal region of the peduncle of the explant. It becomes less intense, eventually disappearing, as the meristematic region is approached. Concomitantly, there is a progressive and gradual differentiation of the apical regions from undifferentiated meristems to organized shoot meristems, producing stem and leaves. The leaf primordia are initiated around the apical meristem (Fig. 8) in levels close to one another. A vascular connection was observed between the developing leaf primordia and the corresponding tissues of the new shoot axis, also in development, constituting the procambial strand (Fig. 9). The organs continue their development with the formation of the primary structure of stem and leaves.

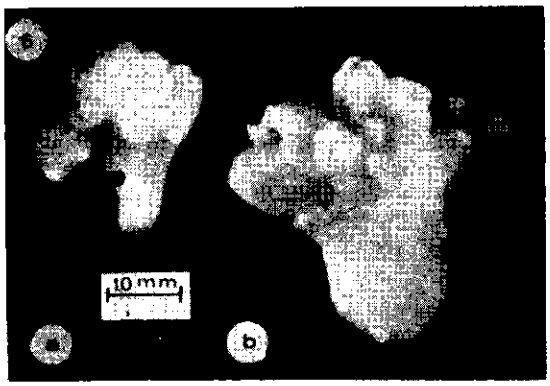

FIG. 6. a. Explant of cauliflower curd collected at harvest time.
b. Explant, 6 days after placed onto the culture medium.

In synthesis, the differentiation and development of shoots on explants of cauliflower are due to the activities of the meristematic cells, which, according to D'amato (1977), are responsible for the maintenance of the genetic stability in vegetative propagation *in vitro*. Trimboli et al. (1977) and Walkey & Woolfitt (1970), working with *in vitro* culture of cauliflower using respectively internodal explants and meristematic apices, obtained initially the formation of callus and from this callus the buds (Trimboli et al. 1977) and leaves (Walkey & Woolfitt 1970) developed. Crisp & Walkey (1974), using the same methodology as Walkey & Woolfitt (1970) described the differentiation of cauliflower buds from the apical meristems and emphasized the inconvenience of passage through a callus stage in the clonal propaga-

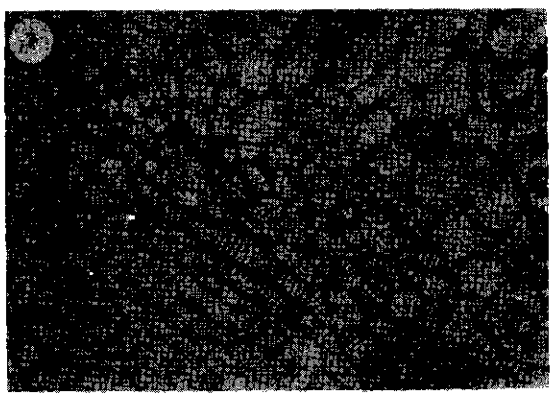

FIG. 7. Transverse section of the base of the peduncle of the cultured explant of cauliflower, showing unorganized vascular tissue.

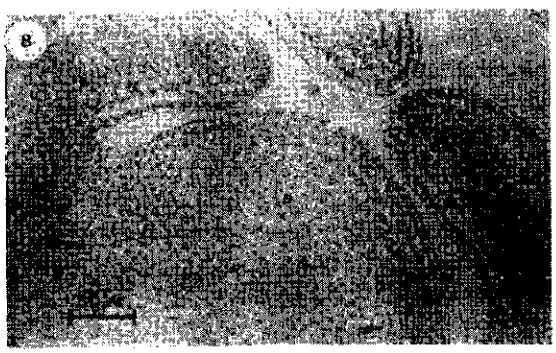

FIG. 8. Differentiation of leaf primordia around the apical meristem in cultured explant of cauliflower.

tion of cauliflower. In the present study, some callus may have formed at the base of the explant and a certain degree of vascular disorganization in the apical region was noted; however, we do not believe that a true callus developed in the apical meristem region or contributed to the formation of shoots.

When apical segments of 2 to 3 cm were taken from these shoots and transferred to the nutrient medium consisting of Murashige and Skoog (1962) inorganic salts and vitamins with addition of 10 mg/l of indolebutyric acid, 6.5 g/l of agar, 30 g/l of sucrose with the pH adjusted to 5.7 ± 0.1, the differentiation of a root system was obtained (Fig. 10) and normal polar development of the root and shoot followed.

In addition to the development described above, approximately 10% of the cultures established in this study demonstrated a pronounced development of the bracts, present in the explant, which came in contact with the nutrient medium. From the highly developed blade of these bracts, shoots were observed to develop (Fig. 11). However, these shoots were not used in the clonal propagation of cauliflower, since it is highly probable that they originated from the dedifferentiation of bract tissues.

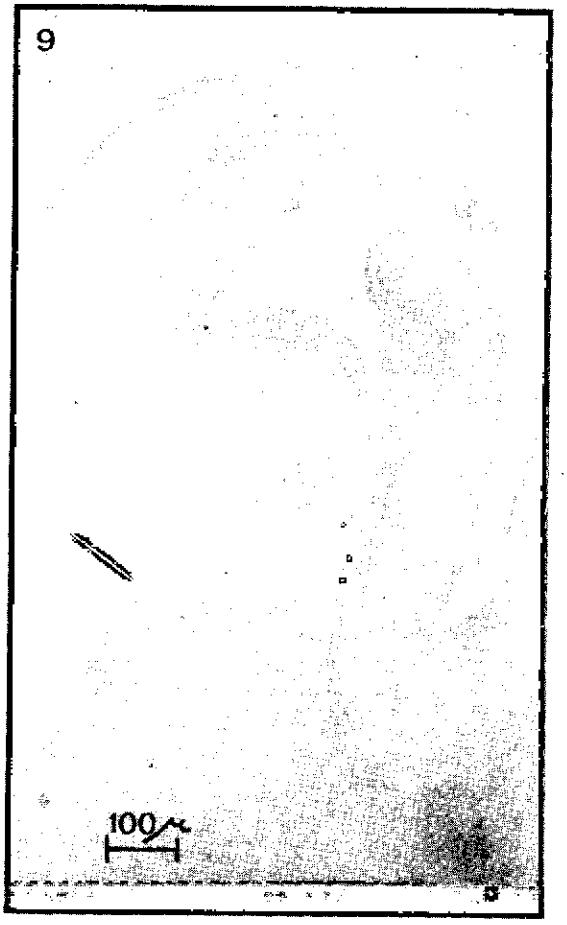

FIG. 9. Vascular connection between the developing leaf primordia and corresponding tissue of the new shoot apex.

FIG. 10. Development of shoots from a bract.

FIG. 11. Growing plantlet after being transplanted to pot.

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