The Process during Shoot Regeneration in the Receptacle Culture of Chrysanthemum (*Chrysanthemum morifolium* Ramat.)

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Experiments were carried out to determine the position where the regenerated shoots originated in the receptacle culture of chrysanthemum. The culture medium, used in this experiment, was composed MURASHIGE and SKOOG's (1962) inorganic and organic components supplemented with 3% sucrose, 0.8% agar, 1 mg/l 6-benzylaminopurine and 0.1 mg/l 1-naphthylacetic acid. After the receptacles were cultured, the inoculants were sampled at regular intervals, immediately fixed with FAA, and processed for microtome sectioning by ordinary paraffin method. After staining with DELAFIELD's haematoxylin, the samples were observed by light microscope. Based on the observations, the process of shoot regeneration after receptacle culture are summarized as follows. The receptacle constituted of two types of epidermal cells, large cells covered with thick cuticle and relatively small cells covered with a thin layer of cuticle and positioned at the junction between receptacle and floret residue epidermis. After 6 days of incubation, cell divisions of the small cells were observed. These cells continued cell divisions actively. Subsequently, these cells formed meristematic cell masses which protruded over the epidermis. About 2 weeks after incubation, the cell division continued and the protrusions became conspicuous. By 16 days after incubation, the appearance of regenerated shoot was confirmed. Within 20 days after incubation, perfectly organized and regenerated shoots were eventually formed. It was clarified that these regenerated shoots were formed from small epidermal cells positioned at the junction between the receptacle and the floret residue epidermis. Thus, the shoots were directly formed from the epidermal cells (direct shoot regeneration) without any intervening callus formation. It is suggested that the receptacle culture will be a useful method to eliminate the periclinal chimera.

**KEY WORDS:** *Chrysanthemum morifolium*, receptacle, periclinal chimera, adventitious bud, epidermis, direct shoot regeneration

Introduction

Cultivated chrysanthemums (*Chrysanthemum morifolium*) belong to the family *Compositae* and have mainly 2n = 6x = 54 chromosome number (DOWRICK, 1953). Ordinarily, chrysanthemum cultivars are vegetatively propagated from rooted cuttings because they have genetically controlled self-incompatibility (DREWLOW et al., 1973, ZAGORSKI et al., 1983, STEPHENS et al., 1984). During vegetative propagation, many sport mutants are induced by spontaneous mutations. WASSCHER (1956) reported that approximately 30% of the cultivars originated as sports. Moreover, these sport mutants showed periclinal chimeras in many cases.

LANGTON (1980) reported that in F1 progenies derived from crossing various varieties, disagreement between genotype and phenotype on the segregations of yellow and white flower color was sometimes encountered. This result suggested that periclinal chimera existed in the petal tissue.

STWART and DERMEN (1970) investigated the flower color variations of the sport family derived from cultivar Indianapolis and interpreted that they were the result of periclinal chimera formation because the petal consistent of a three-layer-structure of different genetical origin.

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It is indispensable to consider chimerical structure in breeding programs and the genetic analysis of vegetatively propagated plants like chrysanthemum. Moreover, it is also significant to develop useful methods to eliminate the periclinal chimeras.

According to Broertjes and Van Harten (1988), chimeric structures were broken by adventitious bud technique in many vegetatively propagated ornamental crops, in which the adventitious bud was originated from one epidermal cell.

Plantlets can be obtained in chrysanthemums through the formation of adventitious buds in various tissues cultured in vitro (Roest and Bokelmann, 1975, Broertjes et al., 1976).

In this study, the author observed the process of shoot regeneration in receptacle cultures of chrysanthemums and discussed the possibility of overcoming chimeric structure.

Materials and Methods

About 20 varieties of chrysanthemum maintained in the Laboratory of Theory of Agronomy and Plant Breeding, Faculty of Agriculture, Nagoya University were preliminarily tested for receptacle culture. From the result, Yellow Delaware, one of the potunum varieties, was selected because it possessed a relatively high ability to produce shoot from the receptacle tissue. Moreover, the receptacle explants began to expand as early as 2 days after incubation and a green spot, sign of shoot regeneration was visible 2 weeks after incubation.

The chrysanthemum was propagated from rooted cuttings and grown in pots to flowering stage.

Half opened flowers were sterilized in 70% ethanol for a few seconds, then a 1% sodium hypochlorite solution for 15 minutes and subsequently rinsed with sterilized water three times. After removing ray and disc florets, only parts of the receptacle were cut and vertically divided into two segments. Subsequently, the segments were cultured on medium based on Murashige and Skoog (1962) containing 0.8% Difco Bacto-agar, 3% sucrose, 1 mg/l 6-benzylaminopurine and 0.1 mg/l 1-naphthylacetic acid. The cultures were incubated under continuous light exposure (fluorescent light, 5,000 lux) and at 23°C.

For the histological observations, the explant samples were harvested every day for the initial 10 days and then every two days until 30 days after incubation. The explants were fixed in FAA (70% ethanol : formalin : acetic acid = 90 : 5 : 5 v/v). After dehydration with graded alcohol series, the samples were embedded in paraffin and sectioned at 10 μm with a rotary microtome. Serial sections were stained with Delafield's haematoxylin and observed using a light microscope.

Results

The tissue organization of receptacle before culture is shown in Figs. 1A and 1B. At lower magnification (Fig. 1A), the parts where florets had been attached were perpendicularly connected to the vascular bundle extending from the parenchyma tissue in the receptacle. This vascular bundle was connected with other vascular bundle and passed horizontally through the portion not so deep in the receptacle. Moreover, the subepidermal parenchymatous cells were smaller than the inner parenchymatous cells. The epidermal cells were relatively large and externally covered with a thick cuticle layer.
Fig. 1. Longitudinal section of the terminal portion of the receptacle before incubation.
A: At lower magnification  B: At higher magnification
VB: Vascular bundle  PT: Parenchyma tissue
LEC: Large epidermal cell with thick cuticle
SEC: Small epidermal cell with thin cuticle
Arrow-heads show the region where the floret has been attached.
Bars indicate 100 μm.

At higher magnification, the epidermal cells appeared to be regularly arranged (Fig. 1B). The epidermal cells contained two types of cells, namely, large cells covered with thick cuticle and small cells with thin cuticle. These small cells were located at the region where the florets had been attached. This area appeared somewhat hollow.

After 2 days of incubation (Fig. 2A), the cells of the epidermal layer and the outer parenchyma became enlarged. This enlargement was induced in the outer region of the vascular bundle passing horizontally through the deeper layer of the receptacle. The large epidermal cells covered with thick cuticle enlarged considerably, while the small cells covered with thin cuticle enlarged slightly. After 5 days of incubation (Fig. 2B), the epidermal cells, especially the large cells, underwent division without cell elongation. Few small epidermal cells divided. At the area where the florets had been attached, very big balloon-like cells were formed. These balloon-like cells covered the entire area where the florets had been attached.

Longitudinal sections of the receptacle 6 days after incubation (Figs. 3A and 3B), showed that the small epidermal cells divided conspicuously. The divisions extended toward the area where the florets were once attached. These small epidermal cells covered with a thin layer of cuticle elongated longitudinally at first, and then divided perclinally. In addition, the outer cells of divided small epidermal cells repeatedly divided in various directions. After 8 days of incubation (Fig. 3C), these cell divisions derived from small epidermal cells led to the formation of meristematic cell masses, which afterwards protruded from the epidermal tissue, forming a dome.

The meristematic cell masses that were formed at the terminal portion of the receptacle
are shown in Figs. 4A and 4B. These sections were obtained from samples 16 days after incubation. The status of the section on lower magnification is shown in Fig. 4A. Based on the observations, it was considered that the meristematic cell masses protruded outwards from between the balloon-like cells. In the surface of these meristematic cell masses, some shoot spines were formed. Moreover, huge vascular tissues were newly formed in the deeper parenchyma tissue of the receptacle (Fig. 4A). The vascular tissues sometimes ramified in irregular directions. The boundary between the meristematic cell masses and their surrounding cells was clearly recognized (Fig. 4B). From these results, it was thought that the meristematic cell masses were initiated from a few number of competent cells.

The structure of a shoot apex was observed in the section of 16-day-old culture (Fig. 5A). This structure existed somewhat externally in the terminal portion of the area shown in Fig. 4. The structure appeared somewhat incomplete in shape, because the trichomes on the leaf
primordia and axillary buds were not formed yet.

The completely formed shoot apex was observed in the section of the sample 20 days after incubation (Fig. 5B). The trichomes were already formed on the leaf primordia. Moreover, the primordium of the axillary bud and the vascular tissues were formed at their ordinary position. The cells constituting the apical meristem were relatively small and divided actively.

**Discussion**

Many papers about tissue culture of the family *Compositae* have been reported. For example, the plantlet regeneration of *Gerbera jamesonii* (Pierrick et al., 1973) and *Chrysanthemum cinerariefolium* (Roest and Bokelmann, 1973) was observed from young capitulum culture. In *Chrysanthemum morifolium*, Roest and Bokelmann (1975) reported that young segments of flower pedicels regenerated rapidly large numbers of adventitious shoot in vitro. Broertjes, et al. (1976) also reported that the chrysanthemum cultivar 'Bravo' easily regenerated shoots
from various explants, such as petals, flower heads, tiny leaves and pedicels. In all explants, excluding the pedicels, adventitious shoots appeared after three weeks at the earliest and usually on calli. Pedical explants, however, regenerated adventitious shoots directly from pedical epidermal tissues 10 days after incubation.

In this experiment, it was shown that the regenerated shoots were formed from small epidermal cells covered with a thin layer of cuticle. These small cells were located at the junction region of the florets and the receptacle. So, the regenerated shoots might be initiated from the floret residue.

The periclinal chimerical structure was frequently observed in the vegetatively propagated plants such as chrysanthemum. SAGAWA and MEHLQUEST (1957) suggested that the frequent change of flower colors, mostly into red, might not be due to a genetic change but depended upon alteration of the chimeric nature, in carnation cultivar Pink Sim and White Sim. In chrysanthemums, LANGTON (1980) and HATTORI (1991) suggested that it was very significant in breeding programs to consider the periclinal chimerical structure. Moreover, there are many papers reporting adventitious bud formation combined with mutation induction, in vivo and in vitro, with the objective of obtaining a solid mutant. These experiments showed that many of the obtained mutants had solid, non-chimerical structure in vegetatively propagated plant species such as Streptocarpus (BROERTJES, 1969), self-incompatible Nicotiana alata (De NETTANCOURT et al., 1971), Achimenes (BROERTJES and LEFFRING, 1972), potato (VAN HARTEN et al., 1972), and chrysanthemum (BROERTJES et al., 1976).

Although it could not be clearly defined how many cells were concerned with the shoot formation, the regenerated shoots were formed directly from the epidermal cells only, without any intervening callus formation. This result possibly enables elimination of periclinal chimera structure by the receptacle culture in the chrysanthemum. On the other hand, further studies are needed to regenerate shoots from various tissues other than the epidermis with the objective of producing solid, non-chimerical shoots with different genetical background from chimerical plant.

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Literature Cited

The Process During Shoot Regeneration in the Receptacle Culture


キク（Chrysanthemum morifolium Ramat.）花床培養におけるシュート再生過程

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キクの花床（花托）を培養することにより得られるシュートの再生過程を明らかにするためにいくつかの実験を行なった。本実験に用いた基本培地は Murashige and Skoog（1962）の無機および有機要素に3%蔗糖、0.8%を加えたものであり、これに生長調製物質として1 mg/lのbenzylaminopurine と0.1 mg/lのnaphthalacetic acidを添加した。培養に供試したキクはポットマム品種 Yellow Delaware であり、この品種は、名古屋大学農学部に保存中のキク品種を用いた予備実験の結果、本実験に用いるのに最適な品種の一つとされたものである。本品種を常法によりガラス室内で栽培し、開花期に半開した側状花序を採取し、表面殺菌ののち、舌状花および筒状花を取り去り、花序のみを切り出し、さらに半分に切り分けたのち、培地に置床した。これらの花序を培養後1－10日目までは毎日、その後30日目までは1日おきに採取し、FAAで固定したのち組織観察に供試した。組織観察は通常のパラフィン切片法により10 μmの連続切片を作成ののち、DELAFIELDのヘマトキシリンで染色ののち、光学顕微鏡で行なった。観察結果から花床培養による植物体再生の概要を以下に示す。花床の表皮細胞は大別して2つの型からなる。すなわち、大きく外側を厚いクチクラで覆われたものと比較的小さく、外側を薄いクチクラを覆う細胞を含む細胞が観察された。この小さな表皮細胞は花床表皮細胞と小花の残余部分の表皮細胞の移行部にあり、細胞の大きさからは小花の表皮細胞と考えるべきものであった。培養後6日目の切片の観察によれば、上述の小さな細胞での活动的な細胞分裂が認められ、これらの部分での分裂活性が続続することにより、分裂細胞層が形成された。さらに、これらの分裂細胞層が表皮細胞上に突出した。培養後2週間で、これらの細胞層はさらに分裂を続け、顕著な分裂組織となった。培養後16日目まではシュート形成が確認された。培養後20日目には完全な形のシュートが形成された。以上の観察結果より、これらのシュートは花床表皮細胞と小花の残余部分の表皮細胞の移行部の小さい表皮細胞から形成されることが明らかとなった。また、これらのシュートはカルスを経由することなく表皮細胞のみから直接分化することが明らかとなり、キクの花床培養により、栄養繁殖植物で問題とされる層状に遺伝的背景を異にするいわゆる周縁キメラを効率的に打破することができることが示唆された。