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COLCHICINE-INDUCED DISPLACEMENT OF FLORAL ORGAN REGENERATION SITES IN THE WOUNDED SUNFLOWER CAPITULUM

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ABSTRACT: As previously described a circular wounding procedure was applied to the young capitulum of the sunflower to isolate a 1 mm diameter cylindrical plug of receptacle tissue *in situ* and stimulate the recapitulation on the plug surface of involucral bracts, ray and disc floret initials. Applying 4.4 mM colchicine to the wounded wall of the plug for 5 days, subsequently resulted in a collar of meristematic-like cells forming 20-50 μm from the wound site followed by the appearance of floral initials on the inside of the collar, 100-120 μm distance from the plug wall, demonstrating that dedifferentation and regeneration processes are not confined to the plug rim.

RESUMEN: Una técnica para producir cortes circulares fue aplicada en el capítulo de girasol durante su incipiente formación, a los efectos de aislar un segmento cilíndrico de tejido del receptáculo de 1 mm de diámetro. De esta forma se produce *in situ* sobre la superficie del mismo la recapitulación de brácteas involucrales, flores liguladas y flores fértiles. Aplicando una solución de colchicina (4.4 mM) a la pared del corte durante 5 días, se produjo un collar de tejido meristemático el cual se formó 20-50 μm alejado del sitio del corte. A ello siguió la aparición de las iniciales de las brácteas involucrales y flores sobre el lado interno del collar y a 100-120 μm de la pared del corte. Esto demuestra que los procesos de dediferenciación y regeneración de órganos florales en el receptáculo del girasol no están solamente confinados al borde del corte.

INTRODUCTION

The sunflower capitulum is formed from a sequence of floral organ initials which first appear on the flanks of the flowering apex in FS§* 3, 4 and 5 and then spread inwards across the surface of the rapidly expanding receptacle in FS 6 and 7 (Marc and Palmer, 1981). The initials develop into either involucral bracts, ray or disc florets depending on their position in the developmental sequence. Palmer and Marc (1982) showed by wounding the surface of the receptacle, that the capitulum retains an ability to regenerate involucral bract and ray floret initials until FS 5, when the receptacle is a saucer-shaped disc. Hernández and Palmer (1988) developed a circular wounding procedure which changed the natural commitment of the central region of the receptacle from producing disc floret initials exclusively, to inducing the regeneration of a daughter capitulum, complete with involucral bracts and ray florets, as well as disc florets.

Mature cells at wound sites commonly dedifferentiate and show enhanced rates of cell division. This activity occurred at the wounded edge of the plug leading to the formation of a raised rim. It was concluded that this rim provides a generating impetus for

* §FS, floral stage in the sunflower using the ten-stage classification of Marc and Palmer (1981).

floral organ initials but the method of control was not determined. The rim cells could provide a suitable cellular location and they may be sites for gene activation and an increase in messenger, ribosomal and transfer RNA (Kahl and Wielgat, 1976). Reported here is an experiment in which circular wounding of the receptacle was followed by colchicine application to arrest post-wound cell division at the wound site and its immediate neighbourhood.

MATERIALS AND METHODS

(*Helianthus annuus* L.) plants cv. Hysun 30 were reared in 1 liter pots, containing a sand/peat/vermiculite mixture, and grown in a controlled artificial environment at a constant air temperature of 28°C. Fluorescent tubes and incandescent lighting provided a photon flux density of about 500 μmol s^{-1} m^{-2} and 18 h long-day photoperiod.

The experiment was commenced when the plants were 25 to 32 days old and the shoot apical bud was in an early stage of flowering, (FS 4 or 5), when the capitulum receptacle surface is a flat undifferentiated saucer-shaped disc, 1.2 - 2.0 mm in diameter (Marc and Palmer, 1981). Young leaves and involucral bracts surrounding the capitulum were cut away to expose the receptacle surface.

A modified hypodermic needle (Hernández and Palmer, 1988) was then used to make a circular wound in the receptacle surface, 50 μm wide and about 200 μm deep. This produced a narrow plug of receptacle tissue 1 mm in diameter, which was laterally isolated from the remainder of the receptacle while remaining attached to the subapical meristem.

Aqueous colchicine solutions (Sigma, USA) were prepared in the following concentrations, 4.4 mM, 5.4 mM and 10.9 mM. 10 μl of each solution was applied to a freshly made wound and spread around it with the aid of an eyelash hair. Each solution was re-applied at 6 h intervals over a 5 day period. Controls comprised plants in which 10 μl of deionised water was applied to the circular wound at the same time as the colchicine applications. The wounded apices were protected against desiccation by covering them with aluminium foil. Each treatment was applied to 5 plants and the experiment was repeated once.

Harvesting for measurements was carried out when 20-30% of the plug surface was covered by floret primordia. This state was reached after 4 to 5 days in control plants and 10 to 13 days in colchicine treated plants. After dissection, capitula were fixed under vacuum in ice-cold 8% glutaraldehyde in 25 mM phosphate buffer (pH 6.8) and dehydrated in methoxyethanol, followed by a graded acetone/ethanol series. The specimens were then critical-point dried in liquid CO_2, sputter coated and photographed using a Cambridge S4-10, scanning electron microscope. The mean spacing between regenerating floral organ initials and their mean diameter were determined from measurements of the scanning electron microscope micrographs of the plug surface. Mean values are based on 10 to 15 measurements for floret spacing and 30 to 45 measurements for floret diameter.

RESULTS AND DISCUSSION

In the controls a raised rim formed at the junction of the wound and the plug surface in 1 to 3 days (Fig. 1A). Floral initials then appeared on the surface of the rim 3-5 days after wounding (Fig. 1B). These changes were preceded by the occurrence of periclinal cell divisions in cells at the rim site as previously reported (Hernández and Palmer, 1988). The colchicine treatments suspended development of the plug while they were being applied. 5.4 mM and 10.9 mM colchicine either killed the plug or resulted in irregular callus-like surface growth in the majority of the remainder. Considering the results for 4.4 mM colchicine; during the period of colchicine application there were no visible developmental changes

in any of the 10 treated plugs, but after the colchicine treatment was stopped each showed a consistent growth response. After 1 to 3 days a collar of cambium-like tangentially dividing cells appeared. This ranged in width from 70-90 μm and was displaced from the plug edge by a distance of from 20-50 μm. Regeneration of floral initials then commenced inside this collar in a circular zone which was 100-120 μm distant from the wound rim (Fig. 1C). Once regeneration had commenced production of new initials continued and these spread inwards towards the centre of the plug in the same way as had occurred earlier in the controls (Fig. 1D). Presumably colchicine prevented floral organ initiation in the wound region by suppressing cell division (Holmsen and Hess, 1985) or by causing disruption of microtubule orientation (Murata and Wada, 1989). In the colchicine treatment the mean diameter of the regenerating initials and the spacing between them were not significantly different from the controls (Table 1).

floral apex of the sunflower becomes an organising site for floral initials in FS 3, 4 and 5 and that the raised rim of the plug mimics this organising role by generating a radial developmental gradient which controls the fate of floral initials, directing them to form either involucral bracts, ray or disc florets according to their position in the regeneration process. The colchicine effect can be explained if the arrest of cell division at the plug edge allowed surface cells closer to the centre of the plug to dedifferentiate and divide to form the collar, once the colchicine treatment was stopped. On this hypothesis the collar assumes an organising role leading to the generation of floral initials and is analogous to the natural rim of the receptacle in FS 5. Its location away from the immediate vicinity of wounded cells can be explained if dedifferentiation and regeneration of cells in the receptacle surface occur whenever a barrier to symplastic movement of an as yet unidentified influence arises. Such a barrier would be the wound wall in the control plug, and the zone of non-dividing

TABLE 1. Mean diameter, spacing and initiation time for regenerating floret initials appearing on the plug surface after wounding and in the presence of 4.4 mM colchicine.

Treatment	Time from wounding (h)	Mean diameter (μm)	Distance between initials (μm)
Control	86.4	111.7a[1]	148.9b
Colchicine	220.8	113.1a	154.7b

1. values in the same column followed by the same letter do not differ significantly.

The location of regenerating floral initials away from the wound edge, in the colchicine treatment shows that organ regeneration can commence anywhere on the surface of the plug and that wounded cells are not an essential starting site for the regeneration process. It has been proposed by Hernández and Palmer (1988) that the natural rim of the

cells at the wound site in the colchicine treatment could be another. The organising role of the natural rim of the capitulum which forms in the sunflower in FS 5, can also be explained on this hypothesis if it too functions as a symplastic barrier. Symplastic barriers have not been investigated for the developing sunflower capitulum, but in the stem of *Ege-*

ria densa they have been located between the epidermis and cortex and at the nodes (Erwee and Goodwin, 1985).

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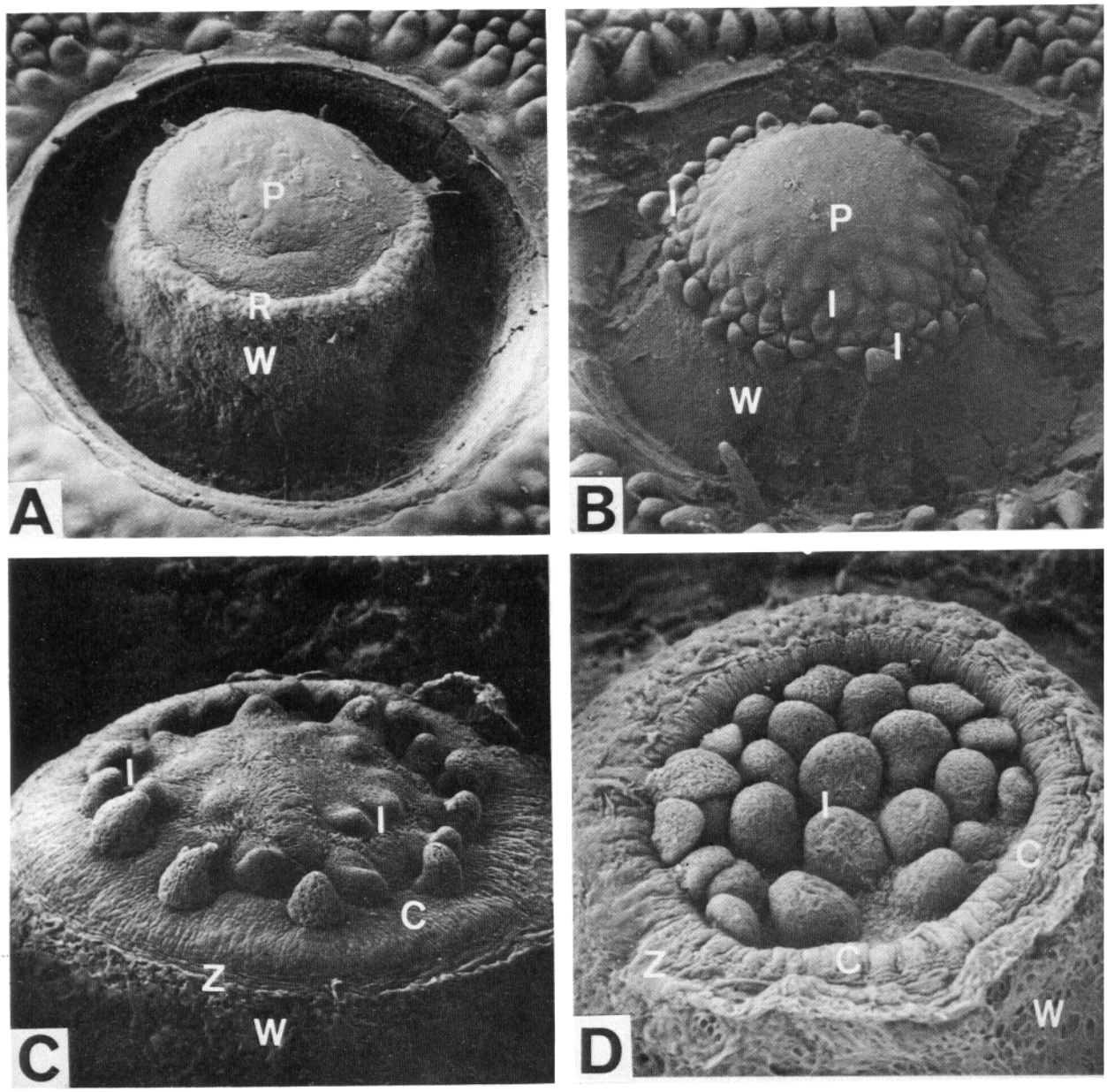
Fig. 1. (A) control; plug after 3 days, showing development of the raised rim at the junction of the wound and plug surface (65 x).

(B) control; plug after 5 days, showing regenerating involucral bract and floret initials (60 x).

(C) colchicine plug; 5 days after colchicine treatment was stopped, showing collar of meristematic cells and regenerating initials forming away from the plug rim (110 x).

(D) 7 days after colchicine treatment was stopped; showing inwardly displaced collar and utilisation of the available space on the plug surface by involucral bract and floret initials (120 x).

C: collar; I: involucral bract, ray or disc floret initial; P: plug surface; R: raised rim; W: wound edge; Z: displacement zone.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