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FLORET DIFFERENTIATION IN THE CAPITULUM OF SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

In the capitulum of sunflower the process of generation of new floret primordia starts at the receptacle rim and continues towards its center. Then, each floret primordium differentiates gradually over space and time, in the centripetal direction. The florets appear in an annular generative zone first as a gentle dome and then become a two-part structure: the floret bract and the floret corolla.

This paper provides a detailed description of the beginning of the floret primordium differentiation during the floral morphogenetic process in the sunflower capitulum.

Key words: Differentiation, floret, *Helianthus annuus*, morphogenesis, pattern, primordium, sunflower.

INTRODUCTION

The order of development and arrangement of organ primordia on vegetative and reproductive apex is defined as phyllotaxis.

The explanation of how floret differentiation is produced and the creation of the complex phyllotactic pattern (floret or seed) in the sunflower capitulum has stimulated a great deal of studies (Mathai and Davis, 1974; Vogel, 1979; Williams and Brittain, 1984; Ridley, 1986; Hernández and Palmer, 1988; Hernández and Green, 1993; Hernández, 1995). Floret primordia in the sunflower capitulum normally form two sets of conspicuous rows (parastichies), the number of which generally conforms to the Fibonacci series, 21, 34, 55, 89, 134, with 55 and 89 being the most common numbers (Hernández, 1988).

The generation of these rows has been interpreted following the basic principles developed for phyllotaxis (Schwabe, 1984), namely that they are the result of developing primordia at a determined divergence angle, in many cases close to the Fibonacci angle (Hernández, 1988). The arrangement of florets on the receptacle has then been regarded as a geometrical phenomenon (Vogel, 1979) and the Fibonacci angle of divergence between consecutively emerging primordia has been

considered as a natural consequence of locating each primordium or groups of consecutive developing primordia in the available space left at the level of the generative front (Palmer and Steer, 1985; Hernández, 1988,1991). More recently a biophysical interpretation of the floret pattern generation has been proposed for capitula (Hernández and Green, 1993, Hernández, 1995; Green *et al.*, 1996).

In any case the beauty of floret primordia pattern represents an ordered arrangement of organs in space and time. This paper provides a detailed description of the beginning of the floret primordia differentiation process in the sunflower capitulum.

MATERIALS AND METHODS

Plant cultivation

Plants of sunflower (*Helianthus annuus* L.) cv. Dekalb G100 (Dekalb Argentina S.A.) were grown in a growth chamber in 2 liter plastic pots containing garden soil. The growth conditions were: 18 h daylenght photoperiod, 360 $\mu mol\ s^{-1}\ m^{-2}$ PPFD at the canopy level and 26/24°C day/night temperature. Plants were watered daily and fertilized to maintain optimal level of nutrients.

Starting at floral stage 5 (Marc and Palmer, 1981), i.e., 32-34 days after seedling emergence, the last formed apical leaves and involucral bracts were removed and the incipient floral meristem (receptacle) exposed. Hernández and Palmer (1988) have shown that this procedure does not affect the subsequent development of the inflorescence to maturity.

Sequential scanning electronic microscopy (SEM) observations of the reproductive meristem

Sequential replicas of a single receptacle surface were obtained during floret formation (Williams and Green, 1988; Green and Linstead, 1990). Then the details of floret differentiation and pattern formation were followed over time (Tiwari and Green, 1991; Hernández and Green, 1993; Hernández, 1995).

Each replica was examined in a JEOL JSM-35CF scanning electron microscope at 10 kV and photographed. This replica method was applied twice a day to ten plants for a period of 18 days.

RESULTS

Floret organogenesis and pattern extension in the receptacle occur together in a ring-like generative region (Figure 1A). This leading circular edge is defined as the generative front, and the annular area of developing florets behind it as the generative zone (Palmer and Steer, 1985; Figure 1A).

The sequential imaging of a single region of the generative zone enables to present a detailed description of the floret initiation process. The florets clearly

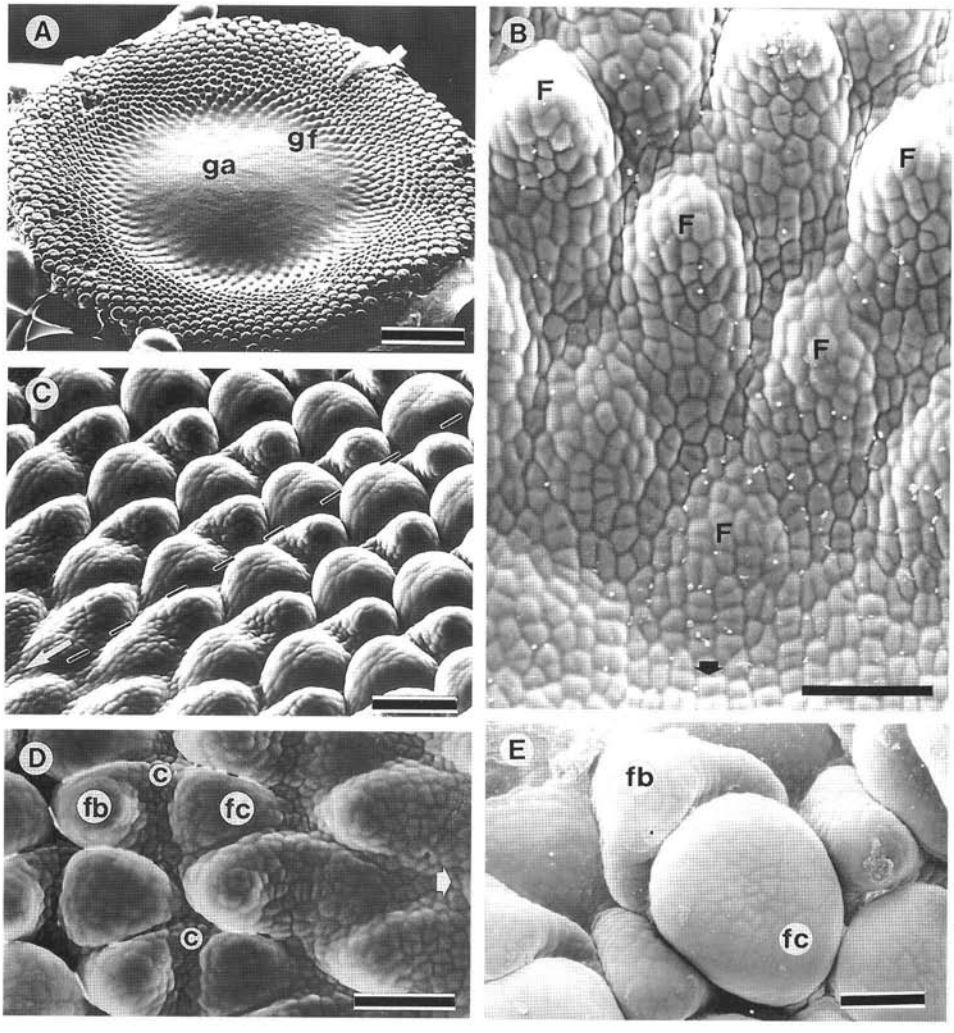

Figure 1. SEM micrographs of the incipient development of florets in the sunflower capitulum showing the developmental progression of floret primordia from undifferentiated tissue to well formed florets

A. *Panoramic view of a capitulum. The generative front (**g f**) is an arc-like region of undifferentiated tissue and the generative area (**g a**) the empty circular region at the capitulum center. Bar = 2 mm*

B. *A high power SEM image of a single field at the generative front of **A**. The incipient formation of one floret is noted (**F**). In the early stages the florets are comet-like with a well defined head (above) and a diffuse tail (below). The arrow indicates the receptacle centre. Bar = 50 µm*

C. *Floret primordia developing at a late stage. Each starts as a parallelogram delimited by parastichy lines (hatched line). The arrow indicates the receptacle centre. Bar = 100 µm*

D. *Top view of **C**. The development of a transverse crease (**c**), is best seen in the upper, older, florets. The upper (abaxial) part of each floret becomes a disk flower bract (**f b**), the lower part becomes a disk flower corolla (**f c**). The arrow indicates the receptacle centre. Bar = 50 µm*

E. *A floret primordia at the end of complete differentiation. The floret bract (**f b**) is at upper left, the floret primordia corolla (**f c**), at the center. This corolla will develop a five-lobed petal symmetry. Bar = 50 µm*

arise in a gradient (Figure 1B). They are formed in tissue which previously had been remarkably quiet (Hernández, 1995). It has been previously shown that very little cell division or tissue expansion occurs in the central region of the generative advancing front (Hernández, 1995).

Floret primordium arises gradually over space and time, in centripetal direction. The floret develop first as a small protuberance at the generative front (Figure 1B). The abaxial extremity of the floret arises first. It is a subtle bump, roughly near the mid-point of two adjacent, slightly older florets (Figure 1B-C). Later a floret has a comet-like appearance with the pointed end towards the receptacle center (Figure 1B). After 24 h in each floret a bisecting crease is distinguishable (Figure 1C-D) and tend to lie normal to the disk radius. Thus, relative to the radius of the head, the floret is usually seen as polygon (parallelogram) bisected by a transverse crease (Figure 1C-D). The floret can occasionally be a rhombus; then it is not skew but centered on a radius of the capitulum (Figure 1D). As seen from the side, the young floret soon becomes a horizontal wedge, the high (abaxial) end being the oldest (Figure 1C). Three days after the beginning of floret differentiation each floret becomes a two-part structure: an abaxial bract and an adaxial flower (Figure 1E).

CONCLUSION

The developmental description of this paper shows in detail how at the level of the receptacle meristem the disk florets are made. The development of the sunflower plant is characterized by consistent cyclic extension of a spiral pattern. There is a remarkable variation in the nature of the organ in the pattern during ontogeny. The spirally arranged leaves developed during the vegetative stage give way to involucral bracts, then to ray florets, and finally to disk florets. The last ones are produced early in the plant's life cycle and are bipartite organs: each floret has an outer (abaxial) bract and inner (adaxial) tubular flower (Figure 1C-E).

The particular structure and pattern of a plant produced during its development is controlled by its genotype which is in turn regulated in some degree by environmental stimuli such as light, temperature, water status and mineral nutrition and internal controls modulated by the level of growth regulators. So it is also possible to consider how the harmony of this morphogenetic process could be distorted if any kind of environmental stress comes up during its occurrence.

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DIFERENCIACION FLORAL EN EL CAPITULO DE GIRASOL (*Helianthus annuus* L.).

RESUMEN

En el capítulo de girasol, los primordios florales comienzan a diferenciarse en el borde externo del receptáculo y continúan desarrollándose hacia el centro del mismo. Cada primordio floral se diferencia entonces gradualmente en el tiempo y el espacio en dirección centrípeta. Las flores aparecen en una región anular, denominada "zona generativa", primeramente en la forma de una delicada protuberancia para luego convertirse en una estructura diferenciada en dos partes: la bráctea y la corola de la flor.

Este trabajo provee una descripción detallada del comienzo de la diferenciación de los primordios florales en el capítulo durante el proceso morfogenl en el girasol.

DIFFERENCIATION FLORALE SUR LE CAPITULE DE TOURNESOL (*Helianthus annuus* L.).

RÉSUMÉ

Sur le jeune capitule de tournesol, les primordia floraux commencent par se diffainsi le dapparaissent graduellement dans le temps et dans l'espace de mani.

Les fleurs apparaissent dans une région appéllée "zone génératrice", d'abord sours la forme d'une faible protubérance, pour se convertir ensuite en une structure différenciée en deux parties: la bractée et la corolle de la fleur.

Ce travail présente une desription détaillée du début de cette différenciation das primordia sur le capitule et montre la fragilité et le synchronisme du processus de morphogénése floale chez le tournesol.