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FLOWER INITIATION AND DEVELOPMENT IN *CUSCUTA AUSTRALIS* R. BR. (CONVOLVULACEAE)

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Abstract: The initiation and developmental features of floral organs of *Cuscuta australis* R. Br. were examined with the scanning electron and light microscopy. The sequence of initiation of the floral organs in a flower bud is that of sepals, stamens, petals and gynoecium. The first sepal is initiated laterally; the remaining four sepals are initiated in helical succession. The five petals are initiated alternating with the sepals. The five stamens are initiated slightly early, and alternating with the petals. The five scales arise at the base of stamen, when two stigma are visible. Two opposite carpels arise with four ovules and fuse toward apex gradually, after all other floral organs are initiated. The five sepals mature first, curve at the tips and enclose the other organs. The five petals are delayed in develop, and elongated later after tetralobed anthers are visible in stamen. The two carpels after subsequent growth and fusion develop into two stigma and two short styles. The four ovules are exposed before the complete fusion of carpels. They are partially separated by incomplete septum afertward.

INTRODUCTION

Despite its cosmopolitan distribution and the biological and agronomic importance of the *Cuscuta*, the initiation and developmental features of floral organs have received, so far, little attention (Tiagi, 1966).

The long flowering season of this common weed in Taiwan is a benefit for a series of research dealing with reproductive biology of flowering plants. The genus *Cuscuta* is also of taxonomic interest, in part because of its taxonomic status still have different opinions (Lawrence, 1951; Wilson, 1960; Cronquist,1988; Hutchinson, 1973; Gandhi et al., 1987; Woodland, 1991).

The scanning electron microscope is a useful tool for study on floral ontogeny and related fields (Chandra Sekahar and Sawhney, 1984; Medan and Hilger, 1992 ; Sherry et al., 1993); Moreover, Tucker (1984) have demonstrated very well the use of development in systematic studies with informations from the floral development of legumes.

In this paper we use the scanning electron and light microscopy to describe the initiation and developmental features of floral organs of *Cuscuta australis* R. Br., the most widespread species of the genus in Taiwan (Chang, 1978; Liao, 1990). These morphological studies should also serve as the basic information for our comparative and systematic studies with other genera of the Convolvulaceae.

MATERIALS AND METHODS

The study materials of *C. australis* were collected in the field, the voucher specimen were deposited at NCKU herbarium, and the flower buds and young inflorescence of various sizes were fixed under aspiration in 5 % glutaraldehyde in 0. 1 M phosphate buffer (pH 7.0), at room temperature for 2 hr. The samples were rinsed in buffer and

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postfixed in 1 % osmium tetroxide (in the same buffer) for 2 hr. The materials were rinsed and dehydrated in a graded alcohol series. The materials were critical-point-dried. Flower buds and inflorescence were mounted on SEM stubs and coated with gold. The materials were observed with a Hitachi s2500 scanning electron microscope at accelerating voltages of 25 kV and the images photographed on Kodak TMY 120 (ASA 400) rolling film. For light microscopy, some other materials were fixed in FAA, dehydrated in alcohol series, embedded in paraffin wax and stained with hematoxylin after sectioning.

OBSERVATIONS AND DISCUSSION

The mature flower of *C. australis* is typically described as being regular, hypogynous and with 5-merous floral parts. The sepals are free at upper parts and united at base. The petals are united into a short-campanulate corolla with 5-lobes. The stamens are exserted. The pistil consists of two united carpels bearing a two-loculed ovary with two ovules in each locule. Two styles with capitated stigma are separated at ovary apex. The fruit is a capsule with large interstylar opening (Hutchinson, 1973; Chang, 1978). The common features of mature flower of *C. australis* (Fig. 13,14,15) , in present studies, generally match the above descriptions. However, some flowers which with 4-merous floral parts are also found (Fig. 9). The variation in number of floral parts is common in this genus (Hutchinson, 1973).

Sepal initiation and development

The first sepal is initiated laterally to the axis of the flower (Fig. 1); the remaining four sepals are initiated in helical succession (numbers in order in Fig. 1, 10). The helical order of initiation of the sepals is generally found in tribe Caesalpinioideae of Leguminosae (Tucker,1984; Table 1) and is very common in Convolvulaceae (author's unpublished data). After inception, the sepals gradually curved inwards to enclose the floral apex (Fig. 10). A shallow calyx cup is formed by zonal growth of all confluent sepal primordia. This type of calyx tube formation is similar to which of tomato (Chandra Sekhar and Sawhney, 1984). Subsequently, the sepals are elongated (Fig.2, 3, 10). At anthesis, the sepals are not reflexed and remain fully erect similar to that of many Cruciferae (Weberling, 1989). The size of sepals is nearly equal and approximately as long as corolla tube at maturity (Fig. 14).

Petal initiation and development

The petals alternated with the sepals, are smaller than the sepals and stamens at early stage (Fig. 2, 3). Subsequently, the petals are visible as flaps (Fig. 3), then reach the height of the stamens (Fig. 4). When tetralobed anthers are visible, the basal region of petal are enlarged gradually and the tips are elongated over the anther (Fig. 7, 8). The five confluent petals and stamens form the corolla tube by zonal growth. (Fig. 8). Five petals are enlarged and covered the inner floral organs before anthesis (Fig. 11). After anthesis, the corolla tube extended with the petals which are slightly reflexed (Fig. 14). The withered corolla tube and petals are persistent in mature ovary. (Fig. 15).

Stamen initiation and development

The five stamens are initiated in positions alternating with the petal (Fig. 2, 3). The stamens develop bilobed anther with extensively gowth of the upper part (Fig. 3, 4). Consequently the stamens with tetralobed anthers are recognizable (Fig. 5, 6, 7, 8). The well developed anther shows a longitudinal division into two equivalent thecae, with longitudinal dehiscence line and inconspicuous connective (Fig. 17). Both thecae face inwards on the ventral side (introse, Fig. 9). The filaments are elongated at anthesis (Fig. 14).

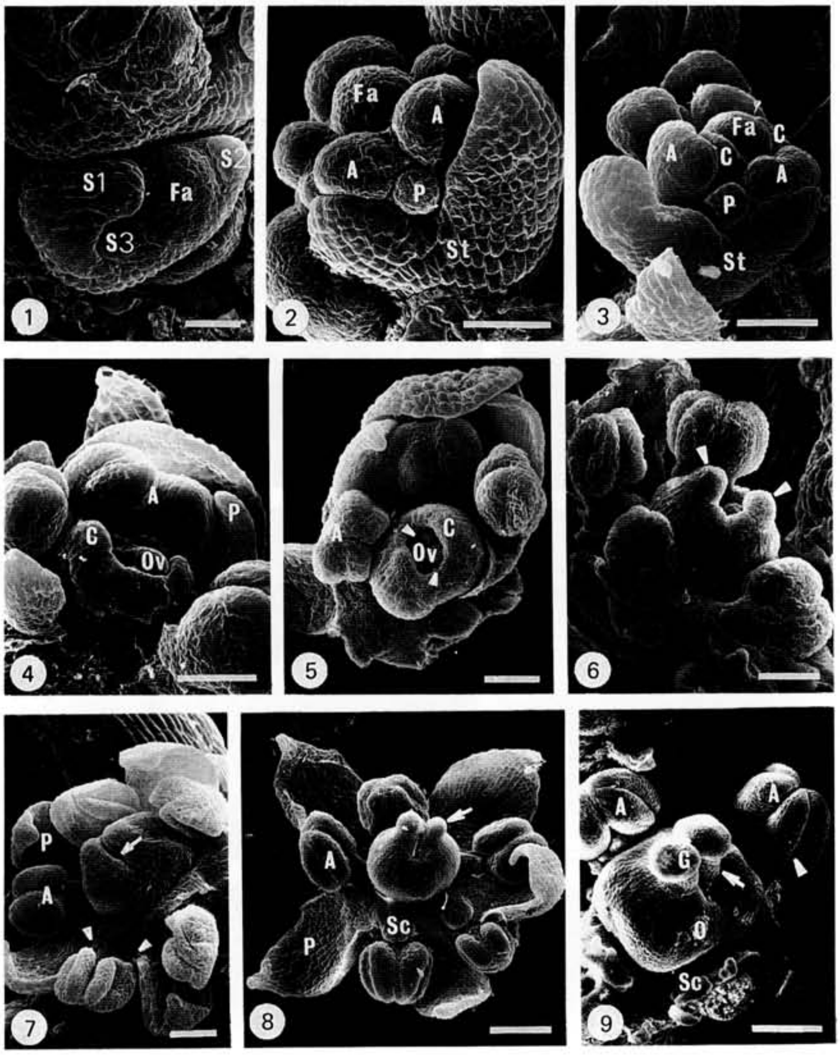

Fig.1-9. Floral organogenesis in *Cuscuta ausalis.* (SEM micrographs). Bar = 50 μm in Fig. 1-2; in Fig. 3-7. Bar= 100 μm, and in Fig.8-9. Bar = 200 μm.

1. Early sepal stage, with three sepals visible. The first sepal (S1) initiated is on lateral side, opposites with the second sepals (S2), and follows by the third sepals (S3). The remaining two sepals are not initiated. The floral apex (Fa) is convex. **2.** Lateral view of flower with calyx tube (St), petals (P) and stamens (A). The stamens are initiated alternating with the petals. Fa, floral apex. **3.** Lateral view of flower with calyx tube (St), petals (P) and stamens (A). Two carpels (C) arise from the edge of floral apex (Fa). The stamens are larger than the petals, and are growing to become biolobed at this stage. **4.** Lateral view of flower in which organogenesis is completed. The petals (P) reach the height of stamens (A). Two carpels (C) are more elevated, and surround four ovules (Ov). The apex of carpels are more elongated. **5.** Flower in which stamens (A) develop well with tetralobed anther. Two carpels (C) form the ring-like ridge, gradually enclose the ovules (Ov), and with two protrusions (arrowhead) which will beccome septa later. **6.** Two carpels are nearly enclosing the ovules, and their apex (arrowhead) are more elongated. **7.** Flower with all sepals removed, five petals (P) and five stamen (A) with their base connected to form the corolla tube (arrowhead). The ovary is formed by fusion of the two carpels which left a linear suture near apex (arrow). **8.** Flower with all sepals removed, five petals (P) with five stamens (A), and the petals are more larger than the stamens at this stage. The scales (Sc) are initiated at the base of the stamens. Filaments are not elongated at this stage. The apex of the carpel is growing to become stigma (arrow). **9.** Flower in which the stamens (A) are well developed with logitudinal dehiscence line on anther sacs (arrowhead). The young scales (Sc) are located at the base of stamens, and with dentation at the apex. The ovary (O) with the global stigma (G) and short style (arrow). [4-merous flower in this sample]

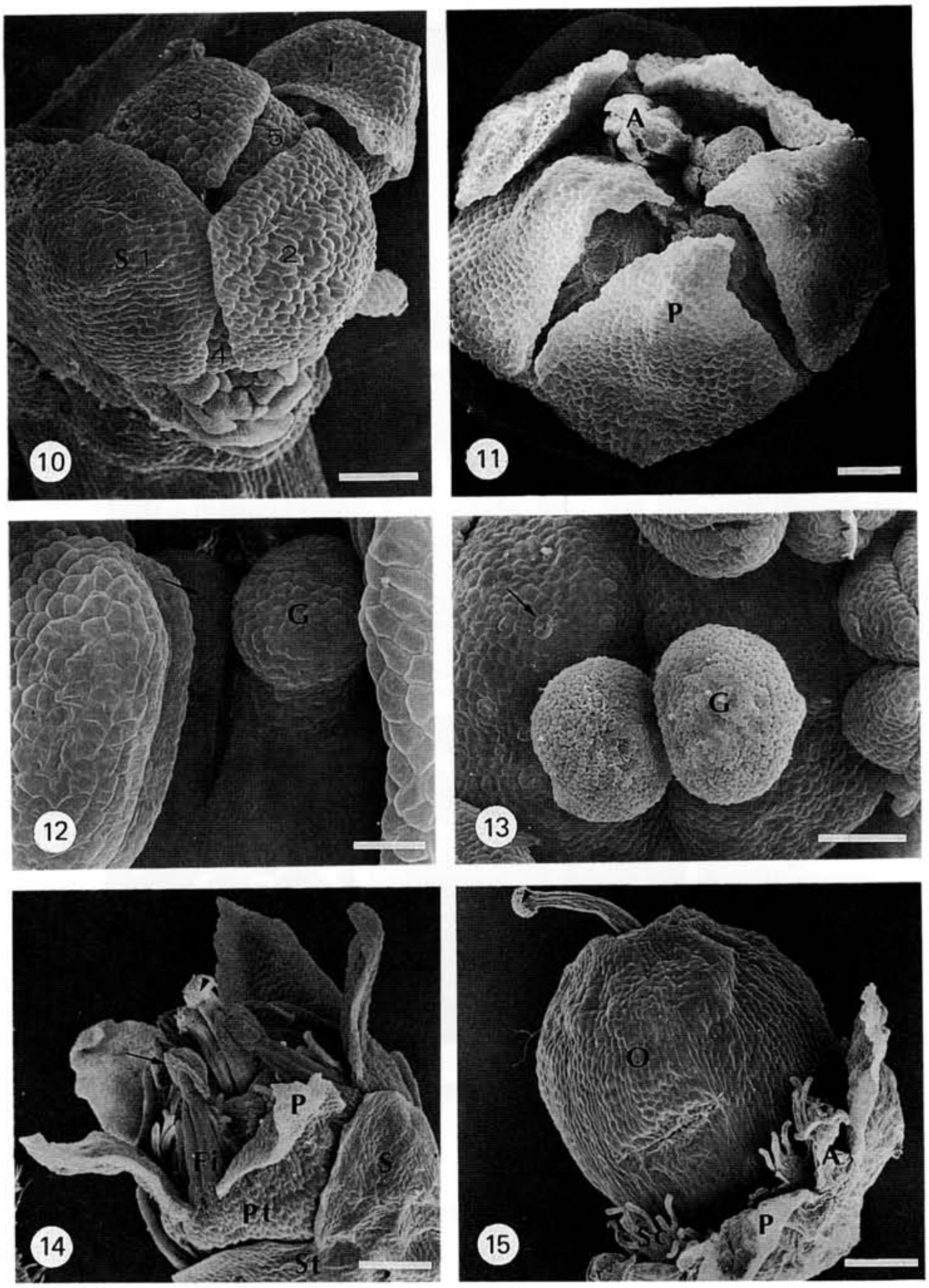

Fig. 10- 15. Some features of floral organs in *Cuscuta australis.* (SEM micrographs). Bar = 200 µm in Fig. 10- 11; in Fig. 12-13 Bar= 50 µm, and in Fig. 14-15. Bar = 400 µm.

10. Five sepals (S) enclose other floral organs. The sepals are numbered in the order of their helical sequence. (the first sepal initiated is numbered 1). **11.** Five petals (P) are fully enlargedand covered the inner floral organs before anthesis. The stamens (A), with elongated filaments, are enable to dehisce. **12.** Close view of the apex of two caprpels, the right carpel with a global stigma (G) is developed faster than the left one (arrow). **13.** Top view of the gynoecium, the surface of the global stigma is papillated (G), and stomata are visible on the ovary surface (arrow). **14.** Anthesis flower with calyx tube (St), corolla tube (Pt) and gynoecium are well developed. Sepals (S) are appressed to the corolla tube and altermating with the slightly reflexed petals (P) . The stamen, with anthers (arrow) somewhat shrinking and the filaments (Fi) are elongated from the corolla tube, The ovary are developed with the stigma on the elongated style (arrowhead). **15.** Pollinated flower with sepals removed showing the enlargement of ovary (O) and the shrinkage of all other floral organs includes the stigma (arrowhead), the fimbriate scales (Sc), the stamens (A) and the petals (P)

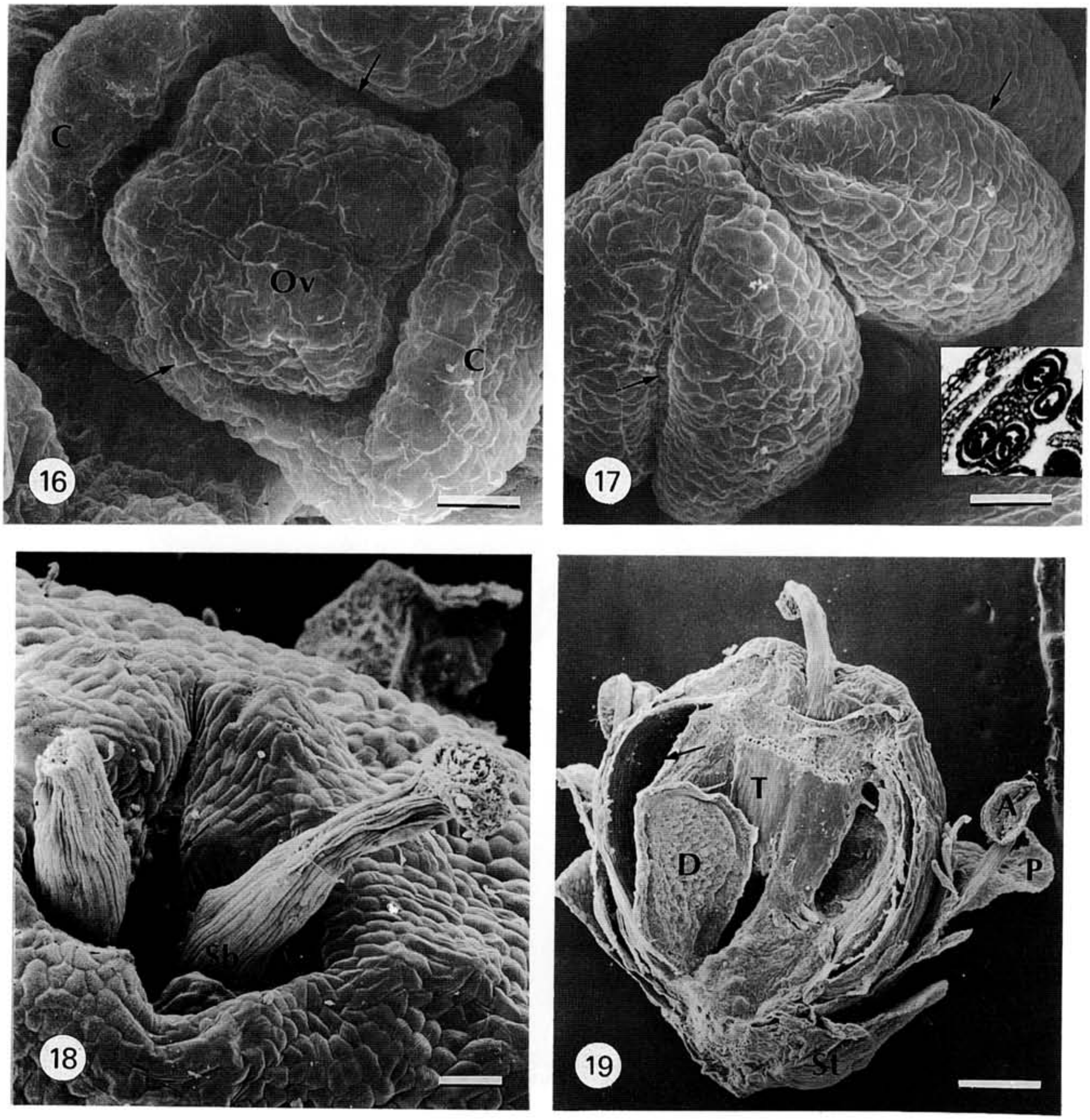

Fig. 16-19. Some features of floral organs in *Cuscuta australis* (SEM micrographs). Bar = 20μm in Fig. 16; in Fig. 17 Bar = 40 um, in Fig. 18 Bar =100 μm, and in Fig.19 Bar = 400 μm.

16. Early stage of initiation of the gynoecium. Two carpels (C) form the ring-like ridge, gradually enclose the ovules (Ov), and with two protrusions (arrow). **17.** The well developed anther shows tetralobed anther sacs with longitudinal dehiscence line (arrow). Inserted is the cross-section of the anther, with pollen grains. **18.** Two style base (Sb, one is labeled) are separated by a stylar canal (arrow). **19.** The septum (T) and inner ovary wall (arrow) is visible in this axial section of a mature flower. One seed (D) is visible at the base of ovary. The other floral organs are persistent but withered. St, Calyx tube; P, Petal; A, Stamen.

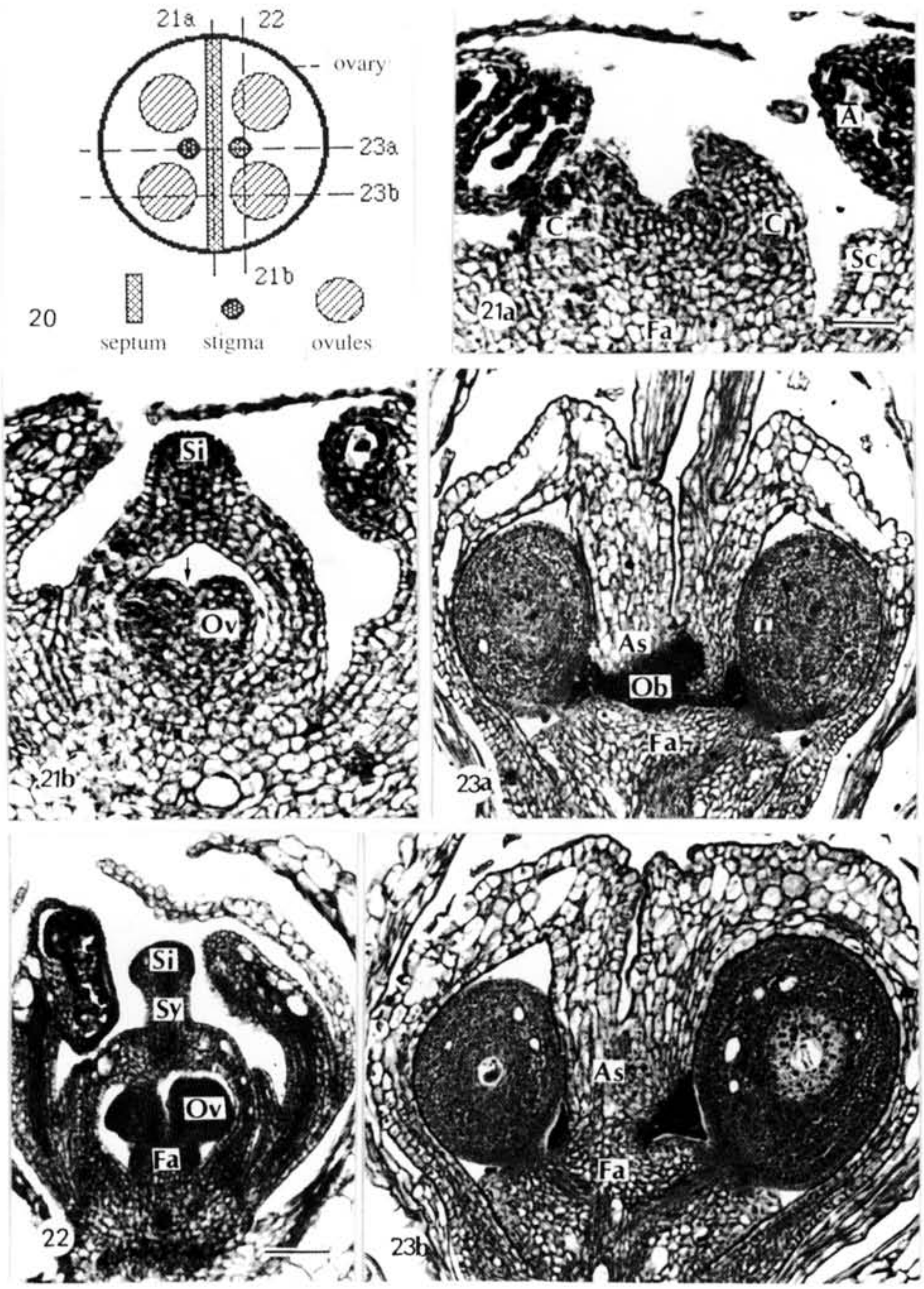

Fig. 20. Scheme to show the location of the corresponding longitudinal sections of Fig. 21-23, through different region of the gynoecium (vertical and horizontal line with number).

Fig. 21-23. Longitudinal section of the gynoecium showing some developmental features of different stage in *Cuscuta australis*, (LM micrographs). Bar = 60 μm in Fig. 21a, b; in Fig. 22-23, Bar= 150 μm.

21a. L. s. through septum of early stage (cf Fig.7), two young ovules are visible from ovary base (Fa) and surrounded by two carpels (C). Note that the congenital septum is formed by the fusion of carpel margin and the placenta. A, stamen; Sc, scale initial. **21b.** L. s.. through stigma and locules of the same gynoecium, the stigma (Si) is just developing, style is not present, and ovules (Ov, one is labeled) are not well developed (arrow). **22.** L. s.. through stigma and locules of slightly late stage of the gynoecium (cf. Fig. 8), stigma (Si) and style (Sy) is visible. The ovules (Ov) are visible at placenta (Fa). **23a.** L. s. across septum and through stigma of the gynoecium near anthesis. There are ectotrophic conducting tissues (Ob) on the placenta as well as the septal margins. The enlarged placenta (Fa) nearly fused with the apical septum (As). **23b.** L. s.. across septum and through locules of the gynoecium near anthesis, the elevated placenta (Fa) is fused with the apical septum (As).

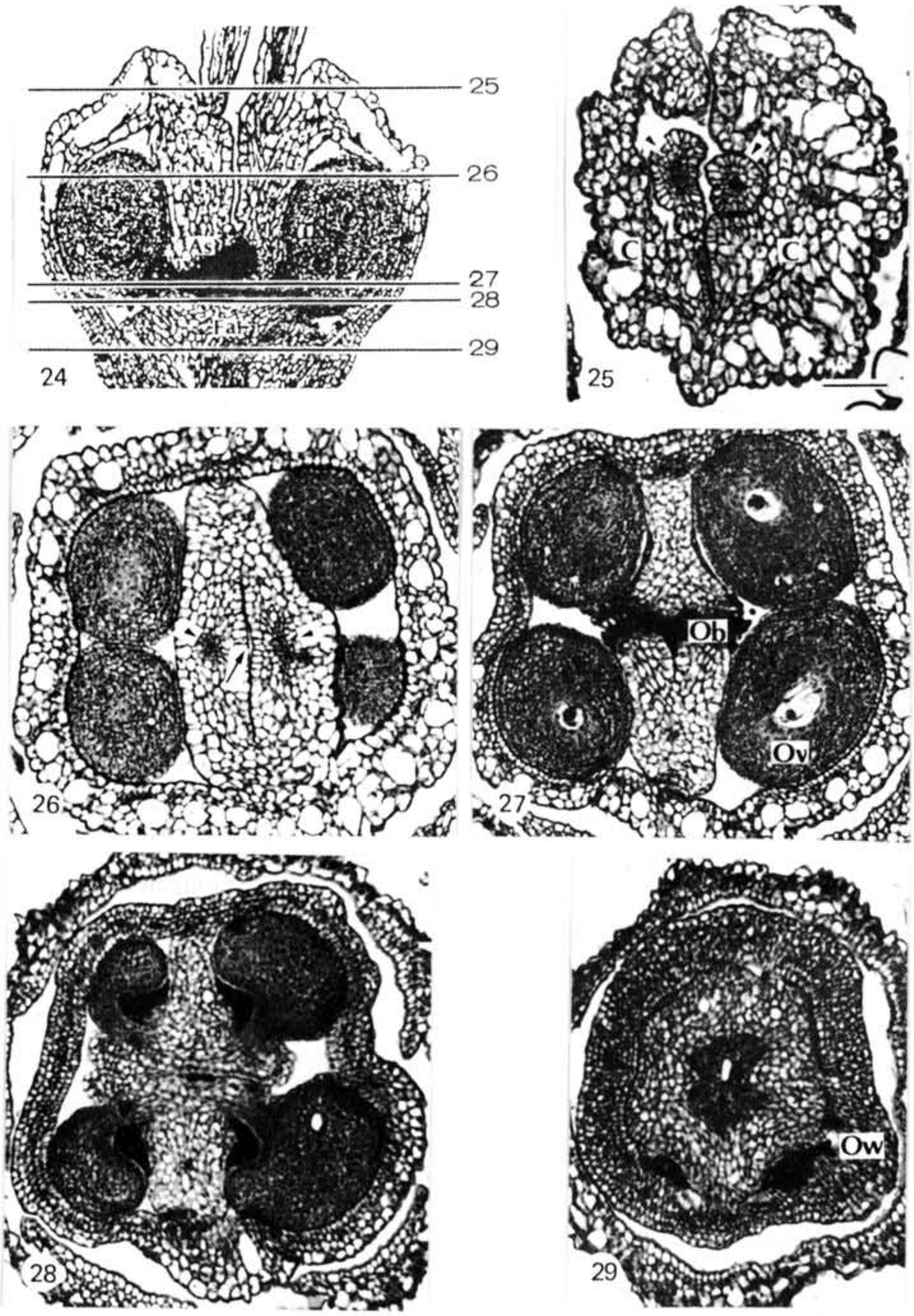

Fig. 24. Scheme to show the location of the corresponding cross-sections of Fig. 25-29, through different region of the gynoecium(horizontal line with number).

Fig. 25-29. Series of cross-sections of a gynoecium.(LM micro graph). Bar = 150 μm

25. Apex of ovary, the two style branches (arrowhead) which lies between two carpels (C) are all free from one another. 26. Further downwards, the carpers that face each other and the styles (arrowhead) are fused into a continuous septum. The stylar canal (arrow) is visible in the center (cf. Fig. 18). **27.** In a zone further down again, the septal margins become visible which with ectotrophic conducting tissue (Ob). Ov, ovules. **28.** Near the base of the ovary, the margins of carpels meet the placeta. **29.** The basal part of ovary, the vascular bundle of the four ovules at center is diverging from the placenta which is surrounded by ovary wall (Ow).

Scale initiation and development

The scales are initiated after all other floral organs are completely initiated. Scale primordia arise at the base of each stamen, from the corolla tube, when the stamens are developed with two thecae (Fig. 8, 21a). After the short style with global stigma, the young scales grow up with dentation at the apex. (Fig. 9). At anthesis, the scales are fimbriate at apex, and expanded (Fig. 14). After anthesis, the scales are still persistent (Fig. 15, 19). Although the presence of scales inside corolla tube is a general character in the genus *Cuscuta* (Hutchinson, 1973), it is not common in other members of the Convolvulaceae (Lawrence, 1951; Hutchinson, 1973) , with exception in *Evolvulus alsinoides* (Tiagi, 1966; author's unpublished data). The features of the scales of *C. australis* are similar to that of *C. reflexa* which was described by Tiagi (1966) with fimbriate margins. The position of the scale is different from the genus description that was described as "scales between the stamens" by Hutchinson (1973, P. 622, Fig. 337). Moreover, the developmental features are quite different from those coronal scales of Boraginaceae, *Gentiana* and Silenoideae (Weberling, 1989) both in shape and position. The function of the scales, which was depicted by Tiagi (1966) as floral nectaries with glandular bodies under them, is not confirmed in the present study.

Gynoecium initiation and development

Two carpels, congenitally united, are initiated with the two adjacent sides somewhat suppressed (Fig. 3,). Four ovules are visible at the early stage of the gynoecium (Fig. 4, 16), then the apex of carpels are more elongated, form the ringlike ridge, and gradually enclose the ovules to forming the ovary with a linear suture left near apex. (Fig. 5, 6, 7, 21b). Consequently, the apex of the carpel is growing to become two separated stigma with short style (Fig. 8, 9, 12, 13, 22). At anthesis, the surface of stigma is papillate and the styles are more elongated (Fig. 13, 14). The stigmata and styles are wilted and persisted on the developing fruit (Fig. 15, 19).

A septum is formed partially by the upgowth of the placenta with two protrusions from the ridge (Fig. 5, 16, 21a, 26) at very early stage, and then this part contact with an apical septum which projects into the ovary from above (Fig. 23a). There are two types of structure of conducting tissue; the ectotrophic conducting tissue which cover the placenta is known as the obturator (Weberling, 1989). The formation of the obturator in two species of *Cuscuta* has been illustrated by Tiagi (1966). In addition, he also descirbe that the epidermal cells of the septal margins can draw out and produce a glandular tissue, simulating the tissue of the obturator (Tiagi, 1966). There are ectotrophic conducting tissue on the placenta as well as the septal margins (Fig. 23a, 27). The apical septum has been described by Hartl (1962) in many members of Sympetalae in Angiosperm including the Convolvulaceae; While the obturator has been interpreted as the sterile end or a mere outgrowth of the floral axis of the Lentibulariaceae and Primulaceae (Weberling, 1989). There are different interpretations on the features of placentation of Convolvulaceae and Cuscutaceae. For examples, Woodland (1991) has illustrated that the ovules borne on basal-axile placentas in Convolvulaceae and on axile placentas in Cuscutaceae. Cronquist (1988) has described in a synoptical arrangement of the families of Solanales that the ovules of Convolvulaceae are basal and erect. On the other hand, Tiagi (1966) pointed out that the placentation in *Cuscuta* is axile and shares some features of the parietal placentation based on vasculature of series cross-section. The ovules of *C. australis* is positioned in the center of the ovary at the base (Fig. 29) and are partially separated by the congenital septum and the apical septum (Fig. 23a, 23b, 26, 27, 28).

The different developing speed of the two carpels (Fig. 12) and the distribution of stomata on the surface of ovary (Fig. 13) are two more features of the gynoecium. The differences in developing speed are also presented more or less in other floral organs of *C. australis*, and are not uncommon in floral development; However, the ovary with stomata is somewhat peculiar in *C. australis* which is rarely mentioned on other species of *Cuscuta*.

CONCLUSION

The initiation and developmental features of floral organs of C. australis are illustrated . The bisexual flower is protandrous becauese the anthers are well developed very earlier than the gynoecium. The features of the fimbriate scales are not common in the members of the Convolvulaceae. Another feature is also not found in the Convolvulaceae. It is that the ovules are exposed before the fusion of two carpel apexes. The combination of an apical septum with a congenital basal septum is a feature similar to some members of the Convolvulaceae. The placentation is basal and it shares some feature of the axile placentation.

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菟絲子（旋花科）花部之發育

郭長生 廖國媖

摘要

以掃描電子和光學顯微鏡檢探究旋花科植物菟絲子 (*Cuscuta australis*) 花部的發育情形。花之各部始原依萼片、雄蕊、花瓣及心皮之順序出現。除記述發育時花部的特徵外並和旋花科及其它植物比較。兩個心皮先端尚未癒合之前，胚珠很早就出現於雌蕊上，此點較爲特殊。又起源爲基生的胚珠因具不完全隔板而看似中軸胎座。