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Morphology and Anatomy of Mature Embryos and Seedlings in Parasitic Angiosperm *Cuscuta japonica*

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Morphological and anatomical features of mature embryos and seedlings were observed at different growth stages in the parasitic angiosperm *Cuscuta japonica* Choisy. The spirally coiled embryos from scarified seeds had no cotyledons but possessed blunt radicles. Seeds germinated at 30°C in the dark. Although most embryo cells incubated for 16 h did not have starch grains, the shoot cells of three-day-old seedlings possessed numerous starch grains. After these seedlings were transferred to a lightened growth chamber, all the shoot apical regions of seedlings grown for 6, 8, and 10 days became greenish and hooked. Most of the shoot cells, including the green apical parts, contained abundant starch grains. The hooks opened only when one seedling made contact with another seedling. This suggested that the green and hooked shoot apical regions played an important role in searching for and twining about their host plants. In some two-day-old seedlings, the massive roots were circular or semi-circular. This enabled the shoot axes to stand erect on some substratum. It would assist the shoots in making contact with the host plant. In eight-day-old seedlings, the green apical regions also were hooked and the roots were considerably degraded.

Keywords: Anatomy, *Cuscuta japonica*, Embryo, Morphology, Parasitic angiosperm, Seedling

The seedlings of *Cuscuta* have chlorophyll that enables photosynthesize (Zimmermann, 1962; Pattee et al., 1965). Although all the *Cuscuta* species still contain chlorophyll a and b while exploiting their host plants (Macleod, 1963; Ismail and Obeid, 1976), the rate of photosynthesis is decreased (Pattee et al., 1965). The excised stem tips of *C. campestris*, grown in vitro, produce a considerable amount of chlorophyll. This probably plays an important role in maintaining the growth of the culture (Loo, 1946).

Fujita (1964) has characterized the mature embryo of *C. japonica* as a spirally coiled, yellow cylindrical structure, with a tapering apex, no cotyledon, and having a blunt radicle without a root cap. Truscott (1966) has suggested that in embryo culture of *C. gronovii*, the loss of both cotyledons and roots was a single embryonic event in the evolution of *Cuscuta* from its autotrophic ancestors. The morphology and anatomy of early seedlings in *C. pedicellata* has been studied by Lyshede (1985), who has also observed the fine structures of the tuberous radicular end (Lyshede, 1986) and the one-week-old seedlings in the same species (Lyshede, 1989). However, the function of organs during their autotrophic growth period is not yet clearly understood. The present study attempts to describe some morphological and anatomical features of *C. japonica* embryos and seedlings at various developmental stages, and to evaluate these features in view of their parasitic roles.

MATERIALS AND METHODS

Seed Germination

We scarified mature, dormant seeds of *C. japonica* Choisy with concentrated sulfuric acid for 25, 35, or 45 min, then rinsed them in tap water and distilled water. Twenty seeds from each scarification treatment were placed on moist filter paper in Petri dishes and incubated in the dark at 30°C. The seeds scarified for 45 min showed the highest rate of germination, consistently exceeded 90%. We dissected the embryos from the seeds incubated for 16 h, and observed them under a dissecting microscope.

Seedling Growth ·

The roots of the three-day-old seedlings were wrapped in wet cotton and placed at the bottom of a 500-mL beaker. These seedlings grew in a growth

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chamber at 30°C for 8 h, and then at 25°C for another 16 h. White light, of about 1370 lux, was maintained during only the 30°C temperature treatment. We collected the embryos soaked for 16 h and the seedlings grown for 2, 3, 6, 8, or 10 days for an examination of their morphological and anatomical features.

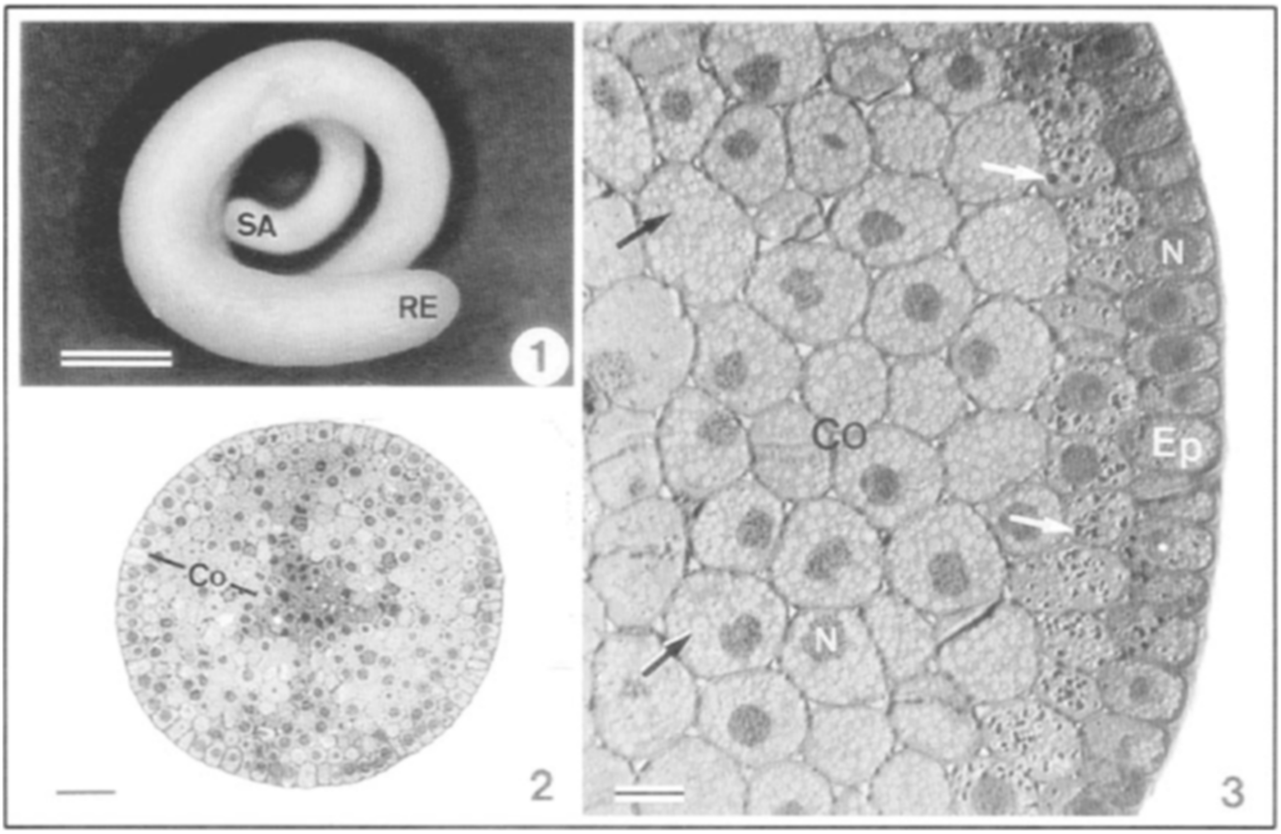
Light Microscopy

The apical- and mid-portions of the shoots, plus the radicles and roots for all sampled embryos and seedlings were cut into approximately 1 mm^3 segments. We fixed these segments in a mixture of 2.5% glutaraldehyde-2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 6.8) for 2-3 h at room temperature. The materials were postfixed in 1% osmium tetroxide, with the same buffer (pH 6.8), for 1 h at room temperature. After postfixation, the segments were washed in buffer, and dehydrated in a graded series of acetone. The tissues were embedded in Spurr's resin (Spurr, 1969) and sections were cut on a LKB-V ultramicrotome. We stained thick sections with 0.05% toluidine blue and examined them with an Olympus BH2 light microscope and BX50 phase differential interference contrast (DIC) microscope.

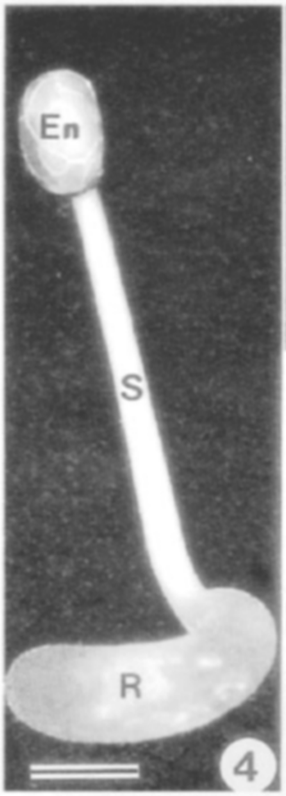
RESULTS

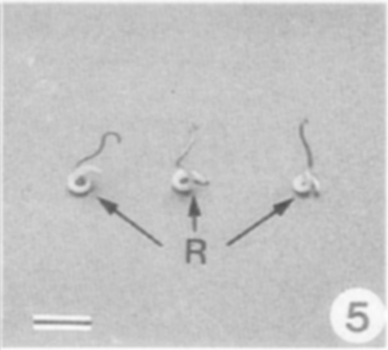
Embryo

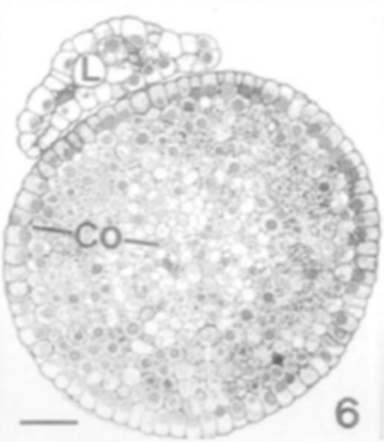
The mature embryo of *C. japonica* was en- closed by a starchy endosperm. Each embryo was yellow and spirally coiled, turned almost two times on itself. The shoot was tapered toward the apex and had a few scale-like leaves, but no cotyledon, while the

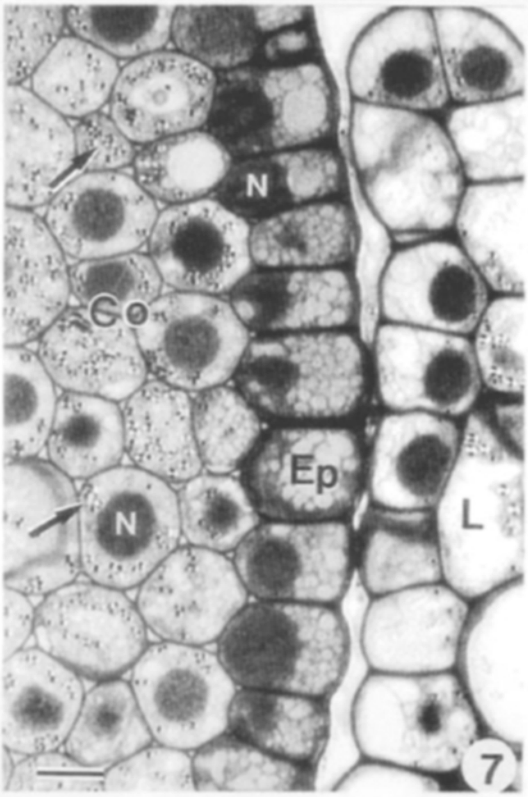


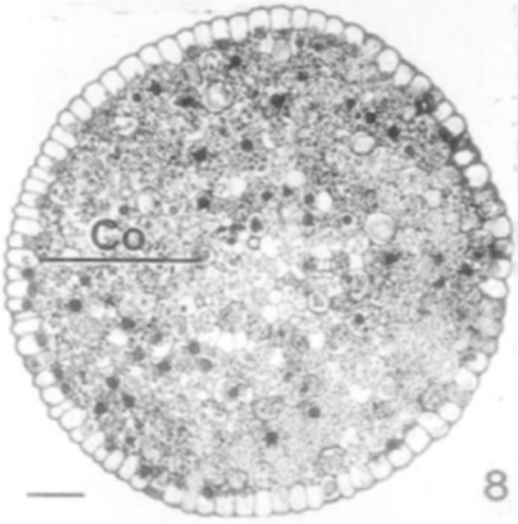
Figures 1-3. Light micrographs of the *C. japonica* embryo from a seed incubated for 16 h. **1.** An embryo, devoid of cotyledons, is spirally coiled with a tapered shoot apex (SA) and a blunt radicular end (RE). Bar = 1 mm. **2.** A transverse section of the shoot apical portion from an embryo shows that the epidermal and cortical cells have relatively conspicuous nuclei and many small vacuoles. The small vacuoles and vascular tissues are not distinguished in this light micrograph. Co, cortex. Bar = 20 μm. **3.** An enlarged view of the mid-portion of hypocotyl segment transectioned; the epidermal and cortical cells have conspicuous nuclei. Note the cortical cells containing numerous small vacuoles (black arrows). The outermost cortical cells have darkly stained particles (white arrows). Co, cortex; Ep, epidermis. DIC. Bar = 10 μm.











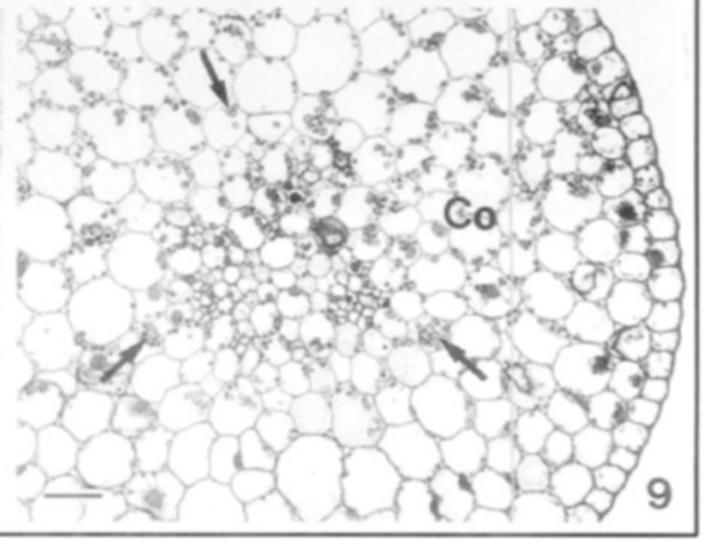


Figure 4. In a side view of a two-day-old seedling grown in the dark, the massive root (R) showing a semi-circular form supports the straightened shoot axis (S). The shoot apical region is covered with the remaining endosperm (En). Bar = 1 mm.

Figures 5-9. The three-day-old *C. japonica* seedlings grown in the darkness. **5.** The shoot apical regions are slightly hooked, and the massive roots (R) are circular or semi-circular. Bar = 5 mm. **6.** The shoot apical segment shows a remarkable feature that the cortical cells (Co) contain many starch grains seen as black dots. L, scale-like leaf. Bar = 20 μm. **7.** In an enlarged view of a part of Figure 6, prominent nuclei in the epidermal cells (Ep) are located at the proximal wall side and several small vacuoles at the distal wall side. Note the cortical cells (Co) contain numerous starch grains (arrows) and many small vacuoles. L, scale-like leaf. Bar = 5 μm. **8.** In the stem at the mid-portion of the shoot axis, the epidermal cells seem to be highly vacuolated, and the cortical cells (Co) possess innumerable starch grains. Bar = 20 μm. **9.** The cortical cells (Co) at the base of the root are enlarged and vacuolated. They contain much fewer starch grains (arrows) than in the cells of the shoot apex as in Figure 6 and of the stem as in Figure 8. Bar = 20 μm.

radicle was blunt and had no root cap (Fig. 1). The embryo within a soaked seed had no distinguishable vascular tissues at the shoot apical region. Epidermal and cortical cells of the shoot apex contained relatively prominent nuclei, and most cells possessed lightly stained cytoplasm (Fig. 2). In the mid-portions of the hypocotyl, most of the cells had a number of small vacuoles; the outermost cortical cells contained darkly stained particles within the small vacuoles (Fig. 3). Several small vacuoles were commonly found in the ground tissue of the radicle. No starch grains were observed within cells of embryonic organs.

Seedlings

After incubating in the dark, two-day-old seedlings had noticeable changes in their morphologies: the shoot axis became straightened compared with the spirally coiled embryonic axis. The roots were massive, but became semi-circular in some seedlings. These roots enabled the shoot axis to stand erect (Fig. 4).

The shoot of three-day-old seedlings were about 0.9-1.8 cm long. Each shoot apical region turned green and became slightly hooked when the seed coat, covering the endosperm, was removed. The root was massive, with a tapered end, and often appeared circular or semi-circular. Therefore, the shoots were able to stand erect on the surface of some substratum (Fig. 5).

Unique features were seen in cross-sections of the epidermis and cortex in the shoot apex (Figs. 6 and 7). The epidermal cells were slightly and radially elongated. Their prominent nuclei were located at the proximal side, with several small vacuoles observed at the distal side. The cortical cells contained numerous starch grains. The features of cortical cells in scale-like leaves around the shoot apex were similar to that seen in the shoot apical region.

Most of the shoot cells in the mid-portion of the seedling also contained numerous starch grains. However, the epidermal cells appeared to be vacuolated; their nuclei were not conspicuous as in the shoot apical regions (Fig. 8). Cells near the root base were considerably enlarged and highly vacuolated, and had much fewer starch grains than in cells of the shoot apex and mid-portions (Fig. 9). Those root cells did not contain starch grains.

The shoots of six-day-old seedlings were about 6-10 cm long. Their morphological and anatomical features were similar to those of the three-day-old seedlings except that the shoot apical region became more curved and greenish (Fig. 10). The cortical cells of the apex also contained numerous starch grains (Fig. 11). Some epidermal cells of the root had expanded and been transformed into root hairs (Fig. 12). The shoots of eight-day-old seedlings were 9-12 cm long. Their apices were also green and highly hooked, like hairpins. The roots were almost degenerated compared with the roots of younger seedlings (Fig. 13).

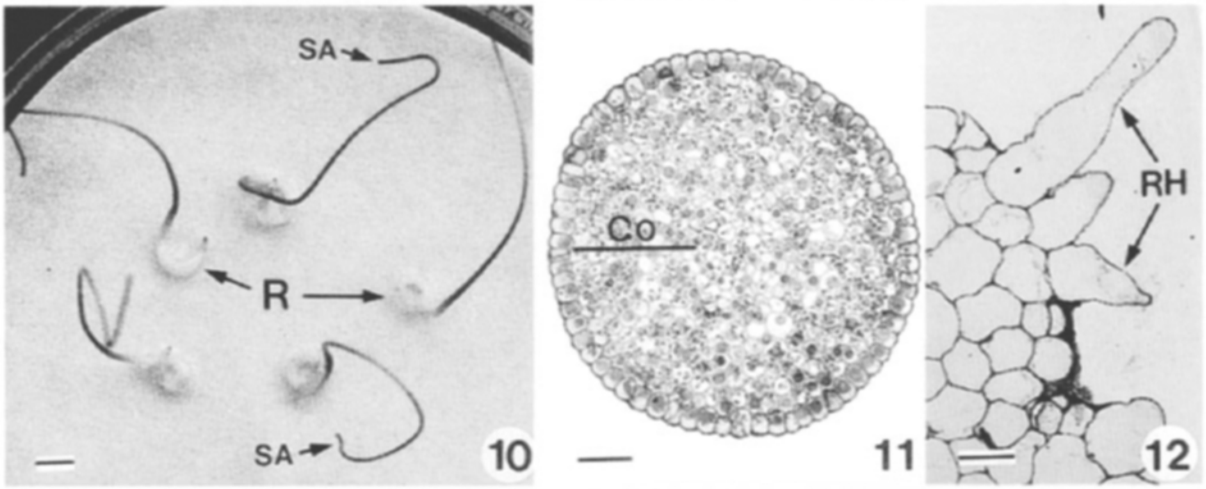
We observed self-parasitism in ten-day-old seedlings. When the seedlings were close to each other in the beaker, one seedling acted as a parasite, twining dextrally around the stem that acted as a host. The green shoot hook of the "parasitic" seedling was somewhat opened (Fig. 14). In contrast, shoot apices that were not in contact with other individuals maintained their hooked shapes. Even though some of the seedlings had been growing for more than 15 days, after germination, without making contact with each other, their green apical hooks continuously maintained their curved shapes.

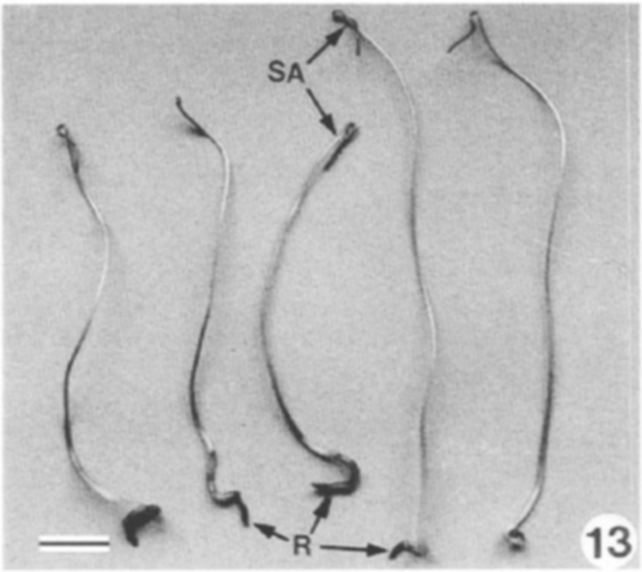
DISCUSSION

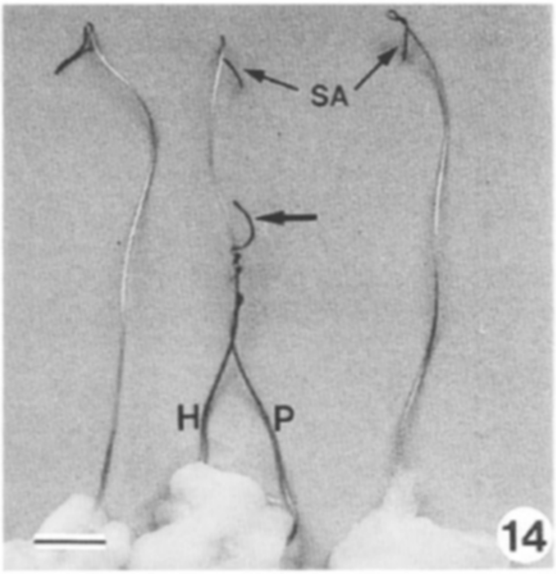
Embryonic Organs

The mature embryos in several *Cuscuta* species are spirally coiled, with no cotyledons, and having a blunt tuberous radicle devoid of a root cap (Macpherson, 1921; Fedortschuk, 1931; Tiagai, 1951; Johri and Tiagai, 1952; Fujita, 1964; Lyshede, 1986). The embryos of most dicotyledonous plants have relatively short and straight axes, but the spirally coiled embryo axes in *Cuscuta* are morphologically unusual. Kuijt (1969) has stated that the embryonic form of *Cuscuta* seemed to foreshadow the parasitic behavior of the older plant. This coiling may, conceivably, help a larger-than-average embryo to better fit within the limited space of a seed. After germination, this extra length may be advantageous when selectively searching for and entwining a host plant, when compared with the limitations of shorter seedlings. The latter would need further growth or elongation of the seedling axis.

None of the *Cuscuta* embryos had cotyledons, unlike the embryos of most dicotyledonous plants. When two cotyledons are attached to the shoot axis or apical region of the seedling, they may act as barriers that disable the seedling from entwining around a host plant. Therefore, the cotyledons, which normally would serve as food-reservoir organs for embryo and seedling growth, may have been lost as *Cuscuta* evolved from autotrophic ancestors (Truscott, 1966).







Figures 10-12. The six-day-old *C. japonica* seedlings grown under light. **10.** Seedlings grown on a wet filter paper in a Petridish. The green shoot apical portions (SA) are also hooked and the massive roots (R) show semi-circular and circular forms. Bar = 5 mm. **11.** A cross section of the shoot apical region shows that the cortical cells (Co) are filled with a number of starch grains. Bar = 20 µm. **12.** Some cells in the root epidermis are enlarged and show some root hairs (RH). Bar = 20 µm.

Figure 13. In eight-day-old seedlings, the green shoot apical portions (SA) are hooked like a hairpin, and the roots (R) are almost degraded. Bar = 10 mm.

Figure 14. Ten-day-old seedlings show self-parasitism: one seedling acting as a parasite (P) twines to the right, counterclockwise, on the other's stem, which acts as a host (H). The shoot apical region of a self-parasitizing stem is opened (arrow); however, the apical portions of other free seedlings are still hooked. Bar = 10 mm.

Shoot Apical Regions in Seedlings

The cells of *Cuscuta* seedlings grown in the light contain starch grains (Lyshede, 1985, 1989). Because light-grown shoot apices are always greenish during the seedling stage, this suggests that the *Cuscuta* seedling can photosynthesize (Zimmermann, 1962; Pattee et al., 1965). The photosynthetic efficiency is highest in seedling, and decreases as the plant becomes parasitic (Pattee et al., 1965). Such photosynthetic ability helps facilitate apical growth or elongation of the shoot until the seedling encounters a host plant.

Rapid shoot growth is caused by active cell division and cell elongation (Lyshede, 1985). Because cellular activity requires an energy source such as starch grains, i.e., products of photosynthesis, the photosynthetic capability of the seedling may ensure its long-term survival. The endosperm enclosing the spirally coiled embryo of *Cuscuta* contains starch (Kuijt 1969). Most cells of embryonic organs in our *Cuscuta* did not possessed starch grain. However, the numerous starch grains accumulated by shoot cells prevented photosynthesis in three-day-old seedlings that had been grown in the dark. This leads to two hypotheses. One is that the source of the starch grains accumulated in the early seedling may be the starch-bearing endosperm tissue enclosing the embryo. It is conceivable that the carbohydrates are digested, transferred, and reserved in the embryo during germination. Another hypothesis is that the cells of the seedling shoot may have a certain biochemical pathway(s) by which the starch grains are converted from reserved material(s) such as protein bodies and lipid droplets. To prove this hypothesis, cell organelles and food materials should be compared between embryonic and seedling cells.

Another morphological characteristic in seedlings is the hooking of the green apical segment. The hook opens under a single saturating red light, and the seedlings became twined (Lane and Kasperbauer, 1968). In our *Cuscuta* seedling, the hook was formed and maintained at the apical region throughout the seedling stage, unless a seedling makes contact with a host. However, the hook was opened when the shoot contacted a "host" seedling as shown in Figure 13. This indicates that the hook may function to seize a host plant at the first stage of parasitism. Therefore, the morphology of the green and hooked apical region of seedling shoots could provide a selective advantage toward parasitism because the apical region may enable *Cuscuta* to continue parasitizing a suitable host for a longer period of time.

Root

The massive or bulbous roots in our *Cuscuta* seedlings comprised highly enlarged and vacuolated cells. Rudimentary roots may function to supply water to very young seedlings while they are deriving nutrients from the seed endosperm (Koch, 1880; Lyshede, 1989). The blunt radicular end of the spirally coiled embryo was transformed into a circular or semi-circular root during seedling development, thereby enabling the seedling shoot to grow erect. These features of the roots in free seedlings of *C. japonica* may be helpful during its searching for and twining with a host plant.

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LITERATURE CITED

Fedortschuk W (1931) Embryologische Untersuchung von *Cuscuta monogyna* Vahl und *Cuscuta epithymum* L. Planta 14: 94-111

Fujita T (1964) Phylloitaxis of *Cuscuta*. Bot Mag Tokyo 77: 73-76

Ismail AMA, Obeid M (1976) A study of assimilation in *Cuscuta hyaline* Heyne ex Roth, *Orobanche ramosa* L. and *Striga hermonthica* Benth. Weed Res 16: 87-92

Johri BM, Tiagai B (1952) Floral morphology and seed formation in *Cuscuta reflexa* Roxb. Phytomorphology 2: 162-180

Koch L (1880) Die Klee- und Flachsseide (*Cuscuta epithymum* und *C. epilinum*). Untersuchungen ueber deren Entwicklung, Verbreitung und Vertilgung, Carl Winthers Universitaetsbuchhandlung, Heidelberg

Kuijt J (1969) The Biology of Parasitic Flowering Plants. University of California Press, Berkeley and Los Angeles, pp 136-143

Lane HC, Kasperbauer MJ (1968) Photomorphogenetic responses of dodder seedlings. Plant Physiol 40: 109-116

Loo S-W (1946) Cultivation of excised stem tip of dodder in vitro. Amer J Bot 33: 295-300

Lyshede OB (1985) Morphological and anatomical features of *Cuscuta pedicellata* and *C. campestris*. Nord J Bot 5: 65-77

Lyshede OB (1986) Fine structural features of the tuberous radicular end of the seedling of *Cuscuta pedicellata*. Ber Deutsch Bot Ges 99: 105-109

Lyshede OB (1989) Electron microscopy of the filiform seedling axis of *Cuscuta pedicellata*. Bot Gaz 150: 230-238

MacLeod DG (1963) The parasitism of *Cuscuta*. New Phytol 62: 257-263

Macpherson GE (1921) Comparison of development in dodder and morning glory. Bot Gaz 71: 392-398

Pattee HE, Allred KR, Wiebe HH (1965) Photosynthesis in dodder. Weeds 13: 193-195

Spurr A (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastr Res 26: 31-43

Tiagai B (1951) A contribution to the morphology and embryology of *Cuscuta hyaline* Roth and *C. palaniflora* Tenore. Phytomorphology 1: 9-21

Truscott FH (1966) Some aspects of morphogenesis in *Cuscuta gronovii*. Amer J Bot 53: 739-750

Zimmermann CE (1962) Autotrophic development of dodder (*Cuscuta pentagona* Engelm.) in vitro. Crop Sci 2: 449-450