CHLOROPLASTS IN TISSUES OF SOME HERBACEOUS STEMS

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(Received: February 9, 1993, Accepted: November 15, 1993)

ABSTRACT

Serial sections of mature stems of ten species of herbaceous dicotyledonous plants were examined by light microscopy and the number of chloroplasts per cell was estimated in epidermis, collenchyma and cortex. Chloroplast identification was made by both light and transmission electron microscopy. Chloroplasts were present in epidermis, collenchyma and cortex tissues of all stems examined. The smallest number of chloroplasts was observed in the epidermis. Collenchyma cells had the largest number of plastids in four of the genera and cortex cells had the largest number in the remaining six genera. The stem epidermis of all genera contained stomates as demonstrated by scanning electron microscopy and aceto-orcein stained epidermal peels.

KEY WORDS: *Xanthium pensylvanicum*, cocklebur, herbaceous dicotyledons, stem chloroplasts and stomates, SEM, TEM

INTRODUCTION

There is considerable information on the number of chloroplasts in leaf tissues (Linsbauer 1962; Butterfass 1979). However, chloroplasts of stem tissues have not been investigated as extensively. Plant anatomy texts by Foster (1942), Linsbauer (1962), and Esau (1965) state that some stem tissues (e.g. collenchyma and cortex) may have chloroplasts; but there is little information as to the number or distribution of these organelles. A search for pertinent references on chloroplasts in stem tissues revealed one on photosynthesis in aspen bark (Pearson and Lawrence 1958) and on the role of the stem in photosynthesis in *Glycine max* and *Spartium junceum* (Nilsen and Bao 1990). Although both reports demonstrated the existence of stem photosynthesis, neither considered chloroplast numbers. Thus we were prompted to study the numbers and distribution of chloroplasts in stems of *Xanthium* and other herbaceous dicotyledons. We also examined stems for the presence of stomates that might support the functioning of these stem chloroplasts.

MATERIALS AND METHODS

Burs of *Xanthium pensylvanicum* Wallr. were germinated in flats of soil in the greenhouse. After the first two foliage leaves had developed, the seedlings were transplanted into 15 cm clay pots. The plants were then grown in the greenhouse during the months of May and June. Natural illumination was supplemented with incandescent light to give an 18-hr photoperiod suitable for vegetative growth. Collection of other plants (see Table 1) was limited to Eastern Montgomery County in Pennsylvania U.S.A. The collected plants were identified by Dr. Carl Keener from the Botany Department of Pennsylvania State University.

Table 1. Average number of chloroplasts in stem tissues of some herbaceous dicotyledons. (Chloroplast Number; 95% Confidence Interval).

SPECIES	COMMON NAME	EPIDERMIS	COLLENCHYMA	CORTEX
<i>Ambrosia artemisifolia</i> L.	Ragweed	0.67±2.10	32.18±9.92	13.51±2.64
<i>Cucurbita pepo</i> L.	Zucchini	0.48±0.48	10.85±2.77	49.52±10.36
<i>Galinsoga ciliata</i> Blake.		7.67±0.84	18.12±2.79	31.45±5.18
<i>Lycopersicon esculentum</i> Mill.	Tomato	10.97±1.82	40.83±5.60	27.13±0.76
<i>Mentha cardiaca</i> Baker.	Mint	16.87±3.11	24.16±4.39	46.41±9.85
<i>Physostegia virginiana</i> L.	False Dragonhead	1.14±0.28	24.05±2.63	26.48±1.82
<i>Rubus fruticosus</i> Brambles.	Raspberry	0.2±0.17	12.91±0.47	12.02±3.54
<i>Saponaria officinalis</i> L.	Soapwort	7.05±1.14	15.65±2.56	32.26±5.18
<i>Solidago nemoralis</i> Verge d'Or	Goldenrod	2.53±0.37	18.13±4.58	12.93±2.21
<i>Xanthium pensylvanicum</i> Wallr.	Cocklebur	9.80±1.95	29.67±2.37	31.01±1.95

Light microscopy – Tissue segments from herbaceous dicotyledons were excised at the midpoint of mature internodes between two axils. The tissue was fixed overnight in a 3:1 mixture of ethanol: glacial acetic acid. Dehydration was performed in a graded series of mixtures of ethanol and normal butyl alcohol. After the tissues were embedded in Tissue Prep (Fisher Scientific Co.), 10 μm sections were cut tangentially to the axis of the stem and stained with Schiff's reagent and Fast Green. Stomates were identified from aceto-orcein stained epidermal peels taken from mature internodes and also by scanning electron microscopy.

Chloroplast counting technique – To estimate the number of chloroplasts per cell, a preliminary counting procedure was established. Photographs were made of serial sections of a number of longitudinally sectioned cortical cells of *Xanthium*. Transparencies were made from photographic prints. A set of transparencies of consecutive sections was superimposed using cell wall contours to identify the same cell until the thickness of the whole cell was covered. Usually 4-5 sections were required to cover the thickness of an average cortical cell. The total number of chloroplasts per cell was determined from transparencies. Although there is the possibility that some chloroplasts might have been sectioned in half thus contributing to a duplicate count, from the superimposed transparencies it was determined that the chance of having a sectioned chloroplast on transparencies of two consecutive sections was less than 1 per cent. In addition, chloroplast counts were made under a microscope using an immersion oil objective at 1000 $\times$ magnification and a Whipple ocular micrometer, from the same serial median or near-median sections used for photography and transparencies. The partial volume of cells was established from cell dimensions and section thickness. To determine total cell volume this figure was multiplied by the number of sections required to cover the whole cell. A sample of 30 cortical cells from a series of sections was used in duplicate counts, from transparencies and direct microscopic counts. Samples of tissues were taken from mature internodes from a single plant of each of the specified genera. Counts obtained from transparencies did not differ significantly from those made directly under the microscope (t-test; $P > 0.2$). Since the method with transparencies was excessively time consuming, direct counts made under the microscope were used for all tissues of other genera. Counts of epidermal cells excluded guard cells. Based on the coefficient of variability, the average counting error did not exceed 5 per cent.

Transmission electron microscopy (TEM) – One millimeter stem segments excised from *Xanthium* tissues were fixed in 3% glutaraldehyde in 0.1M phosphate buffer (pH 6.8). They were rinsed with phosphate buffer and post-fixed in 1.0% osmium tetroxide in phosphate buffer. The tissues were rinsed with buffer and dehydrated in an ethanol series followed by propylene oxide prior to embedding in Embed 812 (Electron Microscopy Sciences). Polymerization was performed at 60°C in an embedding oven. Other plant tissues were fixed as above but using 3% glutaraldehyde in NaOH-PIPES (piperazine N-N' bis-2 ethanol sulfonic acid) buffer (pH 6.8) (Salema and Brandao 1973). Sections were cut on an LKB V ultramicrotome using a diamond knife, stained with uranyl acetate and lead citrate and were examined with an Hitachi H-600 transmission electron microscope.

Scanning electron microscopy (SEM) – Strips of tissues from mature stem internodes were removed and fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 6.8), dehydrated in a graded ethanol series (70-100%) and critical-point dried

in a Denton DCP-1 critical point drying apparatus or air dried following immersion in hexamethyldisilazane (Sigma Chemical Company). They were mounted on stubs, coated with gold on a Denton Desk 1-A sputter coater and observed on a Hitachi S-570 scanning electron microscope at 5 or 10 KV to determine if stomates were present. Numbers of stomates per square millimeter were estimated from micrographs taken at 170 $\times$.

RESULTS

Figure 1A is a photomicrograph of a longitudinal section of *Xanthium pensylvanicum* stem illustrating the appearance of sections from which counts were made. The file of cells at the extreme left is epidermis (ep.) Adjacent to the epidermis is collenchyma (col) followed by thin-walled cortical parenchyma (cp). Chloroplasts are evident in all 3 types of tissue. This was true for all 10 species as indicated in Table 1. The presence of plastids in each tissue was determined by means of light microscopy and verified by TEM. Figure 1B is a low magnification electron micrograph of epidermal and collenchyma cells of *Xanthium* containing chloroplasts. Figure 1C is a micrograph of an epidermal chloroplast, Fig. 1D of a collenchyma chloroplast and Fig. 1E of a chloroplast in a cortical parenchyma cell. The average size of chloroplasts in all tissues was 5.6 $\times$ 2.5 μm . All chloroplasts contained typical grana in which some thylakoid discs were connected by stroma lamellae. Thylakoids and grana are evident in all of the illustrated plastids.

The average number of chloroplasts per cell in epidermis, collenchyma and cortex tissues with 95% confidence intervals

Fig 1. Light and electron micrographs of *Xanthium pensylvanicum* stem. A – Light photomicrograph of a longitudinal section of *Xanthium pensylvanicum* stem with chloroplasts in epidermis (ep), collenchyma (col) and cortical parenchyma (cp). Bar = 50 μm . B – Transmission electron micrograph showing portions of two epidermal cells and one collenchymal cell in a longitudinal section of stem of *Xanthium pensylvanicum*. Two chloroplasts are evident (bar = 10 μm). C, D and E – Longitudinal sections through chloroplasts in epidermis (C), collenchyma (D) and cortical parenchyma (E) (m – mitochondrion, s – starch grain, bars = 1 μm). F – Scanning electron micrograph of a portion of stem with stomates (bar = 20 μm).

is listed in Table 1 for each species of the genera. In the sample of 10 randomly selected herbaceous stems, the smallest number of chloroplasts per cell was found in epidermis ranging from an average of 0.48 per cell in *Cucurbita* to 16.87 per cell in *Mentha*. The highest average number of chloroplasts in collenchyma (40.8 per cell) was found in *Lycopersicon* stem and in cortex tissue in *Cucurbita* stem (49.5). *Xanthium* stems were selected for more detailed studies because this plant has been the primary focus of research in our laboratory. Distribution of chloroplasts within stem cells of *Xanthium* plants is presented (Fig. 2) separately for epidermis, collenchyma and cortex. In the epidermis there were more than 20 cells in the class interval containing 0-5 chloroplasts, with about 40 cells containing 6-12 chloroplasts and a few cells which had 25-30 chloroplasts. The most frequent number (26-34) of chloroplasts was found in collenchyma cells. Cortex also had a high proportion of cells containing 20-29 chloroplasts.

Fig. 2. Distribution of chloroplasts in cells of epidermis, collenchyma and cortex (thin-walled parenchyma) in *Xanthium pensylvanicum* stem.

In order to ascertain the presence of stomates, paradermal stem sections of the investigated plants were examined by SEM and by preparation of aceto-orcein stained epidermal peels for light microscopy. Stomates were observed both by light and electron microscopy in all of the species. Estimates of the number of stomates per square millimeter made from scanning electron micrographs (170x) ranged from 3 in *Rubus* to 39 in *Xanthium*. Such counts were made difficult by the presence of waxes on the surface of the stems as exemplified by *Xanthium* (Fig. 1F).

DISCUSSION

Pearson and Lawrence (1958) made determinations of chlorophylls a and b content in aspen bark and leaves. Rates of photosynthetic activity were also ascertained. Since chlorophyll can only be found in chloroplasts in higher plants, it is reasonable to assume that aspen bark as well as leaves contains chloroplasts. However, no identification and electron microscopic studies of these chloroplasts were made. More recently, Nilsen and Bao (1990) measured stem and leaf photosynthesis in *Spartium junceum* (Spanish broom). Plants with darkened stems had fewer leaves and shortened stems as compared with controls. In addition the dry weights of leaves and shoots were lower in plants with darkened stems. Root weight was similarly affected. Their experiments support the hypothesis that photosynthesis of stem chloroplasts enhances survival and growth of herbaceous plants.

Chloroplasts in epidermis, collenchyma and cortex tissues of mature herbaceous stems in our studies were ellipsoidal discs approximately $5.18 \times 2.5 \mu\text{m}$. These dimensions agree with average chloroplast sizes reported in the literature for leaf cells. Clowes and Juniper (1968) gave an average chloroplast size of $5 \times 2 \mu\text{m}$ and Esau (1965) listed $4 \times 6 \mu\text{m}$. Linsbauer (1962) listed $5 \mu\text{m}$ as the average larger diameter of discoid shaped chloroplasts. Holowinsky et al. (1965) studied chloroplast size in relation to *Xanthium* leaf development. At maturity average chloroplast size was about $6 \mu\text{m}$. A change in the shape of plastids from circular to ellipsoid accompanied their growth.

These authors demonstrated the importance of stem photosynthesis by an 18% reduction in whole plant growth when stem photosynthesis was inhibited.

Data in Table 1 provide information with respect to chloroplast distribution in stem cells of 10 herbaceous dicotyledons. The following average numbers of chloroplasts per cell were estimated: epidermis – 5.7, collenchyma – 22.7 and cortex – 28.3. Other studies have centered on chloroplast distribution in leaf tissues. Linsbauer (1962) listed chloroplast numbers in mesophyll cells from leaves of 11 genera of herbaceous plants and estimated an average of 18 chloroplasts per cell for diploid species. Further studies by Ueda and Wada (1964) gave a range of 15-50 chloroplasts per mesophyll cell and 4-35 chloroplasts per leaf epidermal cell of investigated genera of vascular plants. Esau (1965) gave an average of 76 and 24 chloroplasts per cell in palisade and spongy mesophyll respectively. Lastly, Bartels (1964) found an average of 11.8 chloroplasts in *Epilobium hirsutum* leaf epidermis. Our data demonstrate substantial numbers of chloroplasts per cell in stem tissues of herbaceous dicotyledons which are within the lower range of those reported previously for leaves.

We determined that *Xanthium* stem epidermis had an average of 39 stomates per mm^2 . This number is small compared with the average 211 stomates/ mm^2 estimated from 24 dicotyledonous leaves in the abaxial epidermis by Morholt et al. (1958). The average density of stomates in *Xanthium* stem approximates more closely the average number of stomates (77) estimated by Morholt et al. (1958) in the upper leaf epidermis from a sample of 15 dicotyledonous plants. By fulfilling their physiological function of CO_2 supply, it is not unlikely that the stem stomates enhance the growth and possibly the survival of certain herbaceous plants by facilitating increased rates of photosynthesis. On the other hand, the number is so small in some of these plants that they may have little significance in photosynthesis.

ACKNOWLEDGMENTS

We thank Dr. M. Ledbetter, from Brookhaven National Laboratory and Dr. John G. Torrey, from Harvard University for their help in search of pertinent references. We also thank the W.M. Keck Foundation for support in purchasing a portion of the Electron microscopy equipment used in these studies.

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CHLOROPLASTY W TKANKACH NIEKTÓRYCH ROŚLIN ZIELNYCH

STRESZCZENIE

Seryjne skrawki dojrzałych łodyg dziesięciu gatunków dwuliściennych roślin zielnych badano mikroskopowo i oszacowano liczbę chloroplastów w komórce epidermalnej, kolenchymy i kory. Identyfikację chloroplastów przeprowadzono mikroskopem świetlnym i transmisyjnym. We wszystkich badanych łodygach stwierdzono obecność chloroplastów w tkance epidermalnej, kolenchymy i kory. Największą ich liczbę stwierdzono w skórze. Komórki kolenchymy zawierały największą liczbę plastydów w czterech z badanych rodzajów, podczas gdy sześć pozostałych gatunków największą liczbę plastydów zawierało w komórkach kory. Skrawki łodygi wszystkich rodzajów zawierały aparaty szparkowe, stwierdzone w łuskach epidermalnych barwionych orceiną, a także mikroskopem skaningowym.

SŁOWA KLUCZOWE: *Xanthium pensylvanicum*, zielne dwuliścienne, chloroplasty łodygi, aparaty szparkowe, mikroskopia skaningowa i trasmisyjna