HOSTED BY

ELSEVIER

CrossMark

ORIGINAL ARTICLE

Development of the successive cambia in *Sesuvium verrucosum* Raf (Aizoaceae)

Ola H. Abd Elbar

Agric. Botany Dept., Fac. Agric., Ain Shams Univ., 68 Hadayek Shoubra, 11241 Cairo, Egypt

Abstract The structure and development of successive cambia and its products were studied in the stems of the halophyte *Sesuvium verrucosum* Raf. The young stem has several collateral vascular bundles arranged in a circle and separated by interfascicular zones. The secondary thickening begins with the differentiation of fascicular and interfascicular cambia which give rise to secondary xylem, phloem and lignified cells. This normal cambium ceases to divide after a limited period of activity. A new segment of anomalous cambium was developed from the phloem parenchyma cells outside the normal previous cambium. These cells are served as a site for the origin of the anomalous cambium and subjected to repeated periclinal divisions. This cambium has fusiform cells with semi-storied appearance. The activity of this anomalous cambium produces secondary xylem, phloem, fibers and soft parenchyma as conjunctive tissues. The formation of subsequent cambia followed a similar pattern of development and causes vascular increments in the old stem. So, the old stem of *S. verrucosum* is constructed of concentric fibrovascular bands separated from each other by cylinders of conjunctive parenchyma tissue. This internal structure has a great adaptive potential to the halophyte *S. verrucosum*. This can be detected by the following points: (1) Production of large number of vessels and sieve tubes elements increases the conductive activity; (2) Occurrence of fibers alongside the vessels increases the mechanical strength that helps and protects water columns from embolism and ensures this water to store and transport in the succulent leaves; and (3) Thin walled parenchyma conjunctive tissue offers flexibility to the plant stem which forms a mat like and able to bend toward the ground without harm. Perhaps these features matched well with the *S. verrucosum* plant habitat.
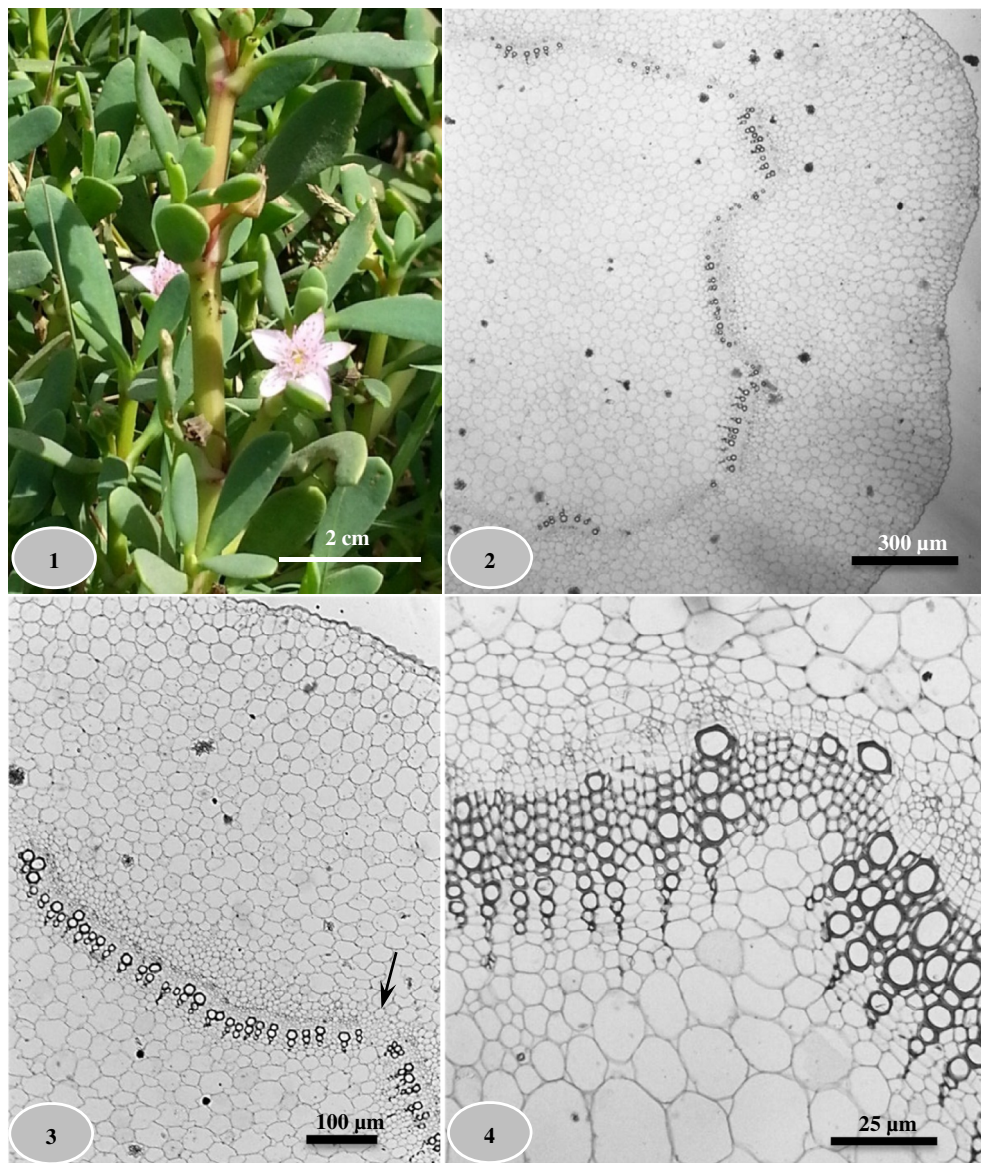
Introduction

The formation of Successive cambia was early recorded in different plants (Schenck, 1893; Pfeiffer, 1926). Recently, many authors studied the initiation and activity of these cambia, Fahn and Zimmermann (1982) in *Atriplex halimus*, Carlquist (2003, 2007a,b), Rajput et al. (2008) in some plants of

Figs. 1–4   Fig. 1: Morphology of *Sesuvium verrucosum* plant. Fig. 2: Transection in a young stem reveals its structure. Note the distribution of the vascular tissues, wide parenchymatous cortex and pith. Fig. 3: An enlarged view of Fig. 2 showing the primary vascular tissues. A narrow interfascicular region separating each two vascular bundles (arrow). Fig. 4: Development and activity of the fascicular cambium. Note some lignified cells produced from the interfascicular cambium.

Aizoaceae. This phenomenon was considered a characteristic feature of some families as Aizoaceae (Pax and Hoffman, 1934; Rao and Rajput, 1998; Carlquist, 2007a,b). It has been considered that, during the course of evolution, different groups of plants have undergone various modifications, which may be biochemical, morphological or structural. These modifications helped the plants to adapt to particular climatic or ecological conditions. Among these structural modifications the patterns of secondary thickening include formation of successive cambia, rayless xylem and paedomorphosis, and the formation of included phloem or of internal phloem Rajput et al. (2008). Stems and roots with successive cambia have great adaptive potential. The relative amounts of parenchyma, fibers, vessels, and sieve tubes can easily be reallocated by this ontogenetic system so as to provide more

mechanical strength, more flexibility, or more storage capacity. Aizoaceae have a wide range of diversity in this respect (Carlquist, 2007a). Therefore, studying successive cambia in Aizoaceae could potentially offer important information on this phenomenon. The present study aims to follow the initiation and the products of the different successive cambia in *Sesuvium verrucosum* and to explain the correlation between the pattern of secondary growth and habit of the plant as well as to elucidate its xylem structure.

Material and methods

Stems of various ages were collected from an identified population of *S. verrucosum* Raf growing in a greenhouse of Department of Agricultural Botany, Faculty of Agriculture,
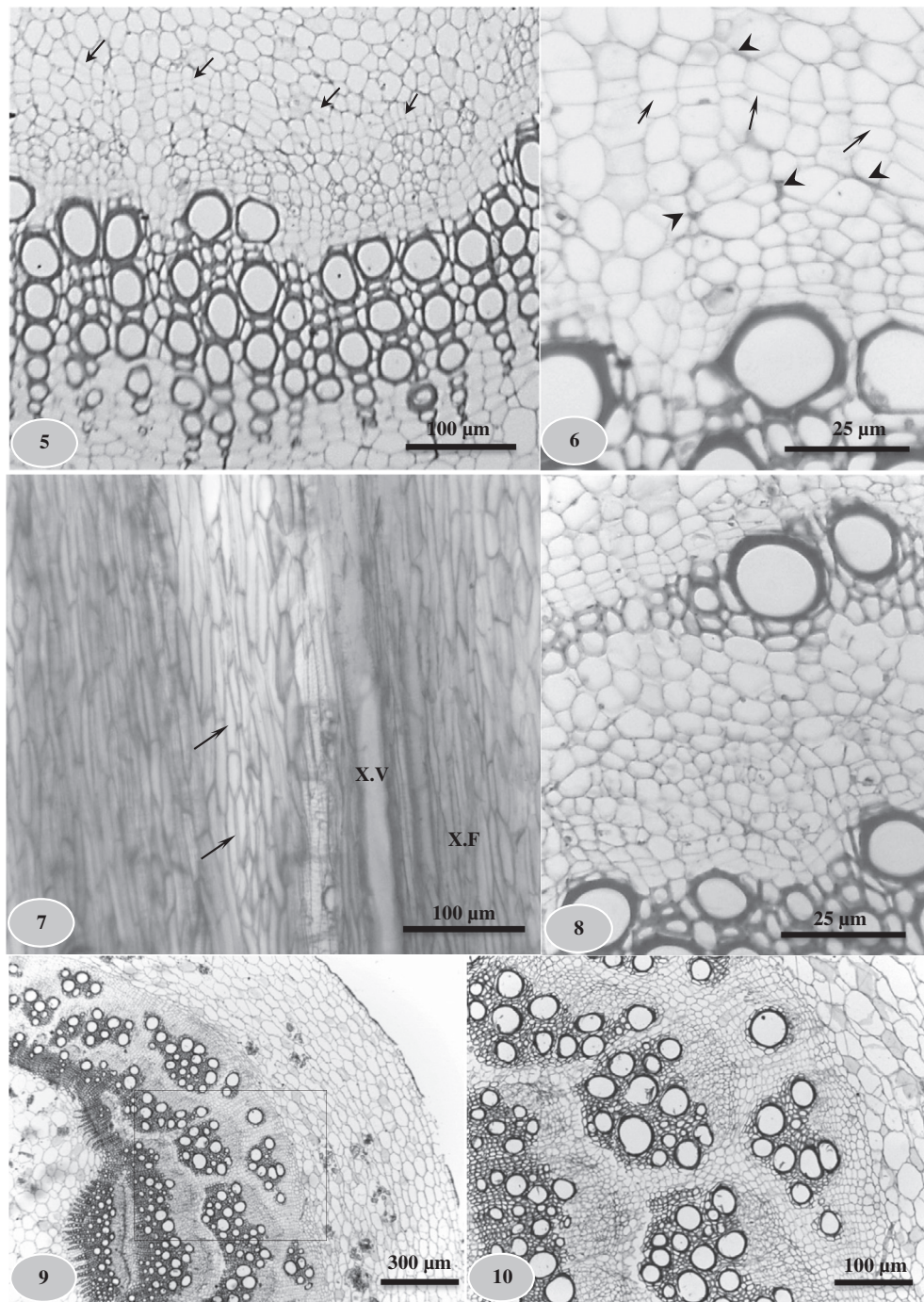
Figs. 5–10 Fig. 5: The initiation of the first anomalous cambium from the phloem parenchyma cells outside the normal cambium. Fig. 6: An enlarged view of Fig. 5 indicates to the periclinal divisions (arrows) and the arrowheads point to crushed sieve elements. Fig. 7: The semi-storied appearance of the vascular cambium as seen in tangential section (arrows). Fig. 8: Differentiation of some xylem, phloem elements and lignified cell from the first anomalous cambium. Figs. 9 and 10: Transections of old stem showing three increments of vascular strands embedded in parenchymatous conjunctive tissue. Dark spots in cortex and pith are druses. Note the variation in diameter between the secondary xylem vessels of the first and successive increments. Abbreviations: X.F = xylem fibers, X.V = xylem vessels.

Ain Shams University, Cairo, Egypt, during 2014. Segments (3–5 mm) were taken from the median part of the internode at different levels of the stem starting from the tip till the internodes adjacent to the ground level. Samples were fixed in FAA (formalin, acetic acid and 70% ethyl alcohol, 5:5:90/100 ml) for 24 h at room temperature. Then it dehydrated and processed using the schedule of the paraffin method. Transverse, tangential longitudinal sections (10–12 μm) in thickness were made by LEICA rotary microtome model RM 2125 RTS. Sections were stained with safranin fast green combination (Johansen, 1940). Pieces of the outermost xylem adjacent to the cambium of approximately
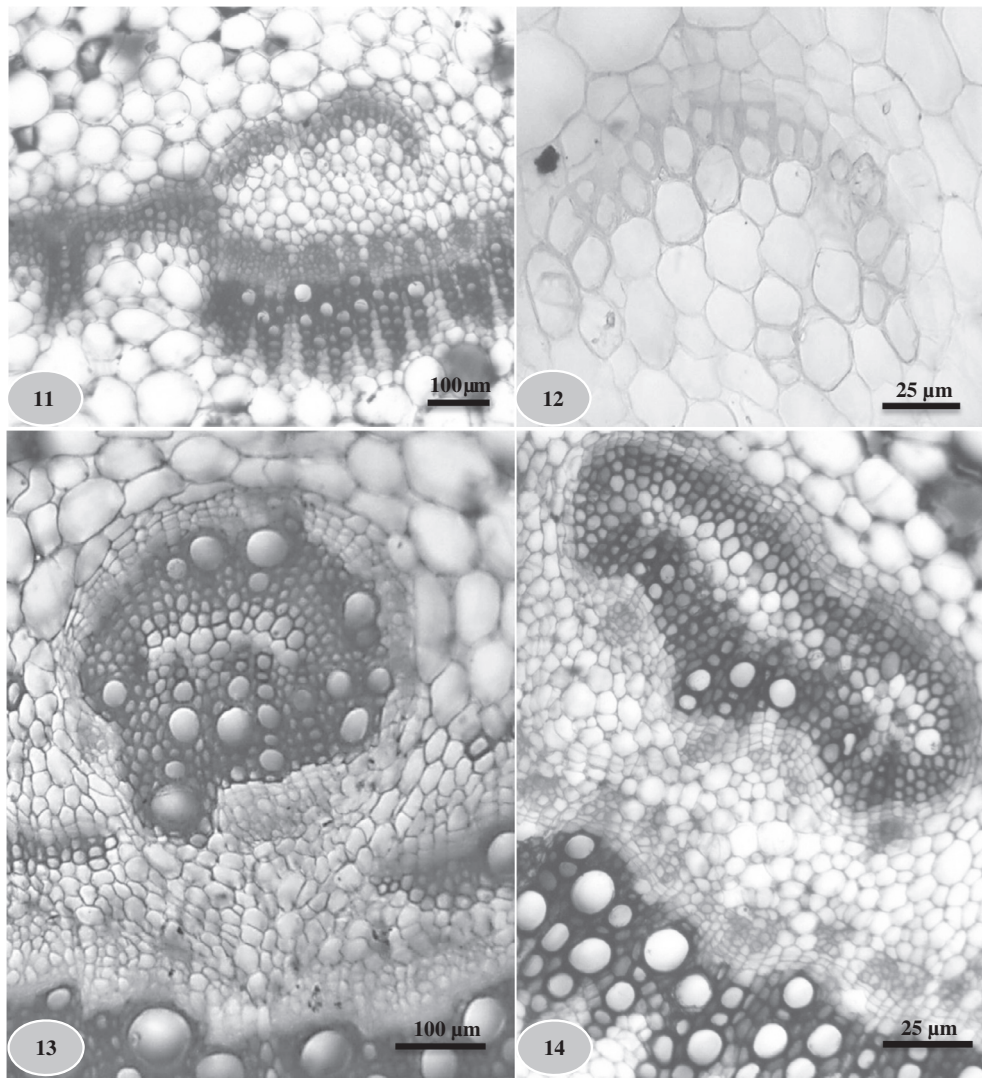
Figs. 11–14   Figs. 11 and 12: Initiation of small anomalous cambium rings. Figs. 13 and 14: Initiation of small anomalous cambium rings.

1-mm^2 thick were macerated using Jeffrey's solution (Berlyn and Miksche, 1976) at 55–60 °C for 24–36 h to study the general morphology and size of the vessel elements and fibers. Anatomical examination and measurements were achieved using a Leica light Research Microscope model DM-2500 supplied with a digital camera.

Results

Anatomy of the young stem

The halophyte *S. verrucosum* is a perennial herb with prostrate to erect stem forming mats of 2 m in length. It has simple opposite leaves with succulent texture (Fig. 1). The stem in transection has a circular outline. The structure of the young stem, in general, is similar to that of dicotyledons. It has one-layered epidermis covered with thick cuticle layer. The cortex consists of thin-walled large parenchyma cells. Several primary collateral vascular bundles surround a wide pith.

Two of them are wider than the others and occur slightly to the interior on both sides of stem. These collateral vascular bundles were separated by interfascicular zones (Figs. 2 and 3).

The secondary thickening and development of anomalous cambium

The secondary thickening begins with the differentiation of interfascicular cambia, connects with the fascicular one and joins the different bundles with each other. The activity of the previous normal cambium gives rise to secondary tissues. The differentiation of conducting elements of xylem and phloem remained restricted to the fascicular segments, in contrast to the interfascicular cambium which produces only thick-walled tissues centripetally and thin walled cells centrifugally (Fig. 4). This cambium ceases to divide after a limited period of activity. Then a new anomalous cambium developed from the parenchyma cells was derived from the normal one, the first developed cambium. These parenchyma cells are
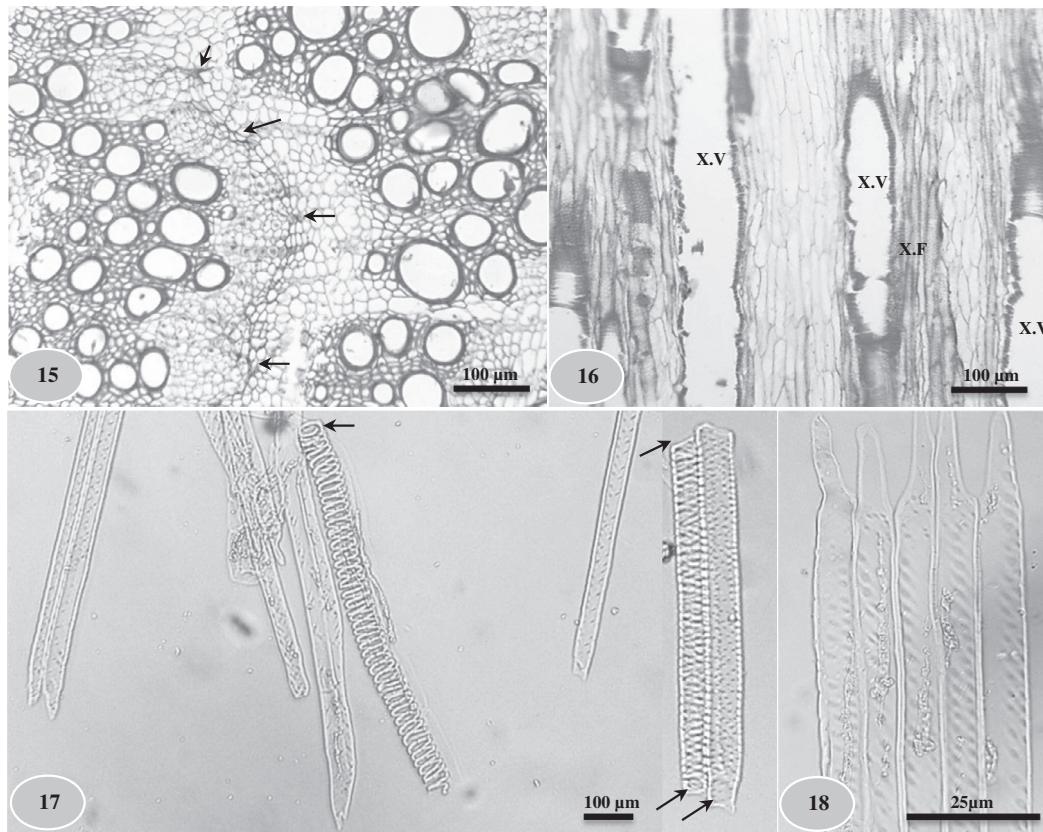
Figs. 15–18 Fig. 15: A portion of the old stem showing the crushing phloem elements (arrows). Fig. 16: Structure of xylem in tangential section. Fig. 17: Xylem vessel elements with spiral and pitted secondary walls and simple perforation plate (arrows). Fig. 18: Xylem fibers with thick pitted walls. Note the living protoplast. Abbreviations: X.F = xylem fibers, X.V = xylem vessels.

subjected to repeated periclinal divisions to form a wide band of cells. These cells served as a site for the origin of new anomalous cambium. This new cambium lies at a distance of about three to six cell layers outside the secondary phloem (Figs. 5 and 6). The cambium has semi-storied appearance in tangential section (Fig. 7).

These cambial strands extend tangentially forming two wide arcs occur perpendicular to the long diameter of the stem. The derivatives of the anomalous cambium are different. Some wide strips of these derivatives differentiate into conducting elements of secondary phloem and xylem (Fig. 8). These strips are 10–30 cells in width. The remaining narrow strips intervening the anomalous cambia produce a lateral meristem. This meristem gives rise to thin-walled conjunctive tissue centripetally and centrifugally forming radial files extending outwards. The formation of subsequent cambia followed a similar pattern of development. So, the old stem of *S. verrucosum* is constructed of concentric fibrovascular bands separated from each other by bands of conjunctive parenchyma tissue (Figs. 9 and 10). As the bands age, the earlier produced phloem becomes crushed (Fig. 15). The number of anomalous bands of vascular tissues was greater along the long diameter of the stem. Occasionally, an anomalous cambium appears as a small ring outside the previous bands. The rings divide producing secondary xylem inwards and secondary phloem outwards. These products appear as small circular or oblong cylinders (Figs. 11–14).

Structure of fibers and xylem elements

The xylem in tangential longitudinal sections consists of vessel elements, parenchyma and thick-walled cells (Fig. 16). The maceration of this tissue revealed that the thick-walled cells are fibers. The vessels have simple perforation plates. Their width ranged between 15 and 55 μm in diameter and between 120 and 200 in length. The width of the anomalous secondary vessels is larger than that of the vessels produced from the normal cambium and have spiral and pitted secondary walls (Fig. 17). Xylem fibers retained their living protoplast and sometimes few accumulate contents observed. It varied in length from 180 to 400 μm with pitted secondary walls with intrusive growth in their ends (Figs. 16 and 18).

Discussion

Stems and roots with successive cambia have great adaptive potential. The relative amounts of parenchyma, fibers, vessels, and sieve tubes can easily be reallocated by this ontogenetic system so as to provide more mechanical strength, more flexibility, or more storage capacity. Aizoaceae have a wide range of diversity in this respect (Carlquist, 2007a).

It was observed that the first cambium initiated and produced secondary vascular tissues as shown in the normal secondary thickening. The initiation of the first anomalous

cambium takes place in outer phloem parenchyma of the first vascular increments. Contrary to the present findings Kirchoff and Fahn (1984), Carlquist (2007b) reported that this cambium originates from cortical cells. The difference between the cortical cells and the phloem parenchyma is distinctly observed. The cortical cells are characterized by their large size while the phloem parenchyma cells are so smaller. In the present study, the crushed sieve elements of the primary phloem were easily observed on both sides of the first anomalous cambium (Figs. 5 and 6). Each new successive cambium has its origin from the outer derivatives of the preceding one. This was deduced from the arrangement of cells in radial files extending through the different increments of vascular tissues. Carlquist (2007b) considered the first anomalous cambium as a "master cambium" since its derivatives are the origin of the next cambium and the derivatives of the latter constitute the successive one and so on.

The increments of stem diameter of *S. verrucosum* caused by successive cambia activity produce numerous functional vascular strands scattered throughout the old stem. Thus, a much greater area of the studied stem is probably available for conduction by secondary phloem and secondary xylem than in a dicotyledon with a single cambium. So, the prolonged conductive activity in these vascular increments is increased. This is an adapted feature of the halophyte *S. verrucosum* which grows in saline habitat and subjected to water stress. This result is matched well with Hargrave et al. (1994) in *Salvia*.

Transections in *S. verrucosum* stem show that the fibers are often organized as sheaths around the individual vessels or intervened the clusters of vessels; perhaps this is a mechanism that helps in the protection of water columns from embolism. This result agrees with Carlquist (2007a) who suggested that the addition of fibers alongside vessels assures increase in mechanical strength as well as prolonging the activity of the vascular pathways formed earlier in the stem and safeguards the integrity of water columns by preventing rupturing of vessels which consider the primary function of these vessel sheaths. Jacobsen et al. (2005) found that the presence of fibers around vessels contributes to cavitation resistance. Maintenance of water column in stems of *S. verrucosum* is useful and necessary to ensure water transferring to storage in branches and leaves which characterized succulence. This result was confirmed by Grigore and Toma (2007) who observed that storage water in leaves is obviously a defensive strategy of halophytes. However, the preservation of water columns in perennial axes and the loss of which would result in significant diminution of the plant body are doubtless aided by the succulence of the stems. The plan of successive cambia, in which vascular strands can be scattered throughout a water storage structure, predisposes species with successive cambia to efficient storage plans, in which conducting strands are not far from any parenchyma cell in which water can be located Carlquist (2007b). Also, the same author found these

results in the roots of *Trichodiadema* and in both the roots and stems of *Marlothistella* (Aizocaeae).

The present results indicated that the conjunctive tissue disposed as soft parenchyma cells occurred between successive vascular increments and this offers flexibility to the sprawling stems of *S. verrucosum*. Carlquist (2007a) studied the structure of sprawling stems in *Aptenia*, *Carpobrotus*, and *Tetragonia* and observed that these plants have strong but very flexible stems which is constructed of concentric cylinder of alternating thin-walled and fibrous conjunctive tissue. The same author added that some of these genera grow on sand dunes or other places subject to soil level shift. Another correlation of this kind of stem structure in Aizoaceae is with the tendency of sprawling plants in this family to be able to bend toward the ground without damage and thereby reroot.

References

Berlyn, G.P., Miksche, J.P., 1976. Botanical Microtechnique and Cytochemistry. The Iowa State Univ. Press, Ames, Iowa, p. 326.

Carlquist, S., 2003. Wood and stem anatomy of woody Amaranthaceae (*sensu stricto*) and the problem of defining rays. Bot. J. Linn. Soc. 143, 1–19.

Carlquist, S., 2007a. Successive cambia in Aizoaceae: products and process. Bot. J. Linn. Soc. 154, 141–155.

Carlquist, S., 2007b. Successive cambia revisited: ontogeny, histology, diversity and functional significance. J. Torrey Bot. Soc. 134, 301–332.

Fahn, A., Zimmermann, M.H., 1982. Development of successive cambia in *Atriplex halimus* (Chenopodiaceae). Bot. Gaz. 143, 353–357.

Grigore, M.N., Toma, C., 2007. Histo-anatomical strategies of Chenopodiaceae halophytes; adaptive, ecological and evolutionary implications. WSEAS Trans. Biol. Biomed. 12 (4), 204–218.

Hargrave, K.R., Kolb, K., Ewers, F.W., Davis, S.D., 1994. Conduit diameter and drought-induced embolism in *Salvia mellifera* Greene (Labiatae). New Phytol. 126, 695–705.

Jacobsen, A., Ewers, F.W., Pratt, R.B., Paddock III, W.A., Davis, S.D., 2005. Do xylem fibers affect cavitation resistance? Plant Phys. 139, 546–556.

Johansen, D.A., 1940. Plant Microtechnique. McGraw-Hill Book Co., New York, pp. 126–156.

Kirchoff, B.K., Fahn, A., 1984. Initiation and structure of the secondary vascular system in *Phytolacca dioica* (Phytolaccaceae). Can. J. Bot. 62, 2580–2586.

Pax, F.R.K., Hoffman, K., 1934. Aizoaceae, second ed. In: Engler, A., Harms, H. (Eds.), In: Die Naturlichen Pflanzenfamilien, vol. 16C Wilhelm Engelmann, Leipzig.

Pfeiffer, H., 1926. Das abnorme Dickenwachstum, second ed. In: Hand-buch der Pflanzenanatomie, vol. 9 Gebrüder Borntraeger, Berlin, pp. 1–272.

Rajput, K.S., Patil, V.S., Shah, D.G., 2008. Formation of successive cambia and stem anatomy of *Sesuvium sesuvioides* (Aizoaceae). Bot. J. Linn. Soc. 158, 548–555.

Rao, K.S., Rajput, K.S., 1998. Rayless secondary xylem of *Trianthema monogyna* (Aizoaceae). Phyton-Horn 37, 161–166.

Schenck, H., 1893. Beitriige zur Biologic und Anatomie der Lianen. II. Schimpers. Biol. Mit-theil. Trop. 5, 1–271.