Structure of Shoot Apical Meristem and Leaf Development in Seedlings of *Thuja orientalis* L.

Hong, Sung Sik and Woong Young Soh

Department of Biology Education, Chonnam National University, Kwangju
1 Department of Biology, Chonbuk National University, Chonju, Korea

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Abstract

The structure and leaf development of the shoot apical meristem in embryos and seedlings of *Thuja orientalis* L. were observed by using SEM, and the area of the shoot apical meristem was studied by an image analyzer. The shoot apex of embryos was of a round tip cone shape with flat sides; it was transformed into an inverted boat shape. In seedlings, the shoot apical meristem was of a convex-rectangular shape during the early stages of development of two decussate 1st leaf primordia; but in the later stage of the development, it turned into a flat square shape. The shoot apical meristem was of a convex diamond shape during the early development of whorled four 2nd leaf primordia; but it turned into a flat diamond shape in the later stage of this process. In the early development of the 3rd leaf primordia, it was transformed into a convex square shape. Both the minimal and maximal areas of the shoot apical meristem gradually decreased as the plant developed from an embryo into a seedling. In seedlings, the 1st leaves showed a decussate phyllotaxis, while the 2nd leaves and later leaves all showed a four-leaf whorl phyllotaxis. All the leaves developed into needles.

Key words: Shoot apical meristem, Leaf primordium, Phyllotaxis, *Thuja orientalis* L.

Most research on the shoot apical meristem of the seed plants has been carried out by median longisectional views (Sussex, 1955; Tolbert, 1961; Gifford and Corson, 1971; Gregory and Romberger, 1972), because this method afforded an easy distinction of characteristically arranged groups of cells. A serial transectional view made it possible easily to trace the cell lineage of the tunica layer and the initiation of successive leaf primordia in the apical region (Hara, 1961, 1971a,b, 1991). However, a comprehensive understanding of the organization and of leaf primordia initiation of the shoot apical meristem was strongly called for three dimensional analysis (Hara, 1971b, 1980, 1991, 1995).

In the meantime, a stereomicroscopic observation revealed that various changes arose morphologically in the shoot apical meristem of the seed plants (Hara, 1980). It has been pointed out that (i) there are prominent differences in the shapes and sizes of the shoot apical meristems of the dicotyledons though they were in somewhat convex shapes (Fahn, 1982) and that (ii) the shapes of shoot apical meristems also showed differences depending on the phase of their plastochron (Sterling, 1945; Tolbert, 1961; Hara, 1980, Fahn, 1982).

Since the shoot apical meristem of the gymnosperms was explained by the cytohistological
zonation through a median longisectional view of *Ginkgo biloba* (Foster, 1938), lots of research has been carried out about it (Foster, 1939a, b, 1940, 1943; Cross, 1943; Parke, 1959; Tepper, 1963; Vanden Born, 1963; Fosket and Mische, 1966; Hanawa, 1966). But little results are known of the research on the shoot apical meristem of the gymnosperms in terms of its three dimensional structure. Therefore, a developmental morphological study on the structure and leaf development of the shoot apical meristem of the gymnosperms is requested.

In the present study, the results of an SEM observation of the structure and leaf development of the shoot apical meristems in embryo and early seedlings of *Thuja orientalis* L. will be reported.

**Materials and Methods**

Seeds of *Thuja orientalis* L. were collected on the campus of Chonnam National University in Kwangju, Republic of Korea in October, 1993. Out of them, seeds of weights heavier than 17 mg/piece were chosen for examination. These seeds were sown in pots with sand and placed and grown in a growth chamber. The growth chamber was conditioned to be lighted for 16 hours (3,000 Lux, 25±1°C) and be left dark for 8 hours (20±1°C) each day, and the moisture was maintained at 60~70% of relative humidity. Plants for examination were collected by 30 pieces, respectively, on the 9th, 11th, 14th, 17th and 20th days after sowing, and the growth of plumule, hypocotyl, root and cotyledons was checked.

For SEM observations the materials were killed and fixed in Craf III, dehydrated in t-butanol, dried in critical-point dryer and coated with gold. The microphotographs were prepared with SEM (ISI Model SR-50) at 10Kv. The diameters of long and short axes and the minimal and maximal areas of the shoot apical meristem were respectively measured from 5 individuals by using an image analyzer (IBAS 2000, KONTRON).

**Results**

*Thuja orientalis* L. bloomed in March or April and its seed fully riped in September. From mature embryos in seeds to young seedlings, observations were divided in convenience into four developmental stages of the shoot apical meristem: (i) shoot apex of embryo, (ii) development of 1st leaf primordia, (iii) development of 2nd leaf primordia and (iv) development of 3rd leaf primordia.

**Shoot Apex of Embryo:** About 9 days after sowing, some of the seeds had ruptured seed coats and some not. Embryos were protected by the female gametophyte inside seeds. The lengths of two cotyledons were about 2.2 mm, and those of the plumule, hypocotyl and radicle were about 36 µm, 2 mm and 1 mm, respectively (Table 1). The plumule was projected vertically between the two cotyledons. Among the materials collected around this period, seeds with unruptured seed coat marked the intermediate area phase of the shoot apices of the embryos inside them (Fig. 2), while those with ruptured seed coat marked the maximal area phase (Fig. 3). The minimal area phase was presumed the early stage of development of two cotyledon primordia.

The diameters of the long and short axes of the shoot apex at the intermediate area phase in superficial view were approximately 330 µm and 77 µm, respectively; and the area was about 25,503 µm² (Table 2). The three dimensional structure of the shoot apex at this stage showed a round tip cone shape with flat sides (Fig. 2). The superficial view of the shoot apex resembled the shape of a convex lens (Fig. 1A), and the side view from the direction of the cotyledons showed a curve...
with a convex center (Fig. 1A').

The diameters of the long and short axes of the shoot apex at the maximal area phase were about 430 μm and 105 μm, respectively; and the area amounted to 40,625 μm² (Table 2). Compared with the intermediate area phase, the shoot apex increased about 100 μm in the long axis, about 28 μm in the short axis and about 15,122 μm² in the area. The three dimensional structure of the shoot apex was of an inverted boat shape which was resulted from the enlargement of both ends of the long axis until they became as high as the central component of the three dimensional shape (Fig. 3). The superficial view of the shoot apex showed a boat shape (Fig. 1B), and the side view from the direction of the cotyledons yielded a trapezoid (Fig. 1B').

Development of 1st Leaf Primordia: When measured on the 11th day after sowing, the cotyledons were 3.3 mm long showing a minor growth. However, the plumule, hypocotyl and radicle rapidly grew and their lengths reached 92.1 μm, 5.2 mm and 4.0 mm, respectively (Table 1). During this period, the female gametophyte surrounded by seed coat was stuck to the cotyledons of seedlings, and the upper part of the hypocotyl was sinuous. The 1st leaf primordia were decussately developed from the two leaf buttresses formed at both ends of the long axis of the shoot apex at the embryo stage (Fig. 4). As a result, the contours of the shoot apical meristem were clearly distinguished, and surface cells on the border of the 1st leaf primordia and the shoot apical meristem continued a series of anticlinal divisions in a vertical direction.

The diameters of the long and short axes of the shoot apical meristem at the minimal area phase in superficial view were about 156 μm and 141 μm, respectively; and the area was about 21,027 μm² (Table 2). The minimal area of the shoot apical meristem at this period was reduced by 19,598 μm² which was about half the size of the maximal area at the embryo stage. The long axis of the shoot apical meristem was reduced by some 274 μm than that in the case of the maximal area phase at the embryo stage, whereas the short axis grew bigger by some 36 μm. The three dimensional structure of the shoot apical meristem at its minimal area phase was of a convex-rectangular shape (Fig. 4); the superficial view looked like a rectangle (Fig. 1C); and the side view from the direction of the cotyledons looked like a somewhat convex curve (Fig. 1C').

The diameters of the long and short axes of the shoot apical meristem at its maximal area phase were about 160 μm and about 159 μm, respectively; and the area amounted to 25,630 μm² (Table 2). Compared with the minimal area phase, almost no change was found in the long axis of the shoot apical meristem, but the short axis grew bigger by some 18 μm and, therefore, the area was by 4,603 μm². The three dimensional structure of the shoot apical meristem at its maximal area phase showed a flat square shape (Fig. 5); the superficial view looked like a square (Fig. 1D); and the side view from the direction of the cotyledons looked like a trapezoid curve (Fig. 1D').

Development of 2nd Leaf Primordia: Fourteen days after sowing, cotyledons and plumules respectively approximated to 7.9 mm and 235 μm which reflected a rapid growth (Table 1). During this period, the female gametophyte is separated from cotyledons, and the upper part of the hypocotyl was either bent in a right angle or erected upright. A pair of 1st leaf primordia was also extended further.

The 1st leaf primordia developed surrounding the shoot apical meristem at the minimal area phase. The minimal area of the shoot apical meristem was about 13,560 μm², and the diameters of long and short axes were about 118 μm and about 116 μm, respectively (Table 2). Compared with the maximal area phase of the 1st leaf primordia, the area and long and short axis of the shoot apical meristem were all decreased, and they were about 12,070 μm², 42 μm and 43 μm, respectively (Table 2). The three dimensional structure of the shoot apical meristem showed a
convex diamond shape (Fig. 6). The superficial view was of a diamond shape (Fig. 1E) and the side view from the direction of the cotyledons showed a curve with a convex center (Fig. 1E').

The area of the shoot apical meristem at the maximal area phase was 16,420 $\mu$ m$^2$, and the diameters of long and short axes were about 128 $\mu$m and 127 $\mu$m, respectively (Table 2). Compared with the cases at the minimal area phase, the area, long axis and short axis all grew bigger, and they were 2,860 $\mu$m$^2$, 10 $\mu$m and 11 $\mu$m, respectively. Four axes were clearly formed along with the extension of the four 2nd leaf primordia in a whorl phyllotaxis and the protrusion of the shoot apical meristem. The three dimensional structure of the shoot apical meristem showed a flat diamond shape (Fig. 7), which was resulted from the protrusions of the components around the shoot apical meristem. The superficial view showed a diamond shape (Fig. 1F), and the side view from the direction of the cotyledons showed a trapezoid curve (Fig. 1F').

Development of 3rd Leaf Primordia: Seventeen days after sowing, the plumule, cotyledons, hypocotyl and root were all rapidly growing in seedlings and they were 532 $\mu$m, 14.9 mm, 38.7 mm and 40.9 mm long, respectively (Table 1). The hypocotyl was upright and cotyledons were spread out.

During the minimal area phase of the shoot apical meristem, four 3rd leaf primordia were almost simultaneously developed in whorl from the four corners of the convex diamond shape at the maximal area phase of development of the 2nd leaf primordia (Fig. 8). The area of the shoot apical meristem at this period was about 12,490 $\mu$m$^2$, and the diameters of long and short axes were about 111 $\mu$m and about 110 $\mu$m, respectively (Table 2). Compared with the maximal area phase of development of the 2nd leaf primordia, the area got smaller by 3,930 $\mu$m$^2$ and both long and short axes were reduced by 17 $\mu$m. The area reduced by 1,070 $\mu$m$^2$ when it was compared with that at the minimal area phase of development of the 2nd leaf primordia. The three dimensional structure of the shoot apical meristem showed a convex square shape (Fig. 9). The superficial view was of a square shape (Fig. 1G) and the side view from the direction of the cotyledons was of a curve with a somewhat convex center, showing similarity to that at the minimal area phase of development of the 2nd leaf primordia (Fig. 1G'). The form of the shoot apical meristem of seedlings alternated to a diamond shape, and, further, to a square shape. Thus, since the whorl development of four leaf primordia continues ever. All decussate or whorled leaf primordia of seedlings developed into needles as they grew further (Fig. 9).

Discussion

In the seed plants, the shoot apical meristem of vegetative shoot is generally of a dome shape (Hara, 1961), but it was pointed out that its shape differed depending on the plastochron (Sterling, 1945; Tolbert, 1961; Fahn, 1982). This phenomenon was similar to the developmental process of the shoot of Aucuba viewed in three dimension (Hara, 1980). The shape of the shoot apical meristem of Aucuba changed into a flat shape, concave shape and dome shape in successive developmental stages of foliage leaves, scale leaves and bracts. In Thuja orientalis, the three dimensional structure varied as the plant developed from the embryo stage to seedlings. The shoot apex of the embryo showed a round tip cone shape with flat sides; but the shape turned into an inverted boat shape (Fig. 3). This phenomenon was explained in terms of the fact that the shoot apex lied between the two cotyledons and developed in parallel with them. At the minimal area phase of the 1st leaf plastochron, or at the early stage of development of two 1st leaf primordia, the shoot apical meristem showed a rectangular shape with a convex center; at the maximal area phase of the 1st leaf plastochron, or the late stage of development of 1st leaf primordia, it turned into a flat square shape. The transformation of an inverted boat shape to a convex rectangular one at the
The three dimensional view of the shoot apical meristem was of a convex diamond shape at the early stage of development of four whorled 2nd leaf primordia, and it later changed to a flat diamond shape at the late stage of development of 2nd leaf primordia. This phenomenon seems to have resulted from the almost simultaneous initiation of four 2nd leaf primordia from the four corners of the flat square shape. At the following early stage of development of four 3rd leaf primordia, the shoot apical meristem further changed to a convex square shape which was resulted from the whorl initiation of four 3rd leaf primordia from the four corners of the flat diamond shape. After that, the shape of the shoot apical meristem alternated to a diamond shape and, further to a square shape according to initiation of leaf primordia. On the other hand, the superficial view of the shoot apical meristem from the embryo stage to seedlings changed in the order of a convex lens shape, a boat shape, a rectangular shape, a square shape, a diamond shape and then back to a square shape. When it was seen in the side view from the direction of the cotyledons, the shoot apical meristem at all stages showed a convex curve during the minimal area phase of plastochron and a curve with a flat center during the maximal area phase, showing a difference from Sequoia (Sterling, 1945). In the case of Sequoia (Sterling, 1945), the median longitudinal view of the shoot apical meristem normally forms a parabola, but it also changed into a half-circle and triangle depending on plastochron. The shoot apical meristem of Thuja orientalis showed a significant structural changes in close relationships with the position and the number of leaf primordia and plastochron.

The area of the shoot apical meristem has a close relationship with plastochron (Hara, 1980); normally, the maximal area phase happened just before the initiation of leaf primordia and the minimal area phase happened after the initiation. The area of the shoot apical meristem of Thuja orientalis from its embryo state to seedlings marked the biggest; the area then gradually got smaller with initiation of two 1st, four 2nd and four 3rd leaf primordia in the given order.

In many dicotyledons, the diameter of the shoot apical meristem usually ranges from 130-200 μm. The diameter of conifers is similar to that of dicotyledons (Fahn, 1982), but that of some gymnosperms was reported to be normally bigger than that of dicotyledons (Kemp, 1943). At the maximal area phase in the 1st leaf plastochron of Thuja orientalis, long and short axes of the shoot apical meristem in superficial view were 160 and 159 μm respectively; at the maximal area phase in the 2nd leaf plastochron, they were 128 and 127 μm, respectively; and at the minimal area phase in the 3rd leaf plastochron, they were 111 and 110 μm, respectively, which proved to be smaller than those of dicotyledons.

The seed plants are known to have their own original forms of phyllotaxis. In the case of Thuja orientalis, the 1st leaves of seedlings had a decussate phyllotaxis, while the 2nd leaves and later leaves had a four-leaf whorled phyllotaxis. Though the 1st decussate leaves of seedlings were big, they all developed into needles later. The result of the observation of the present study has almost the same with the report that, the 1st and 2nd leaves of seedlings were of a decussate phyllotaxis and the 3rd and 4th leaves were of a whorled phyllotaxis in Thuja occidentalis L., although it was not based on developmental examination (Yamanaka, 1975).

A continuous and further study is expected to be carried out, in terms of developmental morphology, on the structure of the shoot apical meristem, leaf development and axillary bud development in the seedlings and the adult plants of Thuja orientalis.
Acknowledgement

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References


Fig. 1. Diagrams showed the changes in structure of shoot apical meristems in embryos and seedlings of *Thuja orientalis*. A-G. Superficial views. A'-G'. Side views from the direction of cotyledon at the same stages of A-G. A. Plumule apex of embryo showed convex lens shape. A'. Curve with a convex center. B. Shoot apex of embryo at maximal area phase in ruptured seed coat showed boat shape. B'. Curve with a trapezoid. C. Shoot apical meristem at minimal area phase of development of 1st leaf primordia showed rectangular shape. C'. Convex curve. D. Shoot apical meristem at maximal area phase of development of 1st leaf primordia showed square. D'. Curve with a trapezoid. E-F. Shoot apical meristems at minimal and maximal area phases of development of 2nd leaf primordia showed both diamond shapes. E'. Convex curve. F'. Curve with a flat center. G. Shoot apical meristem of minimal area phase of development of 3rd leaf primordia showed square. G'. Convex center. C; cotyledon, SA; shoot apical meristem, 1LP; 1st leaf primordia, 2LP; 2nd leaf primordia, 3LP; 3rd leaf primordia, 1L; 1st young leaf.

Figs. 2-9. SEM micrographs of shoot apical meristems in embryos and seedlings of *Thuja orientalis*. Fig. 2. Shoot apex of embryos with intermediate area showed round tip cone shape with flat sides. Embryo collected on 9th day after sowing. Fig. 3. Shoot apex of embryo with maximal area in ruptured seed coat showed inverted boat shape. Fig. 4. Shoot apical meristem of seedling with minimal area at the stage of development of 1st leaf primordia showed convex rectangular shape. Two-1st leaf primordia differentiated a decussate phyllotaxis. Seedling collected on 11th day after sowing. Fig. 5. Shoot apical meristem with maximal area at the stage of development of 1st leaf primordia showed flat square shape. Fig. 6. Shoot apical meristem of seedling with minimal area at the stage of development of 2nd leaf primordia showed convex diamond shape. Four-2nd leaf primordia differentiated a whorled phyllotaxis. Seedling collected on 14th day after sowing. Fig. 7. Shoot apical meristem with maximal area at the stage of development of 2nd leaf primordia showed flat diamond shape. Fig. 8. Shoot apical meristem of seedling with minimal area at the stage of development of 3rd leaf primordia showed convex square shape. Four-3rd leaf primordia differentiated a whorled phyllotaxis. Seedling collected on 17th day after sowing. Fig. 9. All decussate or whorl leaf primordia developed into needles. Seedling collected on 20th day after sowing.

Bars = 100 µm. C; cotyledon, 1; 1st leaf primordia, 2; 2nd leaf primordia, 3; 3rd leaf primordia, 1L; 1st young leaf.
Table 1. Measurement of embryo and during growth of seedlings of *Thuja orientalis* measured with 30 seedlings, respectively

<table>
<thead>
<tr>
<th>Stage(days)</th>
<th>Measurement</th>
<th>Plumule (µm)</th>
<th>Hypocotyl (mm)</th>
<th>Root (mm)</th>
<th>Cotyledon (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo(9)</td>
<td>36.17 ± 6.72</td>
<td>2.07 ± 0.75</td>
<td>1.07 ± 0.31</td>
<td>2.21 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>1st leaf primordia initiation(11)</td>
<td>92.15 ± 11.17</td>
<td>5.25 ± 1.62</td>
<td>4.03 ± 1.99</td>
<td>3.36 ± 0.71</td>
<td></td>
</tr>
<tr>
<td>2nd leaf primordia initiation(14)</td>
<td>235.78 ± 50.78</td>
<td>18.73 ± 4.54</td>
<td>25.22 ± 5.12</td>
<td>7.92 ± 2.38</td>
<td></td>
</tr>
<tr>
<td>3rd leaf primordia initiation(17)</td>
<td>532.34 ± 60.1</td>
<td>38.71 ± 7.56</td>
<td>40.92 ± 8.15</td>
<td>14.91 ± 2.75</td>
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</tr>
</tbody>
</table>

Table 2. Structural change of shoot apical meristem of embryos and seedlings during growth of *Thuja orientalis*

<table>
<thead>
<tr>
<th>Stage</th>
<th>Measurement</th>
<th>Area(µm²)</th>
<th>Diameter(µm)</th>
<th>Three Dimensional Shape</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Long Axis</td>
</tr>
<tr>
<td>Embryo</td>
<td>25,503 ± 2,250</td>
<td>40,625 ± 2,330</td>
<td>330 ± 20</td>
<td>77 ± 6</td>
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<tr>
<td>1st Leaf Primordia</td>
<td>21,027 ± 2,320</td>
<td>25,630 ± 2,400</td>
<td>156 ± 14</td>
<td>141 ± 10</td>
</tr>
<tr>
<td>2nd Leaf Primordia</td>
<td>13,560 ± 1,150</td>
<td>16,420 ± 1,420</td>
<td>118 ± 10</td>
<td>116 ± 9</td>
</tr>
<tr>
<td>3rd Leaf Primordia</td>
<td>12,490 ± 1,050</td>
<td>111 ± 9</td>
<td>110 ± 8</td>
<td>Convex Square</td>
</tr>
</tbody>
</table>

*Intermediate area: the minimal area phase was presumed the early stage of development of two cotyledon primordia.*