*Proc. Indian natn. Sci. Acad.*
Vol. 43, Part B, No. 3, pp. 57–65 (1977)

# EMBRYOGENY OF PINACEAE

## II. LATE EMBRYOGENY IN NON-CLEAVAGE FORMS : *Abies pindrow* and *Picea smithiana*

*by* P. N. MEHRA, FNA, *and* P. D. DOGRA,* *Department of Botany, Punjab University, Chandigarh* 160 014

(*Received* 25 *June*, 1977)

The embryogeny of *Abies pindrow* differs from that of *Picea smithiana* and other genera of Pinaceae in the structure of the suspensor system which lacks $E_2$, $E_3$, etc., segments. $E_1$ elongation in *Abies* is followed directly by $E_t$. *Abies* is a non-cleavage form showing instability of non-cleavage expressed as frequent unitary lobing, persistent unitary lobing of the terminal embryo and as partial cleavage occurring in 15% of cases. *Picea smithiana* shows very little unitary lobing especially in late stages of development. Separation of lobes or partial cleavage is not common. *Picea* therefore differs from *Abies* in showing stability in its non-cleavage character. The embryogeny of *Abies* and *Picea* are summarised by formulae given below :

*Abies :*

$USE_1$................ $E_t$ ................(e) 85%

$USE_1$................)$E_t$(................ e 15%

*Picea :*

$USE_1$... $E_2$... $E_3$... $E_4$.... $E_t$... (e)

## INTRODUCTION

*Abies* and *Picea* are non-cleavage forms (Buchholz, 1920), but Hutchinson (1924) discovered cleavage to occur in 10% of cases in *Abies balsamea*. Sugihara (1947) recorded 20% of cases in *Abies firma* in which separation of embryonic cell masses occurred in the embryo. Buchholz (1920, 1942) studied whole-embryo mounts and demonstrated that embryos of *A. pinsapo*, *Picea abies*, *P. mariana*, *P. smithiana* and *P. omorika* do not show cleavage. He showed that the embryo of *Abies* differs from that of *Picea* in showing dormancy of terminal embryonal tier during $E_1$ (substitute suspensor) elongation and that the early suspensor system in *Picea* is segmented. Hakanson (1956) studied some stages of late embryogeny of *Picea abies*. Studies on disturbances in late embryogeny in relation to seed sterility were made for *Abies pindrow*, *Picea smithiana* and for some other members of Pinaceae by Dogra (1967).

The proembryogeny of *Abies pindrow* and *Picea smithiana* has been dealt with in a morphological study of the proembryo of Pinaceae (Mehra & Dogra, 1975). The late embryogeny of these two species is presented here and their cleavage

**Present address :* Tree Genetics Laboratory, National Botanic Gardens, Lucknow.

behaviour in relation to suspensor system analysed. The terms used for embryogeny are those proposed by Dogra (1967).

## Material and Methods

Ovules of *Abies pindrow* (Royle) Spach. and *Picea smithiana* (Wall.) Boiss. were fixed in 1 : 3 acetic alcohol during May to July every year from 1954 to 1960 and preserved in 75% alcohol. Acetocarmine in glycerine was used to stain and whole-embryos dissected from ovules as shown by Buchholz (1918, 1938). Cavity slides were used for the study of cleavage behaviour of whole-embryo mounts to avoid mechanical pressure on the embryos.

## Observations

### *Late Embryogeny*

#### *Abies pindrow*

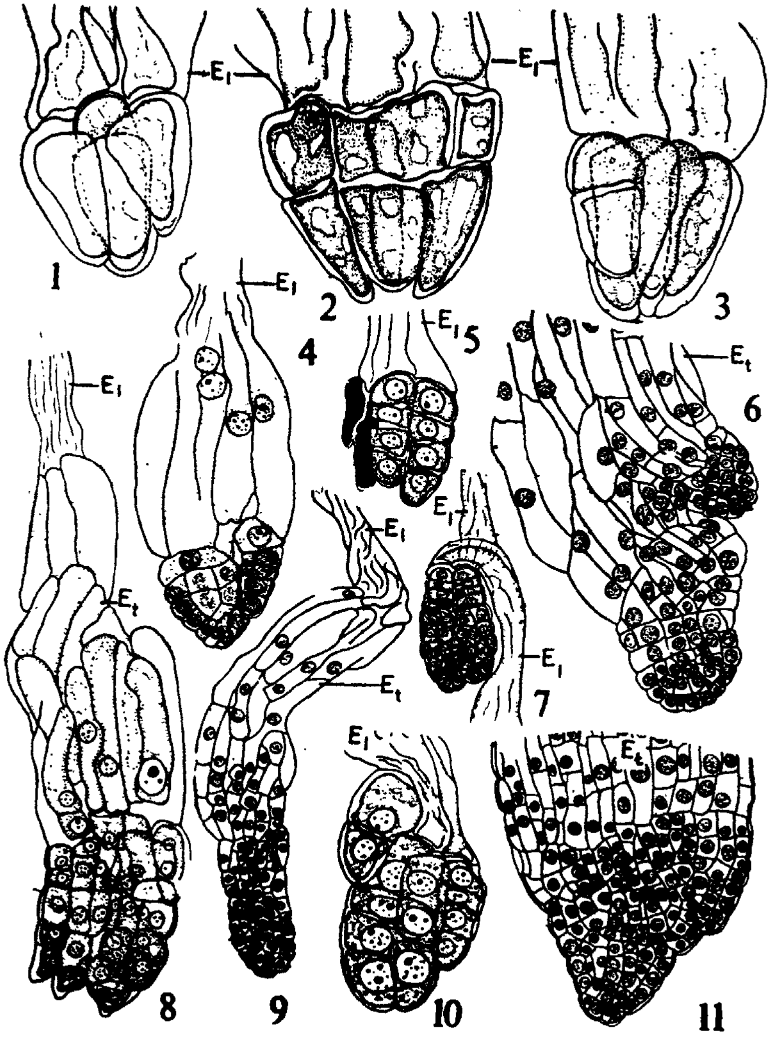
The suspensor S-tier (earlier known as rosette tier) is non-functional. The substitute suspensor $E_1$ is conspicuously long in all the embryos. The terminal embryonal cells remain dormant and do not divide during $E_1$ elongation (Fig. 1). As soon as this elongation is completed the four terminal embryonal cells originating from the four primary embryonal cells of the primary proembryo start dividing. The embryos at this stage fall in two separate groups:

*Group* 1 : The embryos in which all the four terminal cells divide simultaneously at the same levels by transverse walls. These initially contribute equally to the uncleaved terminal embryonal mass (Fig. 2, 8). In early stages the growth of each unit thus formed is linear and the four units are easily discernible from each other in the terminal uncleaved embryonal group of cells (Fig. 8). Later these units change from a linear to a multi-dimensional growth pattern and after many cell divisions the identity of the 4 units is lost (Fig. 9). The proximal cells of the whole compact embryonal mass then elongate to form an $E_t$ elongation (Fig. 9). The embryonal mass forms an embryo which differentiates into a mature polycotyledonous embryo of the seed.

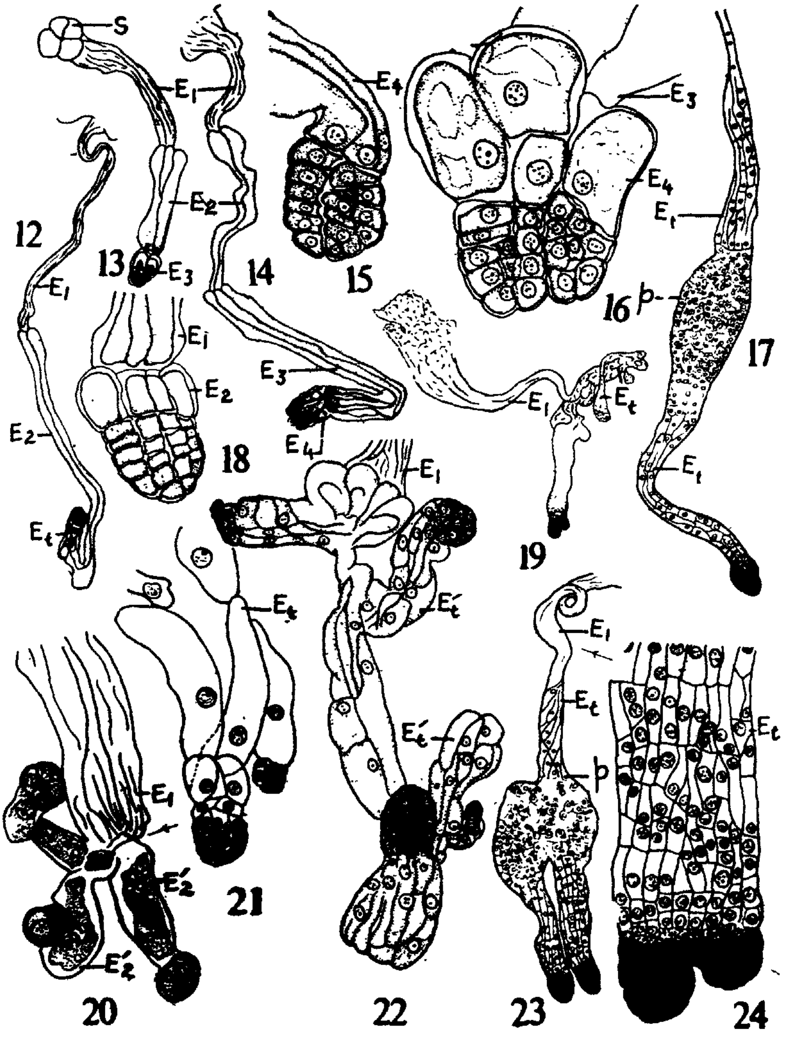
*Group* 2 : The embryos in which the 4 terminal cells, instead of dividing together as in group 1, show independent cell divisions in each unit (Fig. 3, 10). The 4 units therefore show unequal growth. As the cells increase in number in each unit, unitary lobing of the embryonal mass occurs (Figs. 7, 11). The unitary lobing seen in the embryonal mass of cells in most cases is only a transitory phase of embryo development (Fig. 11.)

Differences in growth of the 4 units in the uncleaved embryonal mass do, however, sometimes become pronounced. In such cases the terminal embryonal mass of cells may be constituted of partly joined or fully joined unitary lobes of one, two or three units each (Figs. 4, 7, 21).

Four types of embryos are thus formed as a result of the above phenomena.




Figs. 1-11. *Abies pindrow*. Whole-embryo mounts. 1, Embryo at the tip of a very long substitute suspensor $E_1$ showing 4 dormant, densely staining, terminal embryonal cells. × 200; 2, Embryo in which 4 embryonal units are formed after simultaneous division, at same level, of 4 embryonal cells after period of dormancy. × 200; 3–4, Embryonal units of the embyryo in which one embryonal cell has divided independently of the other three. The other embryonal cells remain undivided. × 200; 4, Embryo in which one unit grows independently of the other three. × 65; 5, Embryo of 4 embryonal units showing degeneration of two units while the other two grow normally. × 105; 6, Embryo showing separation of a unitary lobe due to differences in $E_t$ elongation. × 164; 7, Terminal embryo showing two lobes of two units each growing together. × 45; 8, Embryo formed from linear additions of the cells of the 4 units. The proximal cells of the embryo elongate to form $E_t$. × 105; 9, The terminal embryo where the identity of 4 embryonal units is lost, proximal cells elongate to form $E_t$. × 41; 10, Unitary lobing shown by the terminal embryo because of independent behaviour of 4 units and their unequal growth. × 247; 11, Transitory phase of unitary lobing where it gradually merges to form a homogenous embryonal mass of cells. × 65.




Figs. 12-16. *Picea smithiana*. Whole-embryo mounts. 12, Whole-embryo showing $E_1$, $E_2$ and direct $E_t$ formation. × 15; 13, Whole-embryo showing $S$ and $E_1$, $E_2$ elongations and an elongating $E_3$ × 24; 14, Whole-embryo showing regular tier segments elongating to form, $E_1$, $E_2$, $E_3$, $E_4$ segments. × 24; 15, Embryonal mass showing unitary lobing. One lobe consists of one and other of three units. × 105; 16, Embryonal mass showing unitary lobing. The two lobes are constituted of two units each. × 105.

Fig. 17. *A. pindrow*, whole-embryo mounts. 17, Embryo showing proliferation of middle portion of $E_t$ cells to form a tuberoid swelling. The terminal embryo and its $E_t$ is normal. × 9.

Fig. 18. *Picea smithiana*, whole-embryo mount. 18, Embryo at the tip of $E_1$ showing that all 4 units of the embryonal mass contribute equally by linear additions of cells. × 105.

Figs. 19-24. *A. pindrow*, whole embryo mount. 19, Embryo showing partial cleavage occurring crudely at $E_t$ level in which the terminal embryo is constituted of two units giving rise to persistent unitary lobing. × 95; 20, Terminal portion of an embryo showing partial and well

(*Contd.*)

(a) Embryos in which the unitary lobes persist side by side without separation to late stages of development (Figs. 7, 24).

(b) Embryos in which one or two terminal units may degenerate at early stages (Fig. 5).

(c) Embryos in which unitary lobes may show slight separation but not complete separation because of minor differences in their growth or in their $E_t$ elongation (Fig. 4). Only one of the 4 units in the embryonal mass dominates and others come under its organizing influence in a way similar to that of apical dominance of the vegetative shoot (Fig. 11).

(d) Embryos in which unitary lobes of 1-2 units may separate from the main embryonal mass. This is partial cleavage of the terminal embryonal group. Partial cleavage occurs due to pronounced $E_t$ elongation of some unitary lobes or due to inhibited or slow growth of $E_t$ elongation of some lobes of the embryonal group (Figs. 6, 19, 21).

The type of partial cleavage described under (d) was seen in 15% cases in a study of cleavage behaviour of over 1500 whole-embryo mounts. Cleavage was absent in 85% of embryos studied although transitory unitary lobing seen in embryos described under (c) was common in early stages of their development. This also included not uncommon cases of persistent unitary lobing in which lobing persisted to late stages in 20% of embryos described under (a) but without cleavage of terminal embryonal mass. Unitary lobing of all types was observed to occur in over 25% cases of embryos showing non-cleavage. The embryogeny of *Abies pindrow* can therefore, be expressed by the formulae:

$USE_1$ ............ $E_t$..............(e) 85%
$USE_1$ ............)$E_t$(.............. e 15%

where unitary cleavage occurring only between $E$ unit. derived from the primary pro-embryo is expressed as ) ( , non-cleavage as ( ); and elongation of suspensor-like segments by a series of dots. $E_t$ denotes numerous proximal cells of an embryonal mass which elongate irregularly to a limited extent and do not form tiers.

Total unitary cleavage in which all 4 units of the embryo group separate crudely at $E_t$ level was observed only once (Fig. 22).

$E_2$ formation instead of regular $E_t$ development after $E_1$ and partial cleavage at $E_2$ level was observed in one case (Fig. 20).

The embryonal cells of $E_t$ proliferated in one embryo into a tuberoid structure in the middle of $E_t$ but further down $E_t$ elongation with terminal embryo-formation was normal (Fig. 17). In another similar embryo there was partial cleavage from the tuberoid structure due to formation of separate $E_t$ elongations (Fig. 23).

defined *Pinus* type of cleavage at $E_1$ level. × 65; 21, Embryo showing separation of one unit from the rest by differential elongation of $E_t$s. × 41; 22, Abnormal embryo showing total cleavage occurring crudely at $E_t$ × 24; 23, Embryo showing tuberoid formation due to proliferation of cells of central part of $E_t$ from which two $E_t$s develop independently and separate two lobes of 2 unit each. × 35; 24, Embryo showing persistent unitary lobes growing side by side. × 40.

The non-functional $S$ (suspensor cells, once known as rosette cells), in most of the embryos degenerate in a unicellular condition without any cell divisions. In about 2% cases some of these cells divide once or twice. More than three divisions of a cell were not seen by us in *A. pindrow*. These suspensor cells therefore do not proliferate to form embryo-like structure (once known as rosette embryos) seen commonly in *Pinus* species.

*Picea smithiana*

The substitute suspensor $E_1$ attains a very short length (Figs. 12, 13, 14) in contrast to the long $E_1$ found in *Abies pindrow*. The terminal embryonal tier cells divide early and do not remain dormant as in *Abies* during $E_1$ elongation.

The 4 primary embryonal cells divide to form 4-linear rows of primary units which, in early stages, contribute equally to the growth of the uncleaved terminal embryonal group (Figs. 13, 14, 18). The early divisions in the 4 units occur mostly in tiers (Fig. 18). The successive elongations of cells of the suspensor-system therefore, too, occur in tiers. The suspensor-system of *Picea* thus acquires a segmented structure (Figs. 13, 14). After $E_4$ the non-tiered $E_t$ is formed by elongation of numerous proximal-cells of the uncleaved embryonal mass which differentiates to form a polycotyledonous embryo. Non-cleavage is the rule.

In 10% cases out of 500 embryos $E_t$ is formed earlier, i.e. either after $E_t$ or after $E_2$ elongation (Fig. 12).

In about 15% cases the 4-embryo units although showing a linear growth pattern did not contribute equally to the non-cleaved embryonal mass. In these embryos linear units growing faster over-top and dominate the others in growth. Cases of unitary lobing, due to unequal growth of units, were seen in the embryonal group (Figs. 15, 16). Only in 2% of cases, lobes, consisting of one or two units each, persisted to late stages of embryo development.

Partial cleavage, or separation of one or two primary unit lobes, from the embryonal group, in a manner described for *Abies pindrow* was absent in *Picea smithiana*. *Picea smithiana*, therefore, shows a more consistent and a stable non-cleavage condition than that found in *Abies pindrow*. The embryogeny of *Picea smithiana* can be expressed with the formula:

$$USE_1 \ldots\ldots\ldots E_2 \ldots\ldots\ldots E_3 \ldots\ldots\ldots E_4 \ldots\ldots\ldots E_t \ldots\ldots\ldots (e),$$

where, suspensor-like $E$ segments form tiers of elongating $E_1$, $E_2$, $E_3$, $E_4$ cells attached to the suspensor at one end and to $E_t$ at the other. The non-functional $S$ (suspensor cells) do not usually proliferate. Only in rare cases some of these cells may divide to form irregular degenerated groups of cells.

## Discussion

*Division of the Terminal Embryonal Tier*

In *Abies pindrow* the terminal embryonal tier of cells remains dormant and the embryonal cells do not divide during $E_1$ elongation. This feature is also recorded for *A. vensuta*, *A. pinsapo*, *A. firma* and *Keteleeria davidiana* (Buchholz, 1942; Sugihara 1943, 1947).

In *Picea smithiana* division of terminal embryonal cells takes place early and is not delayed as seen in *Abies*. Early division of these cells is also reported in *Picea smithiana* and in *P. omorika* (Buchholz, 1942). The terminal embryonal cells divide early in *Pinus*, *Cedrus*, *Tsuga* and *Larix* (Buchholz, 1918, 1931; Schopf, 1943; Dogra, 1956, 1962). In *Pseudotsuga* the terminal embryonal tier cells divide neither early nor very late (Allen, 1943).

*Suspensor-System*

The structure of the suspensor system in *Abies* is simple and distinctly different from the other eight genera of Pinaceae.

An exceptionally long $E_1$ followed by direct $E_t$ formation is shown to be characteristic of *Abies pindrow* by us and for *A. vensuta*, *A. pinsapo*, *A. firma* by Buchholz (1943) and Sugihara (1947). The tiered $E_1$, $E_2$, etc., elongations giving rise to a segmented suspensor-system of different genera of Pinaceae are absent in the genus *Abies*.

In *Picea smithiana* a segmented suspensor-system formed by tiered $E_1$, $E_2$ elongations up to $E_4$ level followed by an $E_t$ is shown to be characteristic for *Picea smithiana*. Buchholz (1942) observed a similar situation to be present in *Picea smithiana* and *P. omorika*, and Hakansson (1956) for *P. abies*. Buchholz records $E_5$ to be the last segmented elongation in *P. smithiana* before an $E_t$ develops but we found that $E_4$ was predominately the last such elongation in *P. smithiana*. This is also reported for *P. omorika* (Buchholz, 1942), and *P. abies* (Hakansson, 1956). Such a segmented suspensor-system is absent in *Abies* but is characteristic of other 8 genera of Pinaceae with modifications in relation to their cleavage behaviour.

In some cases of *Picea smithiana* we observed only one or two segmented elongations. In rare and unusual cases where $E_t$ was formed after $E_1$ elongation, the resemblance to the suspensor structure of *Abies pindrow* was remarkable.

*Unitary Lobing in Non-Cleavage Forms*

In *Abies pindrow* unitary lobing was observed in over 25% cases of the total number of embryos showing non-cleavage. Variable degrees of separation of the 4 embryonal units similar to unitary lobing is recorded for *A. balsamea* (Hutchinson, 1924) and for *A. firma* (Sugihara, 1947). Persistent unitary lobing is seen in 20% of cases in *Abies pindrow* by us and perhaps this is present in other species of *Abies* too.

Unitary lobing in *Picea smithiana* was observed to occur only rarely and in early stages of embryo development.

*Non-cleavage and Cleavage*

*Abies pindrow* is predominantly a non-cleavage form despite unitary lobing occurring in over 25% of non-cleaved embryos. 85% of embryos showed non-cleavage, of which 20% showed persistent unitary lobing. Only 15% of the embryos studied showed partial cleavage in this species.

In *A. firma* 28% of cases are recorded where separation of embryonic cells mass occurred (Sugihara, 1947).

*A. balsamea* showed cleavage to occur only in 10% of cases. *Abies* therefore shows a predominantly non-cleavage condition (72% to 85%) as shown for *A. pindrow*, *A. balsamea*, *A. vensuta* and *A. firma* (Hutchinson, 1924; Sugihara, 1947: Buchholz, 1942—present investigation).

Thus all investigations are in agreement that *Abies* is a non-cleavage form but embryos may show a temporary phase of unitary lobing in early developmental stages of many embryos. Persistent unitary lobing seen in 20% of cases of advanced embryos in *A. pindrow* makes each embryo consist of two joined bad twins which may or may not separate. Partial cleavage or separation of unitary lobes of one, two or three units each occurs only in 15 to 28% of embryos in *Abies* (Hutchinson, 1924; Sugihara, 1947; Buchholz, 1942, present investigation). Total cleavage is absent and was found to occur only once in *A. pindrow* where all 4 units separated crudely at $E_t$ level (Fig. 22) as seen in Taxodiaceae (Dogra, 1966).

*Picea smithiana*, on the other hand, is a more stable non-cleavage form than *Abies pindrow*. Buchholz (1926) noted that out of hundreds of embryos only a single instance of separation occurred in *Picea excelsa* and *Picea marianna*. *Picea* therefore, differs from *Abies* in showing an absence of cleavage.

## ACKNOWLEDGEMENTS

We are grateful to Mrs S. Sud, Late Mr R. C. Sud, Mrs I. Singh and Late Mr P. Singh, Mr H. Kaushal, Mr B. Kaushal, Mrs H. Thakur, Mr Sarwan for help in collection of the material from Simla. We also wish to thank Dr (Mrs) A. Pal and Miss S. Tandon for help in preparation of the manuscript.

## REFERENCES

Allen, G. S. (1943).The embryology of *Pseudotsuqa taxifolia* (Lamb.) Brit. *Amer. J. Bot.*, **30**, 655-661.

Buchholz, J. T. (1918). Suspensor and early embryo of *Pinus*. *Bot. Gaz.*, **66**, 185-228.

———(1920). Polyembryony among Abietineae. *Bot. Gaz.*, **69**, 153-167.

———(1926). Origin of cleavage polyembryony in conifers. *Bot. Gaz.*, **81**, 55-71.

———(1931). The pine embryo and the embryos of the related general. *Trans. Ill. State. Acad. Sci.*, **23**, 117-125.

———(1938). Dissection, staining and mounting of the embryos of the conifers. *Stain Tech.* **13**, 53-64.

———(1942). A comparison of the embryogeny of *Picea* and *Abies*. *Madrono*, **6**, 156-167.

Dorga, P.D. (1966). Embryogeny of the Taxodiaceae. *Phytomorphology*, **16**, 125-141.

———(1967). Seed sterility and disturbances in embryogeny in conifers with particular reference to seed testing and tree breeding in Pinaceae. *Studia Forestalia Suecica* **45**, 1-97.

Hakansson, A. (1956). Seed development of *Picea abies* and *Pinus silverstris*. *Meddel. Fran. Statens Skogsforsk* **46**(2), 1-23.

Hutchinson, A. H. (1924). Embryogeny of *Abies*. *Bot. Gaz.*, **77**, 28-289.

Mehra, P. N., Dogra, P. D. (1975). Embryogeny of Pinaceae 1. Proembryogeny. *Proc. Indian Nat. Sci. Acad.* **11**, B, 486-497.

Schopf, J. M. (1943). The embryology of *Larix*. *Ill. Biol. Monogr.* **19**(4), 1-97.

Sugihara, Y. (1943). Embryological observations on *Keteleeria davidiana*. Biessner var. formosana Hayata. *Sci. Rep. Tohoku Imp. Univ.* IV. Series Biol. **17**, 215-222.

———(1947). The embryogeny of *Abies firma* Siebold et Zuccarini *Bot. Mag. Tokyo*, **60**, 58-62.