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THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Beatrice Larson for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

C. O. Rosendahl
Chairman

Josephine E. Tilden

C. A. Morrow

May 27 1918

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Beatrice Larson final oral examination for the degree of Master of Science . We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

May 27 1918

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The Embryology of Zizania palustris L.

A thesis submitted to the
Faculty of the Graduate School of the
University of Minnesota
by
Beatrice Larson
In partial fulfillment of the requirements
for degree of
Master of Science
June, 1918.

The Embryology of Zizania palustris L.

General description.

Zizania palustris (Gray Manual 7 ed.) is a wild plant of great economic value. It is undoubtedly of North American origin, having been used as the "staff of life" for the Aborigines of this country since the earliest records. It is found in portions of North America from the southern part of eastern Canada to Florida and west from the Atlantic thru the states bordering the Mississippi River. It grows in lakes and rivers of muddy bottoms where the water is kept in a slow motion.

It is an annual. When the grain ripens in the autumn it falls into the water, buries itself in the mud and germinates in the spring. Often times, however, if conditions are not suitable, for example if there are floods or droughts, a seed may lie dormant for three or four years. On the return of a favorable season it will germinate and produce a normal plant.

Zizania palustris varies in height from five to ten or twelve feet. In latitude 46 degrees the leaves first appear at the surface of the water about the first of May and the plant reaches full height by the first of September. The seed is ready to be gathered about the second week in September.

The inflorescence is a panicle which appears late in July or the first of August. The panicle has two areas of spikelets. The staminate spikelets are found below and the pistillate, above.

The staminate portion is made up of whorls of spreading branches arising from the joints of the axes. These give rise to numerous pedicels which bear the spikelets. A staminate spikelet consists of two glumes, - of unequal size of a purple color, - and a single staminate flower bears six large stamens. The larger glume has five nerves and bears a soft short awn. The smaller glume has three nerves and is enclosed or protected by the larger glume while the spikelet is still in the bud. The anthers dehisce along their entire length.

The pistillate portion is terminal. It branches in a manner similiar to the staminate portion except that the branches are erect and closely appressed to the main axis. A spikelet has two unequal glumes and two lodicules which surround and almost conceal the small ovary with its feathery stigmas. The larger glume is five nerved and bears a stiff awn of great length. This awn is covered with barbs and bristles and since it falls off bodily with the enclosed seed assists in planting the latter in the mud. Short bristles are also borne along the edges of the glume and a short distance along each nerve. The smaller glume is very delicate and membranaceous. Like the smaller glume of the staminate spikelet it has three nerves. It is almost entirely enclosed by the larger glume and in the ripe seed they are very difficult to separate. The lodicules, at the time of fertilization, expand on the absorption of moisture and force the glumes apart at the base. The stigmas then push out and catch any pollen that may be blown upon them. As soon as fertilization has been accomplished the stigmas begin to wither and the larger glume again closes about the smaller one and the ovary begins to develop.

To insure cross-pollination, the pistillate spikelets of the panicle are produced first and are usually all fertilized before the staminate portion of the same plant has been unsheathed.

I have found in many cases that the spikelets may be said to be hermaphrodites. Many of the "staminate spikelets" have abortive ovaries and likewise many of the "pistillate spikelets" have five rudimentary stamens surrounding their ovaries. Fig. 2.

The abortive ovaries are very minute with microscopic stigmas, therefore nonfunctional. The rudimentary stamens are only five in number while in the normal flower there are six. Without doubt this monoclinous condition is evidence that at one time the flowers were perfect and that this condition has been derived through one cause or another. The spikelets are not considered monoclinous now, however, because the rudimentary parts are non-functional.

Embryology.

The material upon which this embryological study is based, was collected in Kanabec County, Minnesota, from a wild rice field in Ann River.

The pistillate spikelets were killed and dehydrated in alcohol, treated with xylol and embedded in paraffin in the usual way. Microtome sections were cut five microns in thickness. Haidenhain's Iron alum Haemotaxylin proved the most satisfactory stain. The triple-stain was used in some cases; but in the study of the developmental stages of the embryo it was not satisfactory because it was very difficult to sufficiently de-stain.

The ovule.

The ovule of the Zizania, like most grasses, is anatropous, Fig.3, the micropyle facing the point of the origin of the funiculus. The nucellus is straight in this case and is enclosed by two integuments. Fig.4. The integuments are from two to three cells in thickness at their ends, but as they merge into the nucellar tissue at the chalazal end of the ovule, they increase in size. Often they are from six to eight cells in thickness.

No material was obtained of the developmental stage of the embryo sac, but it is hoped that abundant material may be collected this season and that further study may be made. The embryo sac is extremely long, Fig.3, being about five times as long as it is wide. The embryo sac contains the antipodal nucleii and egg apparatus - two synergids and egg as usual. The endosperm nucleus is not found at its customary position in the centre, but is in the proximity of the antipodal cells. The nucellus cap contains two rows of cells, but as the base of the ovule is reached the layer becomes thicker. As the embryo sac develops, the outer cells of the nucellus keep up equal growth. But after a time the tissues of the nucellus and inner integuments are destroyed by the developing endosperm. The outer integument remains to form the seed coat.

The Embryo.

After fertilization a wall is formed about the fecundated egg and the latter elongates. It then divides by a transverse wall into two cells. In Fig.5, the first transverse division is shown to have taken place. One of the synergids persists for some time while the other disappears early and is probably absorbed or disintegrated by the action of the pollen tube. (In no case was the pollen tube observed in the style or in any part of the ovary.) The fecundated egg divides into two cells, a suspensor cell, which lies attached to the micropylar end of the embryo sac, and the terminal cell. The terminal cell is often called the "embryo" cell.

All further development and growth of the pro-embryo is due to the division of the terminal cell.

The suspensor cell in the Zizania shows some enlargement, but remains, perhaps in most cases, undivided Fig.8.

There are great nariations in Monocotyledons. According to Pickett, some of the Araceae have a distinct suspensor cell which divides transversely and longitudially just as the terminal cell. Campbell (5) describe Araceae, on the other hand. Where no suspensor cell is present and the food is received directly from the endosperm which is in very close contact with the embryo from the very early stages. In the Symplocarpus foetidus (Rosendahl 14) a suspensor cell is present in the early stages, but as the pro-embryo rounds off and becomes larger the suspensor disappears entirely. The suspensor cell in Dicotyledons like Senecio (Mottier 11) usually divides into three or four cells, the lower one remaining large.

In Zizania the terminal cell elongates and enlarges until it is greater than the suspensor cell. It is obvious that transverse division as well as longitudinal division takes place to form the embryo.

As a rule in Monocotyledons (Campbell3) the terminal cell under goes a transverse division and the result is a three celled stage of the embryo. I was, however, unable to find such a stage in my material but judging from the more advanced stages this period was undoubtedly passed through.

The suspensor cell may have divided. ~~for~~ In Fig.11 and 12 there is an indication that division may have taken place but probably the usual thing is for the suspensor to remain undivided.

In Fig. 7 and 8, repeated division has taken place and the terminal cell has now become a globular body much enlarged and made up of numerous cells.

The pro-embryo becomes pyriform in shape. In Cross section shown in Fig.9 the pro-embryo is somewhat flattened in one direction but becomes more rounded as development proceeds. (Fig.10) so that in the mature stages (Fig.14) it appears almost circular.

The embryo grows rapidly and after repeated divisions (Fig.7) without any definite sequence or direction, becomes differentiated into definite regions. Fig.12. The epidermal layer is differentiated but there is no external sign of plumule development.

The mature embryo of Zizania has an extremely long scutellum and a very conspicuous epiblast. Fig.15. These two prolongations resemble cotyledons both in origin and appearance. (Bruns 1). The scutellum is spoken of by most investigators as a cotyledon but the nature of the epiblast is still disputed. It is considered by Bruns, van Tieghem and Coulter as a cotyledon which has not been developed fully to possess vascular bundles.

The scutellum develops from the terminal portion and the epiblast develops from the portion below the second transverse wall but still from the peripheral layer.

Coulter (8) says, "In Zizania aquatica (palustris), the so-called epiblast is very conspicuous, arising as distinctly from the peripheral cotyledonary ring as does the so-called scutellum, and attaining at least one quarter to one third of its length. This unusual development of a second cotyledon is associated with the fact that the stem axis above the cotyledon develops a long internode so that the first leaves begin to appear at an unusual distance from the origin of the cotyledon. In fact, in this case the length of the second cotyledon is approximately the length of the first internode and where the leaves begin the cotyledon ends."

The stem bud arises laterally, at the notch or depression on the side. The stem bud or plumule is said to be developed terminally because of the lack of growth of the "second cotyledon." In the Dicotyledons the cotyledons develop alike and the plumule arises between them at the tip. In Zizania the apex of the embryo is at the base of the notch rather than at the tip of the embryo. The stem bud is not protected by a cap as in the Avena or other terrestrial grasses. (Sargant 15) It is wrapped in a leaf-life coleoptile which protects it in its tender stages.

The root originates from the portion nearest the point of attachment of the embryo. I could not determine how many cells went to the building of the various parts. The upper half of the embryo made up the epicotyledonary portion and the lower half of the embryo produced the hypocotyledonary portion.

In the mature embryo (Fig.13) the scutellum remains in the seed in contact with the endosperm. It thus conveys food material to the embryo. The mature seed entirely fills the cavity between the two glumes of the pistillate spikelet and they thus become the "hulls."

The most fundamental differences between the Monocotyledons and the Dicotyledons is found in the embryo according to Coulter (8). Even in the Monocotyledons themselves we find many differences. Zizania differs from some Aroids (Campbell 5) in the form of the pro-embryo. The aroids form a spherical mass of cells for the embryo and the endosperm surrounds the embryo very closely, there being no suspensor cell. In Lilium (Coulter 9) the suspensor cell becomes very massive and acts in its full capacity. In the Orchids there is practically no differentiation of the embryo into the various body regions.

The Endosperm.

The endosperm begins to form before the division in pro-embryo. Fig.5. The endosperm is produced by free cell formation. The primary nucleus goes thru several successive divisions before any walls are laid down. Free cell formation takes place in most all plants whose embryo-sacs grow very rapidly. In Zizania the development of the embryo occupies about three weeks.

The endosperm nucleii are large with two large nucleolii. They are embedded in an alveolar cyto plasm which seems to be spun rather closely about the nucleii like a web. Fig.6. The endosperm tissue is arranged around the periphery of the embryo-sac very scantly, but it is especially abundant in the area immediately about the pro-embryo. (Fig.8) The thin layer of endosperm about the periphery leaves a residual cavity in the embryo-sac which is not filled until in the later stages.

In the earlier stages of development the endosperm entirely encloses the embryo, but as it matures it pushes to the side of the endosperm. It thus comes to occupy the lateral position (Fig.13) so characteristic of Gramineae.

As the embryo matures, the endosperm takes on the shape of the ripe seed. Before maturity, the endosperm tissue becomes thin cell-walled and the contents at first resemble milk or sap.

When the seed is ripe however, this material becomes a mass of starch.

According to Miss Sargant (15) the endosperm of Zizania is very scant compared with the Avena. If the endosperm of the developmental stage is referred to the above statement is correct but in the mature stage (Fig.13) it compares very favorably with the cultivated cereals.

Summary.

1. In Zizania palustris one synergid may persist a considerable time after the first division in the pro-embryo has taken place.

2. The first division produces a suspensor cell and a terminal or "embryo" cell.

3. Divisions in the "embryo" cell are irregular and indefinite. The form of the pro-embryo is at first oblong, later it becomes obovoid or pyriform.

4. The endosperm tissue arises from free cell formation and is very scanty in early stages.

5. The endosperm does not form cell walls until the embryo has progressed considerably.

6. The embryo is differentiated into definite regions early in its development.

7. The mature embryo shows a very conspicuous scutellum and epiblast.

Figures.

Drawings were made from a Bausch and Lomb and a Spencer microscopes together with the projecting drawing machine and also camera lucida.

Fig. 1. Longitudinal section of the ovary and stigma. B.&L. 8 oc, 2 in. obj.

Fig. 2. Cross section of the ovary showing the integuments and the rudimentary stamens. B.&L. 10 oc., 2 in. obj.

Fig. 3. Longitudinal section showing the position of the ovule. B.&L. 10 oc. & 1/16 in. obj.

Fig. 4. Longitudinal section of ovary showing an older stage.

Fig. 5. Showing the first division of the fecundated egg and the persistent synergid. X 750.

Fig. 6. Endosperm tissue. Oil immersion.

Fig. 7. Pro-embryo after division showing very little differentiation. X 750.

Fig. 8. Pro-embryo showing endosperm tissue and nucellus tissue. X 750.

Fig. 9. Cross section, showing the pro-embryo after a few transverse divisions. X 750.

Fig. 10. Cross section of pro-embryo from another region.

Fig. 11. Pro-embryo after divisions showing the beginning of differentiation. Spencer, 10oc. 4 mm. obj. X 490.

Fig. 12. Embryo showing differentiation. X 750.

Fig. 13. Cross section of the mature seed showing endosperm and embryo. The seed coat has been removed. Spencer 10 oc., 2 in. obj. X 32.

Fig. 14. Cross section of mature embryo showing the epiblast some what plainer. X 32.

Fig. 15. Surface view of mature embryo. X10

I wish to express my thanks to Dr. C. Otto Rosendahl, at whose suggestion this work was undertaken, for his kind direction and criticism.

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