

POLYEMBRYONY IN *HIEROCHLOE ODORATA* (L.) BEAUV.¹

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Polyembryony, though far from a rare phenomenon in the angiosperms, is intimately and regularly associated with the reproductive processes in only a few taxa. Among the grasses some species of *Poa* (Nielson 1946a, 1946b), *Tripsacum dactyloides* and *T. lanceolatum* (Farquharson, 1955) are remarkable prolific in their production of supernumerary embryos. Other grasses produce double embryos much less frequently. For example, Webber (1940) in a review of polyembryony, cites twinning frequencies of less than one percent for wheat, oats, rice and rye. Thus, although sporadic formation of more than one embryo per ovule may be encountered among members of *Gramineae*, it is noteworthy when an additional species can be numbered with those few in which the phenomenon frequently occurs.

Several years ago, in the course of an investigation of embryogenesis in some Michigan grasses, it was found that a number of the grains of Seneca grass, *Hierochloe odorata* (L.) Beauv., contained multiple embryos. Recently, in the spring of 1956, collections were begun in two areas of Michigan and examinations of this material confirmed and amplified the earlier findings.

MATERIAL AND METHODS

Hierochloe odorata is an early spring flowering grass of wet meadows and shorelines. Anthesis normally takes place in early May in southern Michigan. Accordingly, collections were made during the second week of May along the Huron River near Ann Arbor, and in June along the shores of Douglas Lake in Cheboygan County. Douglas Lake is near the tip of the lower peninsula of Michigan, where the flowering period of *Hierochloe* is about two weeks later than in Ann Arbor. Although the spring of 1956 was late, the collecting was not started early enough to obtain many specimens showing cytological events leading up to embryo sac formation.

Spikelets were removed without otherwise disturbing the plants, which nowhere were abundant. Some of the specimens were killed and fixed on the spot using a CRAF formula, and later sectioned at thicknesses of 12 to 25 μ . The serial sections were stained either in iron-alum hematoxylin or crystal violet counterstained with fast green. Other material was brought into the laboratory for dissection and study. A number of excised embryos were placed on White's (White 1943) nutrient agar at this time, and eventually whole grains were planted in the greenhouse or placed on wet filter paper in order to study germination and seedling development.

RESULTS

As the examination of material from the two areas progressed it became apparent, first of all, that many of the florets in each inflorescence contained abortive caryopses. Only 29 percent of a sample of 235 central florets were fully developed (the spikelet of *Hierochloe* consists of two lateral, staminate florets and a central, perfect floret). The failures occurred at various stages. Many pistils had not enlarged at all, while others appeared to be full grown but were vesicular and col-

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lapsed when they were pricked. Many more had been attacked and destroyed by thrips, which were quite numerous.

A total of 398 seemingly normal grains were dissected and it was found that 25 percent contained double embryos. The frequencies of this occurrence, in terms of percent, were the same in the Huron River and the Douglas Lake samples. Twelve percent of the total contained no embryos at all although their endosperms looked normal, and two grains contained triplet embryos. Microscopic examination of serial sections of a number of these grains confirmed the observations. In two caryopses the embryos were mere undifferentiated balls of callus. These data are presented in table 1.

The serial sections also showed a number of multiple embryos (fig. 5 and 6). In these, as in the dissected grains, the embryos usually appeared side by side with their coleorhizae in contact at the tips and the cotyledons so closely joined that it was difficult to separate them visually. About half of the twins were of equal size; the remainder consisted of well formed but disproportionate pairs. In most cases the twin embryos were smaller than single embryos.

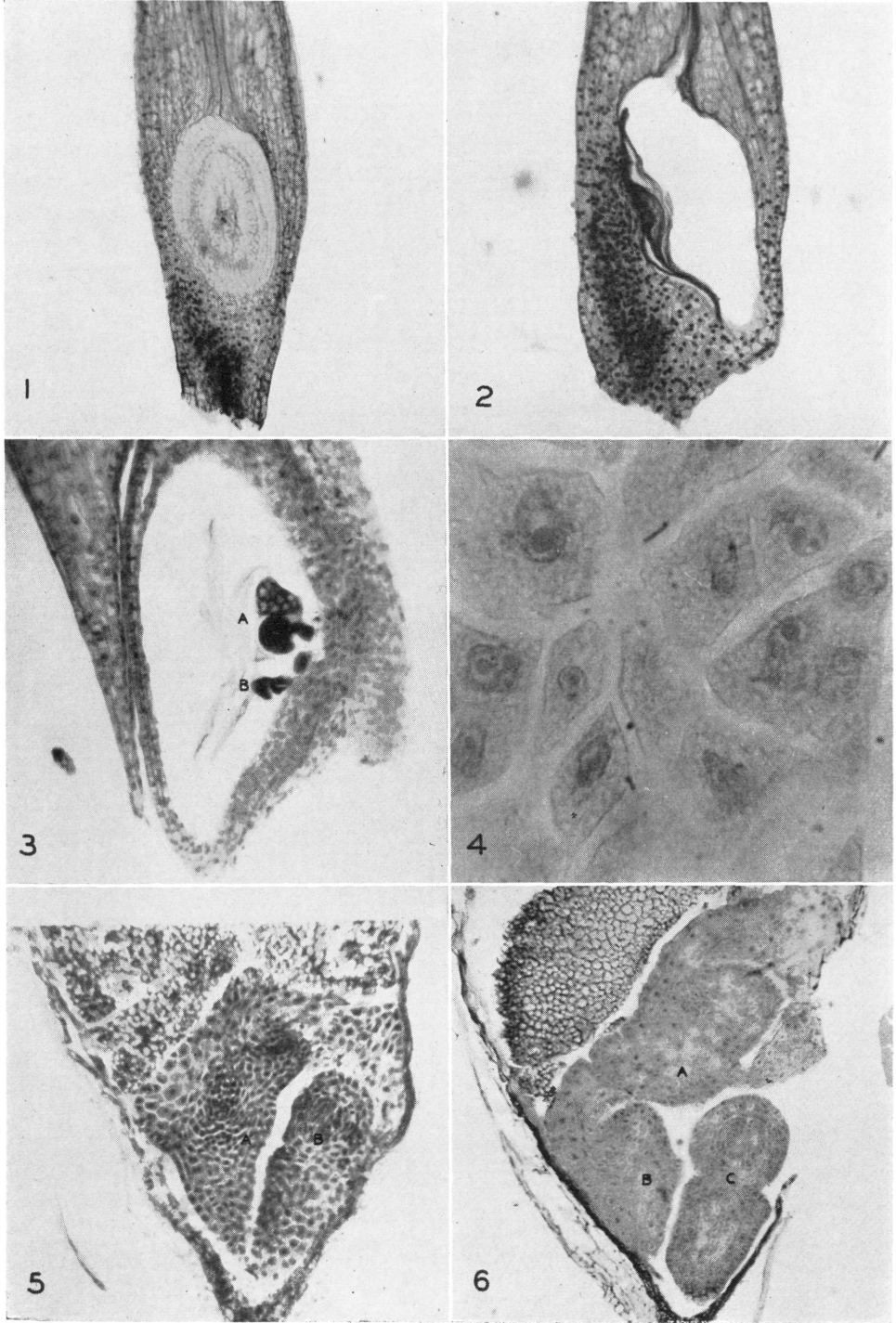
TABLE 1
Embryo development in Hierochloa odorata

Locality	Number of grains containing:				callused embryos
	3 embryos	2 embryos	1 embryo	0 embryos	
Huron River	0	30	86	3	2
Douglas Lake	2	68	178	29	0
Both areas	2	98	264	32	2

Viability of twin embryos.—Ten sets of excised twin embryos were cultured on nutrient agar. Both members of four of these sets grew into small seedlings, while only one embryo in each of the remaining pairs grew. In October, five months after harvesting, a series of germination tests was begun. In the first experiment, 50 grains were planted in individual pots and placed in the greenhouse. Three months later only one of these had germinated and at present (April) no further activity has been noted. Another set of 25 grains was sterilized in 10 percent *Clorox* and placed on nutrient agar. After several weeks had elapsed without evidence of germination, several embryos were excised and replaced on the agar. These, including a pair of twin embryos, immediately grew into small seedlings. The remaining grains in this sample were scarified and replaced on agar, whereupon they also germinated without further delay. A third set, of 100 scarified grains, was then placed on wet filter paper. Germination was slow, but in 30 days 47 had germinated. Only one of these produced twin seedlings (fig. 7). A further trial, this time with selected grains known to contain twin embryos, produced 14 single seedlings and three twin seedlings when cultured on wet filter paper. The remainder of the 23 caryopses did not germinate.

EXPLANATION OF FIGURES PLATE I

1. Caryopsis demonstrating failure in megasporogenesis.
2. Ovule failure following the formation of the embryo sac.
3. Twin embryo sacs. The antipodals of each sac, A and B, are shown.
4. Endosperm cells with lobed and multiple nuclei.
5. Twin embryos, A and B.
6. Triple Embryos, A, B and C.



Embryogenesis.—It is impossible at this time to present a complete report on the formation of multiple embryos in Seneca grass. The embryo sacs, of which 15 were observed, lacked synergids. Two polar nuclei and three large, deeply stained antipodal cells, together with the egg, constituted the most nearly complete embryo sacs observed in this material.

Only one ovule was found in which two embryo sacs (fig. 3) clearly were present, although care was taken that this phenomenon, which has been associated with polyembryony in *Poa pratensis* (Anderson, 1927), should not escape notice if it occurred in *Hierochloe*. Evidences of fertilization were observed in but two instances.

Abortive ovules were commonplace. In many, the abortions had taken place during or soon after megalporogenesis, and the cells of the nucellus and integument in these cases had become enlarged and rather vacuolate (fig. 1). Most of the other failures had occurred after the megagametophytes had formed (fig. 2); in several, a small proembryo was present without endosperm.



FIGURE 7. Single and twin seedlings of *Hierochloe odorata*. Cultured on wet filter paper.

A single ovule, shown in figure 8, contained both an egg and a proembryo. The latter, because of its location, appeared to be of nucellar origin. Origin from a synergid probably may be dismissed, since the synergids were not present at the time of embryo formation. In this specimen it was also observed that the endosperm had failed to develop. Several ovules, one of which may be seen in figure 9, displayed endosperm in the free nuclear stage accompanied by apparently unfertilized eggs. This state seems to be a potential antecedent of the condition in which certain ripened grains lack embryos altogether. In any event, the free nuclear stage was followed by the formation of endosperm cells which contained large lobulate nuclei (fig. 4). Sometimes several nuclei were present in a single cell. These details later were obscured by masses of starch grains.

Although a number of proembryos have been seen in the sectioned material, only in ripe or nearly ripened ovules have multiple embryos thus far been observed. Further observations will be required to ascertain the stage at which the twin embryos appear, as well as the manner of their development.

DISCUSSION

The significance of polyembryony in *Hierochloe odorata*, aside from its frequency, is that it suggests a different origin from that reported for most other grasses. Nielson (1946a, 1946b), citing from the considerable accumulation of literature pertaining to polyembryony and apomixis in the genus, *Poa*, tells of such varied origins as the parallel embryogenesis from multiple embryo sacs, from an egg and a synergid or an egg and a nucellar cell, these being further complicated by different sorts of apomictic developments. The nucellar origin of extra embryos in *Poa*

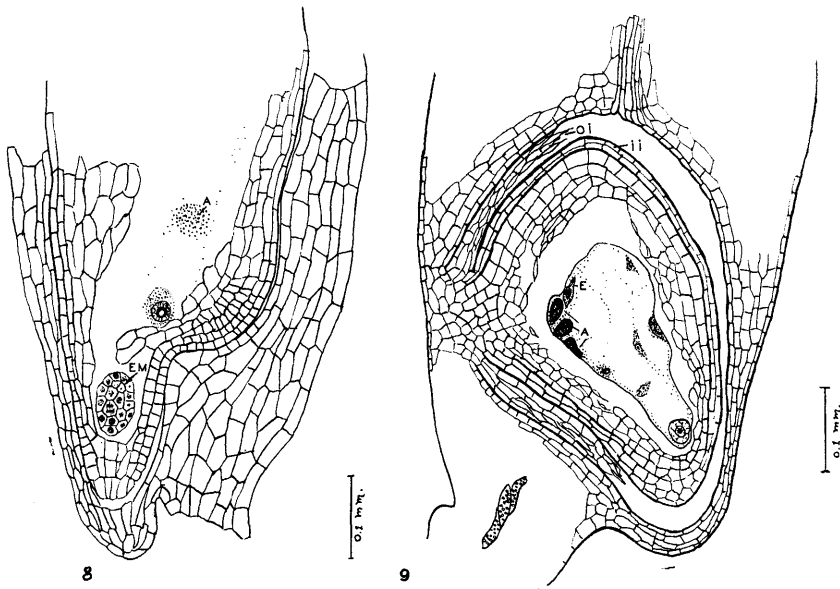


FIGURE 8. A proembryo (EM) and unfertilized egg occupying one ovule. Remaining antipodal cell at A.

FIGURE 9. Ovule containing endosperm in free nuclear stage and an egg; oi-outer integument, ii-inner integument, E-endosperm nucleus, A-antipodal nuclei.

pratensis is the view held by Nishimura (1922), who suggests that several embryos per ovule may be initiated either by nucellar budding or from outgrowths of the suspensor. He also correlates polyembryony with attacks of thrips, although Anderson (1927) later contends that there is no relationship between such infestation and polyembryony. She attributes the formation of multiple embryos to the development of more than one embryo sac. Farquharson (1955) suggests that polyembryony in *Tripsacum dactyloides* may be due to the apomictic development of a synergid together with the egg. In these cases, cited above, the endosperm may develop without fertilization of the polar nuclei, although pollination appears to be necessary in the process.

The twin embryos in *Hierochloe* might be schizogenous in origin. Since proembryos were observed to occur singly while the older, twin embryos were extremely

closely associated, a reasonable conclusion is that they were formed as a result of longitudinal separation of a single proembryonic mass.

The number of grains which were altogether lacking in embryos is another unique feature of this grass and may by inference indicate the occurrence of apomixis.

Although the twin embryos are capable of growing into seedlings *in vitro*, they normally do not both germinate *in vivo*. The difference in growth is probably nutritional rather than genetic, since in many instances one of the embryos appears to be located less advantageously with respect to the endosperm.

SUMMARY

1. Plants of *Heirochloe odorata* from each of two widely separated Michigan locations produced twin embryos in 25 percent of the grains examined. 2. The multiple embryos may have been formed as a result of division of single embryos as evidenced by their orientation and the absence of twin proembryos. 3. The germination of grains was slow even after scarification. This was equally true of those containing normal embryos and those which held twins. The latter grew much better when excised and grown on nutrient agar, where both members of several pairs flourished. Otherwise, usually only one of a pair germinated. 4. Other anomalies observed were triple embryos and the complete absence of embryos. Twelve percent of the grains dissected in this study lacked embryos, although they contained normal endosperm.

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