Rhizoid Initiation in Relation to Gravitation Presentation Time in *Marsilea* Megagametophytes

William W. Bloom and Kenneth E. Nichols
Department of Biology
Valparaiso University, Valparaiso, Ind. 46383

Abstract

The initiation of rhizoid formation in unfertilized megametophytes of *Marsilea* spp. is a response to gravity. Once initiated, rhizoids will grow radially from the plant regardless of the orientation of the plant. The time required for graviperception appears to fall within expected ranges observed in the study of geotropic responses in sporophytes of higher plants. Megagametophytes of *Marsilea* would appear to be excellent material for the study of graviperception in plants.

Introduction

The study of graviperception in plants was reviewed in 1962 by Audus (1). Subsequently, a number of studies have appeared, especially from the Argonne National Laboratory (4, 6, 7). While the earlier studies dealt with presentation time and responses in shoots and roots, more recent studies have concentrated on the mechanisms by which plants are thought to perceive gravitational stimuli. Results from space flight studies with weightlessness have also stimulated many of the recent studies. Audus (1) points out that most studies dealing with graviperception have been concerned with geotropism of the root and shoot.

We have been studying rhizoid formation in unfertilized megagametophytes of the Marsileaceae for some time, and numerous observations lead us to believe that rhizoid formation is auxin-controlled and a response to the force of gravity (2, 3). Once initiated, rhizoids develop and extend more or less radially from the surface of the gametophyte. In our most recent studies we have attempted to determine the age at which the gametophytes are able to perceive the force of gravity, the presentation time required, and the time lapse between perception of the stimuli and the visible response, that is, rhizoid formation.

Methods

Sporocarps of *Marsilea mucronata* were scarified and then hydrated in half-strength Hoagland's No. 2 Solution (5). When sufficient megaspores were free they were transferred with a dropping pipette to agar plates containing half-strength Hoagland's No. 2 Solution with appropriate micronutrients. Following transfer to the agar surface the excess water was drained from the plates. The megaspores adhere firmly to the surface, permitting inversion of the plates as required in the experimental procedures to be followed. Early transfer of the megaspores to the agar surface usually prevents fertilization. The experiments reported here were conducted in an air-conditioned laboratory at 23°C ± 1°. Except for the time during manipulative procedures the

plants were maintained in the dark, as previous studies had shown that rhizoid formation occurred equally well under light or dark conditions.

To determine the age at which the megagametophytes are able to perceive the force of gravity and initiate rhizoid formation, a series of plates was prepared and inverted until the plants reached the following ages: 7, 8, 9, 10, 11 and 12 hours. At the end of each period the appropriately marked plates were turned over so that the original ventral cells were now in a dorsal position.

To determine the presentation time required for rhizoid initiation a second series of plates was prepared as described and held in a horizontal position with gametophytes on the upper surface for 12 hours. These plates were then inverted so that the former dorsally located cells could be subjected to the stimulus for rhizoid initiation in the new position. After exposure for the desired presentation time, the plates were returned to their former position. Presentation times of 5, 10, 15, 20, 25, 30, 35 and 40 min were used. Plants were kept under observation until rhizoids had time to develop on the dorsal surface in response to the gravitational stimuli during the presentation time. All megagametophytes were then examined to determine how many had developed median-dorsal rhizoids and how many lacked such rhizoids.

Results

Plants inverted for 12 hours were definitely sensitive to gravitational stimuli. Those inverted for shorter periods showed little or no graviperception. The age at which the megagemetophytes could perceive gravitational stimuli are shown in Table 1.

TABLE 1. *Dorsal rhizoid formation on megamametophytes of* Marsilea mucronata *inverted until plants reached different ages.*

Age at time of turning in hrs.	Plants lacking dorsal rhizoids	Plants with dorsal rhizoids	Total plants	Per cent with dorsal rhizoids
0	13	0	13	0.0
7	19	0	19	0.0
8	32	0	32	0.0
9	20	0	20	0.0
10	19	0	19	0.0
11	24	1	25	4.0
12	14	12	26	46.2

Plants grown to determine the presentation time were examined after the appearance of rhizoids. No attempt was made to determine the number of dorsal rhizoids formed on each plant. While the shortest presentation time was adequate in a few cases, presentation times of 10 min or longer were adequate to elicit the rhizoid-initiation response in a significant number of cases. The results of this experiment are shown in Table 2.

TABLE 2. *Rhizoid formation on megagametophytes of* Marsilea mucronata *in response to different presentation times.*

Presentation time in min	Plants lacking dorsal rhizoids	Plants with dorsal rhizoids	Total plants	Per cent with dorsal rhizoids
0	27	2	29	6.9
5	25	6	31	19.4
10	23	10	33	30.3
15	17	11	28	42.9
20	21	17	38	44.7
25	15	6	21	28.6
30	12	11	23	42.8
35	13	8	21	38.1
40	11	12	23	52.2

The first visible signs of rhizoid formation were observed about 24 hours after the presentation of the gravitational stimuli. An additional 24 hours was required for rhizoid growth before accurate determinations could be made.

Discussion

Presentation times have been reported of 5-10 min at 20°C for *Heilanthus annuus* hyocotyl; 10-15 min for *Beta vulgaris* hypocotyl; and 22-23 min for *Picea pungens* hypocotyl (1). In these experiments the cells were turned at a 90° angle for the presentation of the stimulus whereas our plants were turned 180°. The presentation times in *Marsilea* gametophytes appear to agree with those of sporophytic tissues of higher plants.

Under the conditions of these experiments the sperm appeared 10 hours after hydration. This would indicate that some plants perceived the gravitational stimulus at about the time the egg cell had been formed and while the gametophyte still consisted of a single layer of cells surrounding the central egg cell. By the time the rhizoids were formed the original perceptive cells would have undergone several cell divisions.

Literature Cited

1. AUDUS, L. J. 1962. The mechanism of the perception of gravity by plants. Symp. Soc. Exp. Bio. XVI: 197-226.

2. BLOOM, W. W. 1962. Some factors influencing rhizoid formation in femalegametophytes of the Marcileaceae. Proc. Indiana Acad. Sci. 72:118-119.

3. BLOOM, W. W., and K. E. NICHOLS. 1972. Rhizoid formation in megagametophytes of *Marsilea* in response to growth substances, Amer. Fern J. 62:23-26.

4. DEDOLPH, R. R., and M. H. DIPERT. 1971. The physical basis of gravity stimulus nulification by clinostat rotation. Plant Physoil. 47:756-764.

5. HOAGLAND, D. R., and D. I. ARNON. 1938. The water-culture method for growing plants without soil. Univ. Calif. Agr. Expt. Sta. Circ. 347:1-39.

6. SHEN-MILLER, J., and C. MILLER. 1972. Intracellular distribution of mitochondria after geotropic stimulation of the oat coleoptile. Plant Physiol. 50:51-54.

7. SHEN-MILLER, J., and C. MILLER. 1972. Distribution and activation of the Golgi apparatus in geotropism. Plant Physiol. 50:634-689.