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Preliminary Studies of the Physiology and Morphology of the Germinating Foliar Embryos of Bryophyllum Calycinum

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PRELIMINARY STUDIES OF THE PHYSIOLOGY AND MORPHOLOGY OF THE GERMINATING FOLIAR EMBRYOS OF *BRYOPHYLLUM CALYCINUM*

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A critical review of the literature pertaining to the germination and growth of the foliar embryos of *Bryophyllum calycinum*, Salisb., has revealed that in most cases the influence of certain physiological and environmental factors upon these structures was not carefully considered. For instance, the history of the plants prior to the experimental period was not fully elucidated and, in absence of statements to the contrary, must have been largely ignored. Yet the work of Dunn (1937) indicates that the influence of "hardening" treatments may extend over as many as three following generations in this species. A common experimental error in most previous work was the lack of simultaneous control of temperature, humidity, and light throughout the time of experimentation.

Another factor usually disregarded was the existence of definite physiological strains or varieties in morphologically similar plants. Mehrlich (1931) found that plants from different clones might differ in their ability to produce roots from the attached leaves of plants kept in continuous darkness and in the faculty of producing roots from the pulvini of detached leaves. From this evidence he concluded that plants from different clones might belong to different physiological strains. Since there are no varietal names for these physiological strains of *Bryophyllum calycinum* the writer will follow the practice of Mehrlich (1931) and Yarbrough (1934) and refer to them as strains identified by the names of their American sources. For example, the plants used in the present study are of the "Chicago" strain since they are all vegetative descendents of plants originally obtained from the greenhouses of the University of Chicago; similarly, those studied by Reed (1923) and Mehrlich (1931) belong to the "Michigan" strain.

It is the purpose of this paper to describe certain preliminary qualitative and quantitative studies conducted by the writer on the physiology and morphology of the germinating foliar embryos of the Chicago strain of *Bryophyllum calycinum* grown under experimentally controlled conditions of light, temperature and humidity.

MATERIALS AND METHODS

Qualitative studies were carried out during the fall and winter of 1940 in the roof greenhouse of the Pharmacy-Botany Building at the State University of Iowa. The radiator thermostat was set at 70° Fahrenheit, and temperature and relative humidity were recorded continuously by thermograph and hygrograph. The plants used were ten months old but had not begun the rapid shoot elongation which characteristically occurs prior to flowering in this species. Their leaves, with few exceptions, were dark green, turgid, and free of

traumata or other blemishes. The average height of the plants was twenty inches. Leaves selected from these plants were divided into three age groups, young, mature, and old. After determining the weight and area of each, ten leaves from each age group were placed on moist sand flats which were then placed under a black sateen screen of the type commonly used in photoperiod experiments. Similarly, ten more leaves from each group were placed on moist sand flats which were exposed to the natural light of the greenhouse. The control plants were also exposed to greenhouse conditions.

To determine the effect of continuous illumination four six-months-old plants, none of whose leaves had been removed, were placed under a thousand-watt incandescent lamp in a reflector which provided four hundred foot-candles of light at the surface of the uppermost leaves. This lamp was operated continuously at night and during cloudy days for a period of ten weeks.

To determine the effects of wounding on proliferation from attached leaves of the Chicago strain traumata of various types were made with a flame-sterilized needle or scalpel on the attached mature leaves of several plants. A single type of wound was applied to each of five different leaves and not more than one type to any one leaf.

Quantitative experiments were carried out in a growth chamber specially devised by the writer in which light, temperature, and humidity could be simultaneously controlled. A detailed description of this chamber will be published elsewhere.

These experiments were conducted during the last three months of the years 1941 and 1942. The plants used were grown under greenhouse conditions from foliar embryos and were seven months old at the beginning of each of the experimental periods. By carrying out the experiments during the same seasons of two successive years the variations of seasonal changes in day length, temperature, and humidity during the pre-experimental period of the plants' history were minimized.

Each typical leaf selected from these plants for experiment was blemish-free, simple, approximately one hundred square centimeters in area, and was selected from the upper half of the middle third of the shoot. Selection of leaves from this region of the shoot insured specimens which were vigorous and almost matured. Each experimental lot consisted of eight to ten leaves.

Within an hour after detachment from the shoot each lot of leaves was placed in the growth chamber where it remained for a period of fourteen days under constant conditions of temperature, humidity and light. The fourteen day growth period was chosen because significant results were obtained within this time and longer periods would have cut down on the number of runs that could be made before the physiological condition of the plants changed too radically from that existing at the age of seven months.

At the end of the experimental treatment the leaves were removed from the chamber and the margins carefully trimmed off and placed

in F. A. A. Subsequently these margins were removed from the preservative and root growth determined by counting and measuring the roots in each notch. The method of measurement was as follows. All roots growing from a given notch were carefully removed with forceps and placed in a drop of F. A. A. on a glass plate. Each root was measured while bathed in the preservative, thereby eliminating shrinkage or deformation due to desiccation. Surface tension caused the root to lie flat against the ruler and reduced the amount of manipulation required to place a given root in position for measurement. A fifteen-centimeter celluloid ruler marked in millimeters was the measuring instrument. Fractions of a millimeter were estimated to the nearest tenth. Measurements were made consecutively, beginning with the basal notch on one end of the strip and working from there to the apex and thence to the basal notch on the opposite side of the blade.

Determinations were made of root growth under the following environments.

1. Temperature, 60° F.; total darkness; relative humidity, 94-100%.
2. Temperature, 75° F.; total darkness; relative humidity, 94-100%.
3. Temperature, 90° F.; total darkness; relative humidity, 94-100%.
4. Temperature, 75° F.; total darkness; relative humidity, 8-13%.
5. Temperature, 75° F.; 16 hours continuous illumination per day from "daylight" fluorescent lamp at intensity of 110 foot candles at the leaf surface; relative humidity, 94-100%.

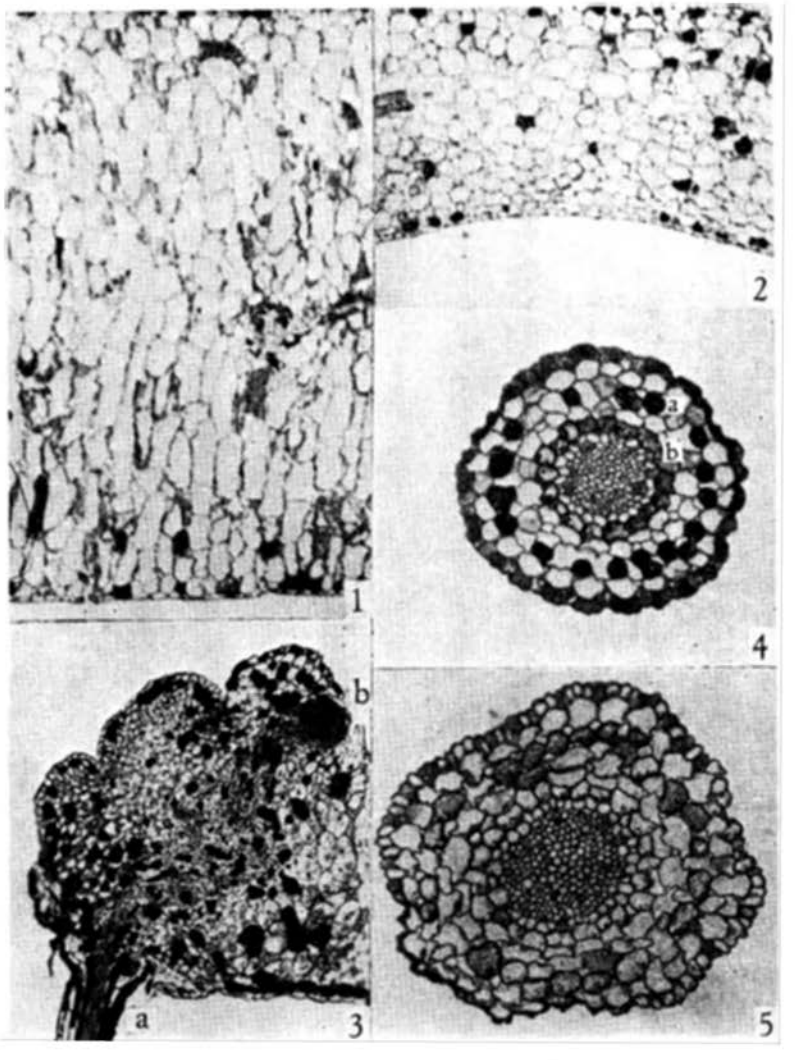
Two lots of leaves were exposed to each of these combinations, with the exception of combination No. 4 which was used on only one lot. As each duplicate was placed in the chamber as soon as the original group had been removed, only one setting of the controls for a given environmental combination was necessary. Frequent checks on the temperature and humidity made sure that the controls were not accidentally changed. Growth of roots under combination No. 4 was so poor and humidity was so far below that encountered under ordinary conditions that it was thought that little would be gained by growing two lots under such conditions.

In addition to the physiological experiments, brief histological studies were made of the following organs in both fresh and paraffin sections.

1. Summer leaves, dormant and proliferating notches in paradermal and vertical sections.
2. Winter leaves, dormant and proliferating notches in paradermal and vertical sections.
3. Air-grown roots, transverse and longitudinal sections.
4. Hypsophylls, paradermal and vertical sections. (Craft, 1942).

RESULTS

The hygrograph and thermograph records showed frequent diurnal variations of 20% in humidity and 10° F. in temperature, these changes being most pronounced on sunny days. During the ten-week



LEGEND FOR FIGURES 1-5

Figs. 1-5.—Fig. 1. Succulent winter leaf of *Bryophyllum calycinum* which is developed in short days. X25.—Fig. 2. Thin summer leaf which develops in long days. X25.—Fig. 3. Vertical section through the foliar embryo showing abaxial root (a) and adaxial root primordium (b). X25.—Fig. 4. Transection through air-grown root 1 mm. back of apical meristem; (a) idioblast, (b) endodermis. X76.—Fig. 5. Transection through air-grown root 2 mm. back of apical meristem. Tannin precipitation is completed in the endodermis and is beginning in the idioblasts. X76.

period the minimum temperature recorded was 59° F. and the maximum 90° F. The extremes of relative humidity were 23% and 79%. Under the dark screen the relative humidity was often as much as 20% higher than that of the room.

Leaves under the screen produced plantlets with shoots 2 mm. long by the end of the fourth day. Shoots of comparable length did not develop from leaves receiving natural light until the sixteenth day and by this time the plantlets in the dark were 15 mm. high. Plantlets grown in darkness showed marked etiolation and contained no anthocyanin. Their average leaf area was about 1 cm.^{2} and the internodes were 10 cm. long. Plantlets developing in light contained considerable anthocyanin and their internodes were too short to be measured with a ruler.

The difference in the percentage of germination in leaves of different ages was negligible, the average of all groups being 85%. However, the plantlets produced from the young leaves in light were only 1 mm. high, whereas those from mature leaves were 2 mm. high at the end of the sixteenth day. Plantlets on the old leaves were not as sturdy as those on mature leaves but their growth rate immediately increased as soon as they took root in the sand. Although the plantlets on the young leaves grew slowly they were sturdier than those produced by old leaves and showed greater survival capacity in air when the roots were unable to reach the sand.

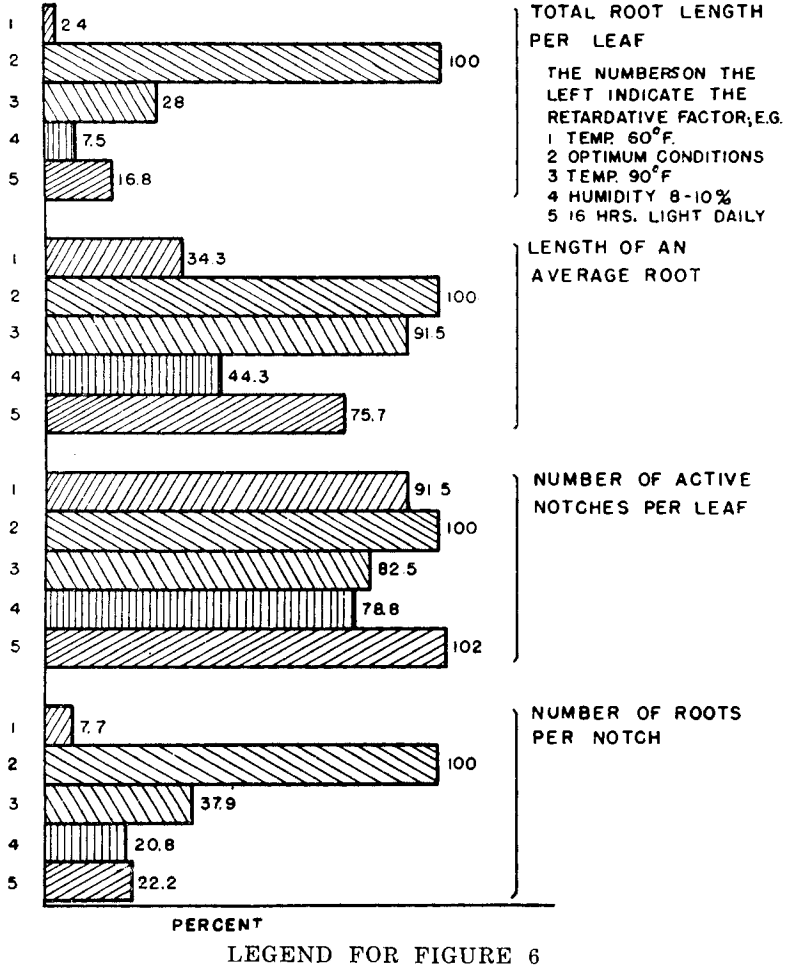
The weight, per cm.^{2}, of the leaves in the three age group was: old leaves 97.55 mg., mature leaves 110.70 mg., young leaves 128.35 mg. Histological examination revealed that the increased succulence of the young leaves, which had grown during short days, was due to an increase in size of all cells and especially to the marked elongation of the central mesophyll cells rather than to an increase in cell numbers (Fig. 1, 2)

Old leaves in sunlight were dried and brittle after eight weeks on the sand flats; mature leaves were brittle at the end of fourteen weeks; some of the young leaves remained on the sand for twenty-one weeks without becoming brittle.

Even thin summer leaves are, when undesiccated, so fleshy that their veins are buried in the mesophyll, but as desiccation proceeds the veins become progressively more conspicuous. Thus by noting the relative prominence of the veins it is possible to gauge roughly the amount of desiccation that has taken place in a given region of the leaf. In leaves with active notches, desiccation first becomes apparent in the region midway between the margin and the midrib. The desiccated area, immediately below the apex in particular, enlarges toward the midrib more rapidly than it approaches the margins. As desiccation proceeds it becomes apparent that the margins lose water more slowly than other parts of the leaf, provided that the foliar embryos are active. In case the foliar embryos remain dormant the desiccated region originates at the margins and encroaches inwards toward the midrib. These results corroborate those of Welch (1938).

About 50% of the foliar embryos produced roots from both the abaxial and adaxial sides (Fig. 3). This response is not found apparently in the Michigan strain studied by Mehrlich (1931), and if adaxial roots occur in strains studied by other workers they have not published this fact. However, more roots grow from the abaxial side than the adaxial side. Root hairs are not produced until the roots come in contact with a body of water or a moist substrate.

Histochemical tests of both fresh and preserved root tips in both transverse and longitudinal sections revealed the presence of pyro-



LEGEND FOR FIGURE 6

Fig. 6. Comparison of several aspects of root growth under different environments. Growth under optimum conditions (75° F., total darkness, relative humidity 94-100%) is evaluated as 100%.

gallol tannin, or closely-related compounds, in the growing endodermis and epidermis as well as in the idioblasts of the cortex (Fig. 4, 5). Tests for anthocyanin in these and other root tissues were negative. The tannin was precipitated only in the mature cells.

The number of xylem arms in the young roots was found to vary between five and seven (Figs. 4, 5).

No proliferation occurred on the attached leaves of any of the plants exposed to continuous illumination. However, a few plantlets appeared from the notches of senescent leaves on the 10-months-old group of plants used as controls in another part of the study. On these plants the proliferating leaves were so easily detached from the shoot that it was evident that an abscission layer had formed.

Wounding of attached leaves was in no case followed by proliferation. This result indicates that attached leaves of the Chicago strain are not as sensitive to traumata as are the leaves of the strain used by Goebel (1902), who found that a single incision through the midrib at right angles was sufficient to cause proliferation. The origin of the strain used in Goebel's study is not known.

The Chicago strain was not observed to produce roots from the pulvinus, this characteristic further separating it from the Michigan strain and from the strain used by Smith (1921).

Results of the quantitative experiments are graphically summarized in terms of percentages by the accompanying histogram (Fig. 6). Since best root growth was obtained at 75° F., in total darkness, in relative humidity of 94-100%, this environmental combination has been termed the optimum and the percentages shown in the histogram are based on the root growth under these conditions considered as 100%.

On an absolute basis, growth under the different environments was as follows:

1. At 60° F., in total darkness in air of 94-100% relative humidity the average leaf had 31.1 active notches. The average number of roots produced by each active notch was 0.69 and the average length of each root was 2.4 mm. Total length of the roots produced by an average leaf was 51.5 mm.
2. Under optimum conditions the average leaf bore 34 active notches with an average of 9.1 roots per notch. The average root length was 7.0 mm. and the total length of roots per leaf was 2189.7 mm. The roots were occasionally branched, the branches usually occuring in whorls of three or four. Few roots less than 10 mm. long displayed branching. Shoot growth was greater in this group than in any other. Under other environments the shoots were usually so small that measurement with the ruler was impossible. In optimun conditions slightly more than half of the shoots growing from the primary notches were measuable and some of them attained a length of 4 mm. The best-developed shoots were usually found in the primary notches. Freeland (1933) has suggested that this distribution

is due to more advanced development of the foliar embryos of the primary notches before the onset of dormancy in these structures.

3. At 90° F., in total darkness in relative humidity of 94-100%, the average leaf bore 28 active notches each of which produced 3.45 roots whose length averaged 6.4 mm. Total root length per leaf was 612.5 mm.
4. At 75° F., in total darkness in a relative humidity of 8-13%, the average leaf bore 26.8 active notches which produced 1.9 roots each. The average root length was 3.1 mm., and the total length per leaf was 163.1 mm.
5. At 75° F., in 16 hours of continuous fluorescent light per day in a relative humidity of 94-100%, the average leaf bore 34.8 active notches each of which produced 2.0 roots. The average length of these roots was 5.3 mm. and the total root length per leaf was 369.0 mm.

Analysis of the data indicated no effect of polarity, from the base of the leaf blade to its apex, on the number or length of roots produced; a possible indication that polar influences do not begin to take effect until the plantlets have reached a later stage of development.

CONCLUSIONS

Results of the writer's qualitative and quantitative experiments add supporting evidence to Mehrlich's (1931) belief that definite physiological varieties or strains of Bryophyllum exist. It is further shown that the Chicago strain of this species possesses marked physiological characters which clearly separate it from the Michigan strain described by Reed (1923) and Mehrlich (1931) and also from the strain or strains of unknown source used by Goebel (1902) and Smith (1921). The mode of origin of these strains is at present unknown.

The differences in the growth rates of the plants of different age groups under similar conditions are believed, in the case of the mature and older leaves, to be due largely to differences in the amount of water available to the plantlets from the leaves whence they arose. However, in the case of the young leaves, which had the highest water content of all groups, this explanation cannot apply. The slow growth of plantlets from leaves of this group may have its explanation in the relative immaturity of its foliar embryos as has been suggested by Freeland (1933). Kakesita (1930) and Mehrlich (1931) found that young attached leaves proliferated much less readily than more mature leaves.

The comparatively slow growth of plantlets in natural light compared to that of the plantlets under the photoperiod screen is believed to be due to the operation of two factors; namely, the retardative effect of light on elongation, and the lower humidity of the air outside the screen. Either one of these factors, as shown by the

quantitative studies, exerts a retardative effect. Under the conditions described it is not possible to say which had the greatest effect.

The results of the quantitative studies indicate that the dormancy breaking mechanism activated by removal of leaves from the plant is not as strongly affected by environment as are the young plantlets. Isolation of the leaf from the parent plant sets up a metabolic pattern in the foliar embryos which is only slightly affected by subsequent environmental conditions during the earliest stages of germination. However, as growth proceeds the young plantlets become increasingly dependent upon the conditions of the external environment. At the same time as its supply of water and nutrients decreases the parent leaf is decreasingly capable of compensating for unfavorable factors in the environment.

It follows then that if the growth of the plantlets, as distinguished from embryo activation, is to be studied from either a quantitative or qualitative standpoint the environmental factors of light, temperature and humidity must be known and reproducible if the results of different investigators are to be correlated. The present study outlines a method by which preliminary investigations of this type have been carried out and presents significant results of these studies.

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