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GEMMAE-CUP PRODUCTION IN MARCHANTIA POLYMORPHA AND ITS RESPONSE TO CALCIUM DEFICIENCY AND SUPPLY OF OTHER NUTRIENTS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 532

PAUL D. VOTH

(WITH SIX FIGURES)

Introduction

Excellent adaptability to nutrient supply and photoperiod has been reported recently for *Marchantia polymorpha* L. (15). Nutrient triangles with relatively small numbers of positions were adequate to effect distinctive growth responses when calcium, phosphate, and nitrate supply was systematically varied. The present study deals principally with the effect of relatively smaller proportions of the six common ions, with particular reference to lower limits of calcium and phosphate supply and to larger concentrations of nitrates. This is accomplished by employing triangles each having fifty-five positions. Similar nutrient triangles have been employed by SCHREINER and SKINNER (11), TRUE (14), and others.

As previously, records were made of changes in gross appearances of the plants, increases in total area, accumulation of dry weight, and formation of gemmae cups. From the data secured it is possible to suggest the relative proportions and concentrations of the commonly used nutrient salts conducive to the greatest vegetative development of this plant. The anatomical details of the apices of plants growing in nutrient solutions deficient in calcium may give clues to the function of calcium in meristematic and differentiating cells and tissues. Comparison of the total number of gemmae cups on antheridial and archegonial thalli seems to offer possibilities in the identification of the sex of vegetative plants of *M. polymorpha*.

General methods

The strains of plants used in this investigation are from the clones used in experiments 8 and 9 (15) and originated as follows:

Culture A: One male plant selected from plants growing in the greenhouses
of the University of Chicago, May 16, 1939.
Culture B: One female plant similarly selected, May 15, 1939.

From the new tips which form dichotomously as the plant grows, these clones have been perpetuated uninterruptedly.

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All experimental work was conducted in the greenhouse, using the culture method of placing six plants on a disk of glass cloth supported by a glass rack and placed in the open half of a moist chamber. Each culture was supplied with about 200 cc. of fresh solution daily (**15**). The plants and apparatus received a daily pressure-rinsing with distilled water, which also served to remove excess algae from the surface of the glass cloth and from the water line in the moist chamber.

All plants grew for 32 days, September 24 to October 27, 1940. Supplementary light was supplied with 200-watt Mazda filament bulbs in reflectors, suspended about 33 inches above the plants. A maximum of 200 foot-candles directly underneath the reflectors and more than 100 foot-candles between the lights at the level of the table top was recorded at the beginning of the experiment, when reflection from the glass cloth was maximum and plant size minimum. These lights were on from sundown until 2:00 A.M., a total photoperiod of 18 hours in each 24-hour period.

Because of experience gained previously, the phosphate supply was considerably decreased, the nitrate supply was increased, and the other ions were given in relatively the same proportions as in experiments 8 and 9. The molar concentration of each ion as well as the amount of each salt used is shown in table 1.

Of the fifty-five units constituting each triangle, only thirty-seven were actually used in the present study. Since the omission of a single ion resulted in striking symptoms in previous experiments (particularly K, Ca, NO_3, and PO_4), many positions along the sides of the triangles were not repeated. The positions actually employed in this study are indicated by squares and the unused ones by circles in figure 1. In a similar triangle, anions were varied and cations remained constant; the NO_3 position was at the top, PO_4 at the lower left, and SO_4 at the lower right.

Male and female plants of cultures A and B, respectively, were grown in separate moist chambers, but the two cultures were always adjacent to facilitate changing of solutions and to provide as comparable a habitat as possible for both sexes.

As in experiments 8 and 9, gemmae cups were counted, all plants were photographed by groups, areas were determined photometrically, fresh weights were recorded, one plant from each dish was preserved in formalin – acetic acid – alcohol preservative, and the remaining plants were weighed when dry. The percentage dry weight of five plants was used to calculate the dry weight of six plants. The present investigation is designated experiment 10.

Investigation

RESPONSE TO OMISSION OF IONS

CATION TRIANGLE.—Each *Marchantia* cutting, when planted on September 24, averaged 3 sq. cm. in area. Plants on positions 10, 28, 1, and 55 received no calcium, and the latter two were given no magnesium and no potassium, respectively. These cultures grew only slightly, with a maximum final area of only about three times the original. A smaller final area than the original may be attributed to disintegration of the dead plants (solution 55). After 4–6 days all plants in cultures deprived of calcium became black tipped and finally died, except a few which regenerated new thalli from the ventral surface of the older plant. Plants on solution 55, lacking calcium and potassium, blackened soonest and were smallest in area.

TABLE 1

CONCENTRATION OF SALTS IN THE SIX STOCK NUTRIENT SOLUTIONS USED IN MAKING UP 74 OTHER COMBINATIONS. TO EACH SOLUTION WAS ADDED 0.2 P.P.M. OF $MNSO_4$, $ZNCl_2$, AND $NA_2B_4O_7$, AND ALSO 0.02 P.P.M. OF $FESO_4$. THE NINE SALTS ARE IDENTICAL WITH THOSE OF EXPERIMENTS 8 AND 9 (15)

MOLAR CONCENTRATION	SALT CONTENT (CC. OF 0.5M SOLUTION) PER L.	MOLAR CONCENTRATION	SALT CONTENT (PER LITER)	MOLAR CONCENTRATION	SALT CONTENT (PER LITER)
CATIONS VARIED					
K SOLUTION		CA SOLUTION		MG SOLUTION	
K......0.0054	....	Ca.....0.0027	....	Mg....0.0029	....
NO_3....0.0032	6.40*	NO_3...0.0032	3.20 cc. of 0.5M	NO_3...0.0032	3.20 cc. of 0.5M
PO_4.....0.0004	0.80†	PO_4....0.0004	0.0504 gm.‡	PO_4....0.0004	0.0698 gm.§
SO_4.....0.0009	1.80	SO_4....0.0009	0.1550 gm.	SO_4....0.0009	1.80 cc. of 0.5M
ANIONS VARIED					
NO_3 SOLUTION		PO_4 SOLUTION		SO_4 SOLUTION	
NO_3....0.0051	....	PO_4....0.0042	....	SO_4....0.00255	....
K......0.0009	1.80*	K.....0.0009	1.80 cc. of 0.5M†	K.....0.0009	0.90 cc. of 0.5M
Ca.....0.0012	2.40	Ca.....0.0012	0.3026 gm.‡	Ca.....0.0012	0.2066 gm.
Mg.....0.0009	1.80	Mg....0.0009	0.1570 gm.§	Mg....0.0009	1.80 cc. of 0.5M

* As KNO_3. † As KH_2PO_4 ‡ As $Ca(H_2PO_4)_2 \cdot H_2O$. § As $MgHPO_4 \cdot 3H_2O$, etc.

When potassium is omitted from nutrient solutions, as in positions 49, 52, 46, and 55 (with the latter two also lacking magnesium and calcium, respectively), the growth responses are as follows: Plants on solution 55 respond to the omission of calcium after a few days. After 18 days, position 46 still possesses small plants (fig. 2), but the characteristic tan color of the older thallus parts—especially along the margins of the wings near the tip—indicates a lack of potassium.

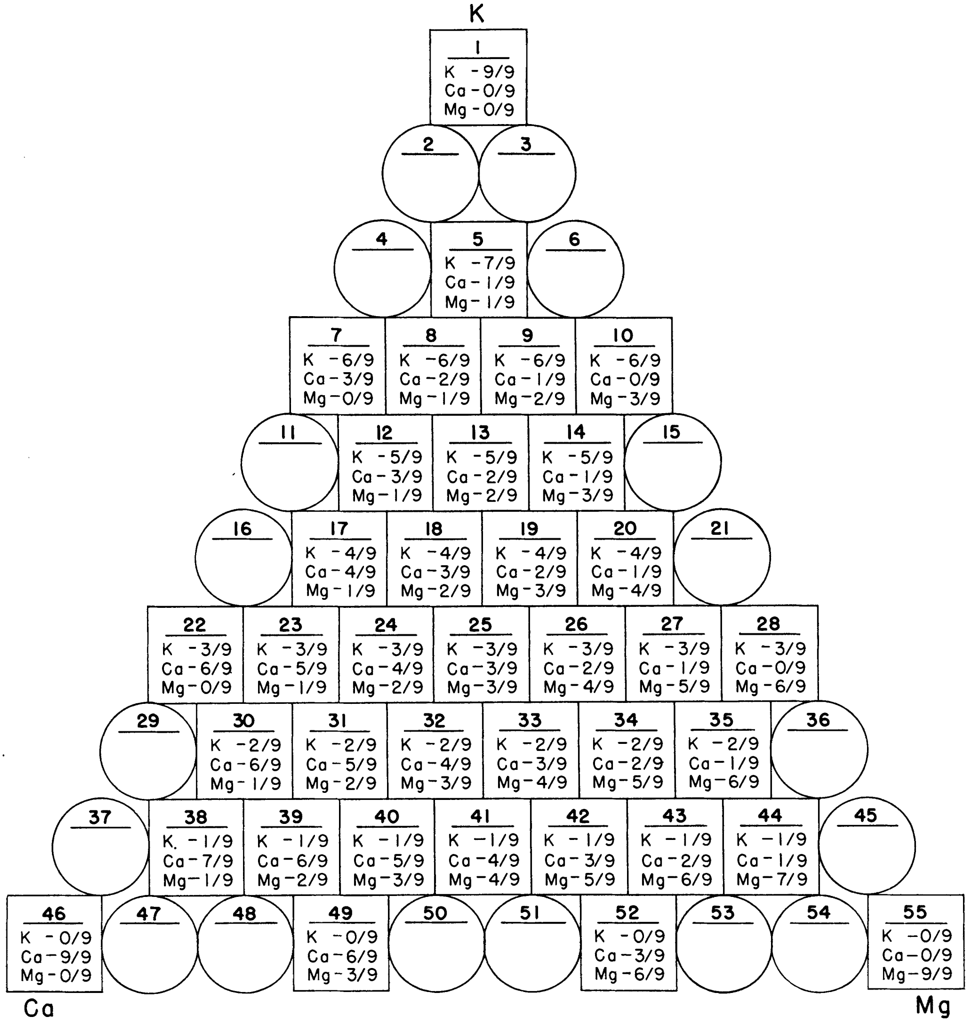
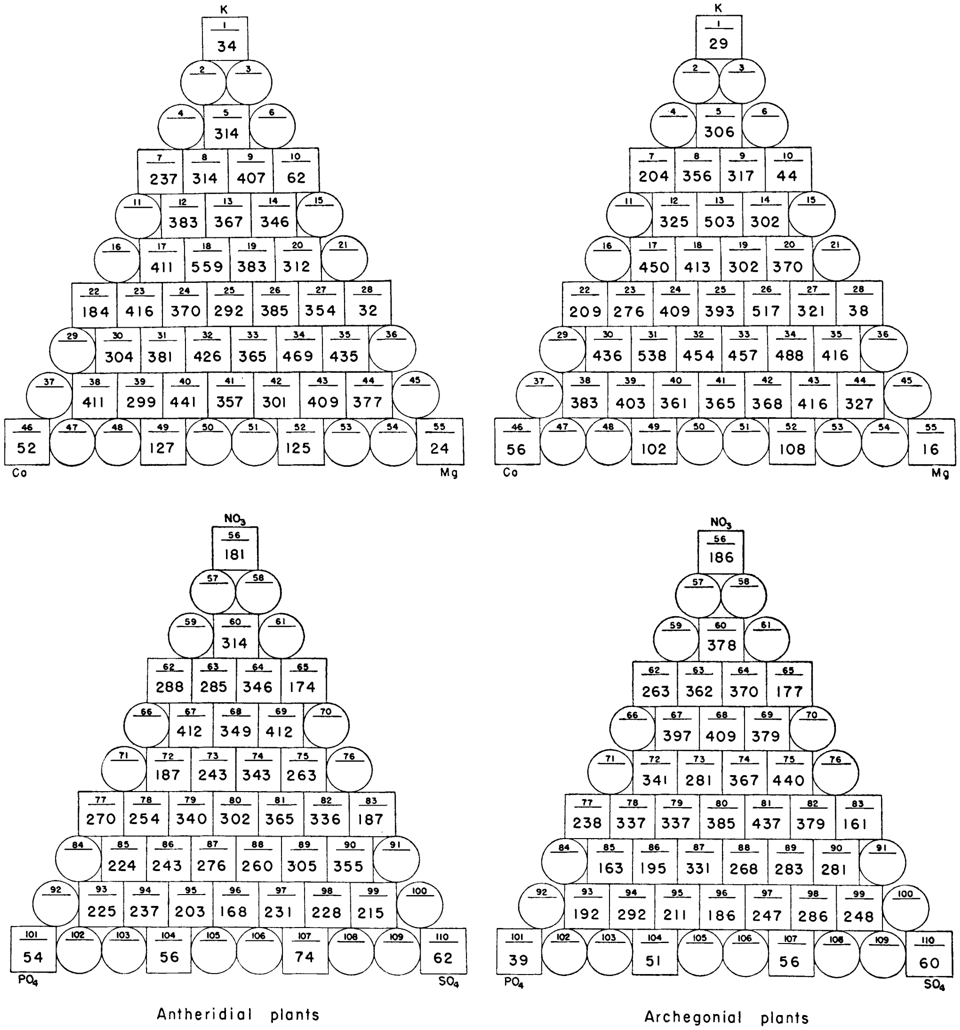


FIG. 1.—Cation triangle showing method of combining stock solutions, on basis of ninths, to secure a range of fifty-five solutions varying in cations and constant for anions. Each apical position lacks two cations; each side of the triangle, one; and the center is supplied with all three in varying proportions. A similar triangle for varying anion combinations was employed: NO_3 at top, PO_4 at lower left, and SO_4 at lower right.

The remaining two cultures lacking potassium develop larger plants without conspicuously brittle margins but with the light brown color in the basal portions of the plants.

The omission of magnesium (solutions 7, 22) affected the total area as well as the dry weights of the plants adversely, but in appearance these plants resembled adjoining cultures receiving a full complement of nutrient ions. The cultures lacking magnesium and another cation responded according to the pattern usual for lack of calcium or potassium (solutions 1 and 46, respectively).



Antheridial plants

Archegonial plants

FIG. 2.—Area in square centimeters of six plants growing in each nutrient combination. Two triangles at left are male plants; at right, female.

ANION TRIANGLE.—When planted on September 25, each cutting averaged 3 sq. cm. in area. Plants growing in solutions containing no nitrate (101, 104, 107), no phosphate (56, 65, 83), or both (110), developed darker midribs; and scales, rhizoids, and lower epidermis became red after about 10 days. In all these positions algae were nearly absent from the surface of the glass cloths and of the solutions during the entire experiment. After about 2 weeks the plants lacking nitrates failed to gain in size, remained relatively narrow, produced practically no new gemmae cups, and forked dichotomously only infrequently, so that apical growing points were relatively few in number. The color of the upper surface of the plants became more yellowish as time went on.

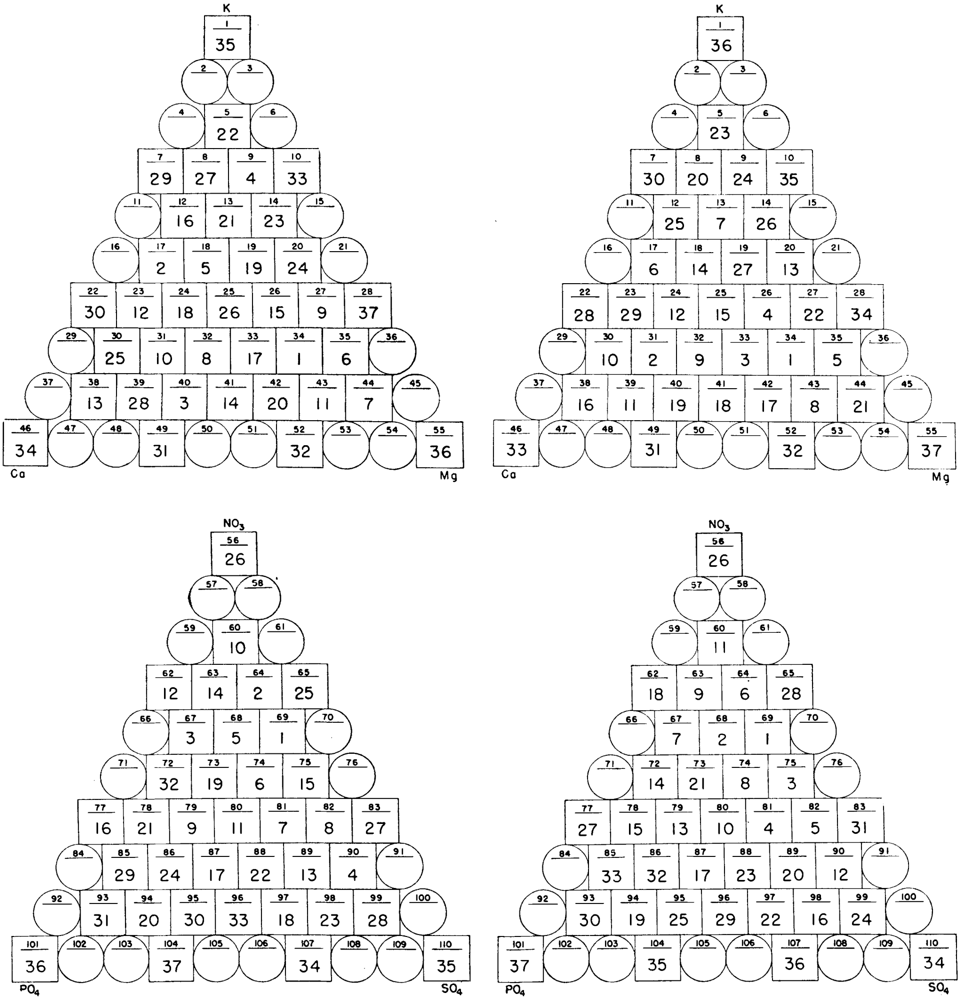
The omission of phosphorus resulted in a different response after 2 weeks. Plants possessed a very dark midrib region, and the intensely green color of the upper surface was superimposed on the red color of the under surface. Dichotomy occurred frequently, giving the plants a rosette-like appearance. The plants were wider than average, and gemmae cups were very numerous (fig. 6). Another characteristic of plants lacking phosphorus was the tendency to be closely appressed to the glass cloth. Whether this is a phototropic or a geotropic response, or influenced by chemical or by mechanical stimuli, has not been determined. Only two antheridiophores formed on plants growing in the entire anion triangle—one each on solutions 65 and 69, the former lacking and the latter low in phosphorus.

Omission of sulphate from the solutions failed to alter the gross appearance of the plants and influenced dry weights only slightly (fig. 3). There may have been sufficient sulphur present in the atmosphere, since no special precautions were taken to exclude greenhouse air from the cultures.

RESPONSE TO COMBINATIONS OF ALL IONS

CATION TRIANGLE.—Plants in the twenty-eight positions in the center of the triangle all continued active growth during the entire period. As in previous experiments, the majority of cultures surrounding the exact center (solution 25 in this experiment) were larger in area, or heavier on the basis of dry weight, or both, when compared with the central position. This observation possibly has no great significance until additional data are available. Dry-weight accumulation by both sexes was greatest in position 34 and had a nutrient supply of $\frac{2}{9}$ K, $\frac{2}{9}$ Ca, and $\frac{5}{9}$ Mg solutions. Each triangle of fifty-five possible positions was treated as a unit in assigning rank. With only thirty-seven positions in use, the greatest dry weight accumulation is designated as rank 1 (position 34 in the cation triangles supporting male and female plants, respectively) and the least dry weight as rank 37 (position 28 in the cation triangle of male plants and position 55 in the cation triangle of female plants—both positions from which calcium is absent). The uppermost triangles in figure 3 show that within the central part of the cation triangle the distribution of rank is fairly diverse, indicating that K, Ca, and Mg may be present in rather wide ranges of concentrations and combinations and still be suitable for healthy growth of *Marchantia*.

ANION TRIANGLE.—Of the twenty-eight centrally located positions, plants in the row receiving $\frac{1}{9}$ PO_4 (solutions 60, 64, 69, 75, 82, 90, and 99) appeared most robust after the first 2 weeks, with the exception of plants on solution 99. Plants on solutions 64 and 69 made observably greater growth during the entire growing period, and on the basis of dry weight the latter ranked highest among male and female cultures in the anion triangle (fig. 3). Toward the base of the triangle,



Antheridial plants Archegonial plants

FIG. 3.—Rank on basis of dry weight. Two triangles at left represent male plants; at right, female. Each triangle is a unit for ranking. Rank 1, greatest dry weight, is in same position (no. 34) in cation triangle for male and also female plants. Similarly, rank 1 in anion triangles is in same position for both male and female plants (no. 69).

where proportions of nitrates decreased progressively, plants were correspondingly smaller (fig. 2) and had the lowest final dry weights of the complete nutrient plants (fig. 3). Male as well as female plants accumulated the greatest dry weight in position 69, with a solution mixture of $\frac{5}{9}$ NO_3, $\frac{1}{9}$ PO_4, and $\frac{3}{9}$ SO_4. When all six ions were present and anions were varied, increased growth in area and greater accumulation of dry weight were definitely correlated with increased nitrate and decreased phosphate supply, even though the former was greatly increased and the latter decreased in the stock solutions of this experiment. These relationships are summarized in table 1 and figure 3.

Response to small amounts of calcium

After 24 days some of the plants in the row of the cation triangle receiving only 1 part of calcium to a total of 8 parts of potassium and magnesium began to develop black tips (fig. 4). Often the wings near the apical notch became black first, followed by darkening of the region of meristematic activity; or at times the region of the apical cell(s) suddenly became dark. A V-shaped zone soon developed progressively in a posterior direction, as shown in a plant tip near the upper center of figure 4, 20♂. On many plants the blackened areas became watery. By the end of the experiment, a few days after the first symptoms were noted, many of the blackened tips were dry, curled upward, and were brittle (fig. 4). No correlation seems to exist between this sudden death of the terminals and the factors which were obviously varied—the concentrations of potassium and magnesium. Since the only common factor in all these cultures was a $\frac{1}{9}$ Ca supply, it may be assumed that the low concentration of the calcium ion is responsible for these symptoms. Injury was observably greatest in male and female plants growing in solution 5. The following solutions are listed in the order of decreasing injury to the respective *Marchantia* cultures: 5♂ and 5♀ > 9♂ > 35♂ and 35♀ > 20♀ > 27♂ > 20♂ > 44♂ and 44♀. No external evidence of "burning" was noted in plants growing in solutions 27♀, 9♀, 14♂, and 14♀. In all the preceding cultures a $\frac{1}{9}$ proportion of calcium with reference to the other cations in solution is equivalent to a calcium concentration of 0.3 millimols per liter, or 12 p.p.m.

Anatomical effects of calcium deficiency

When calcium is absent or deficient in any particular plant, the anatomical effects on the cells and organs of that plant often are characteristic enough to be diagnostic for this element. Death of the stem apex (3, 5, 6, 7, 8), abnormalities and death of root tips (1, 4, 7, 8, 12, 13), hooking downward of the tip of young leaves in the bud (5), and yellowing of the youngest leaves (7) followed by necrosis of leaf tips and margins (5, 6) have all been reported for several flowering plants. Premature vacuolization and eventual loss of all cytoplasm in the elongating cells

FIG. 4.—Portions of archegonial plants (top) and antheridial plants (bottom) of *M. polymorpha* growing on nutrient combination 20, with Ca supply of $\frac{1}{9}$. Several plants of both sexes exhibit blackened tips, most of which turn upward. ×$\frac{3}{5}$.

of the root tips have been reported for Japanese millet, rape, buckwheat (4), wheat, corn (1), pea (12, 13), and other plants. In rape, cells of the cortex of the stem were large and the stele relatively small when compared with the cortex. In buckwheat the situation was reversed. A similar diversity of response was reported in the length of the palisade cells when calcium is withheld from these two plants (4). Roots of peas growing in the absence of calcium lack resistance to overcome infection by microörganisms (13).

When *Spirogyra* and *Zygnema* are grown on minus-calcium solution, the chloroplast loses its conspicuous lobing, becomes narrow, and soon lacks starch. Excessive vacuolization was followed by general cytoplasmic disorganization, although even after 6 weeks nuclear division was reportedly unaffected but the wall between newly formed nuclei failed to form. After 2 months of calcium absence the apical cell of a *Spirogyra* filament was the first to possess a disorganized chloroplast and to contain a dark substance related to the lecithins (9).

The responses of the gametophyte of *Marchantia* to deficiency or lack of calcium were investigated in detail anatomically. Thallus tips from plants receiving only $\frac{1}{9}$ Ca (solutions 5, 9, 14, 20, 27, 35, and 44), as well as tips from initial control plants growing on a complete nutrient stock solution, and a few tips from cultures surrounding the low-calcium triangle positions, were imbedded in paraffin, sectioned at 7 μ, and stained in Flemming's triple stain. Nearly all tips were sectioned longitudinally. Oblique and transverse sections of the tip confirmed observations made on longitudinal ones. Transverse sections of older parts of the plant are of less value in this phase of the study, since nearly all cells a few millimeters back of the growing tip are mature and practically devoid of cytoplasm and much resemble cells of healthy plants. The walls of the cells of the older portions often stain intensely with methyl violet when the cells of the tips of the same lobe are necrotic. DAY (2) found only slight variation between transverse sections of pea roots taken from healthy and calcium-deficient plants, but the amount of elongation was distinctive.

Plants growing as initial controls on a complete nutrient solution possess an apical growing region characterized by much meristematic activity. The apical cell is not clearly distinguishable from many of the surrounding cells (fig. 5, control ♀). The meristematic zone is several cells wide and merges gradually into the zone of cell enlargement and maturation. Cells and air chambers of the dorsal photosynthetic layer differentiated gradually from the surface layers of the apical meristem (fig. 5, control ♀). In the same treatment ($\frac{1}{9}$ Ca supply) not all plants responded alike; some were entirely necrotic whereas others showed slight injury. As a result, tips taken at random might not represent the state of an entire culture accurately. Many sections from cultures showing a deficiency of calcium possessed degenerating cells (fig. 5, 14♀, 9♂, 35♀). Longitudinal sections of black-

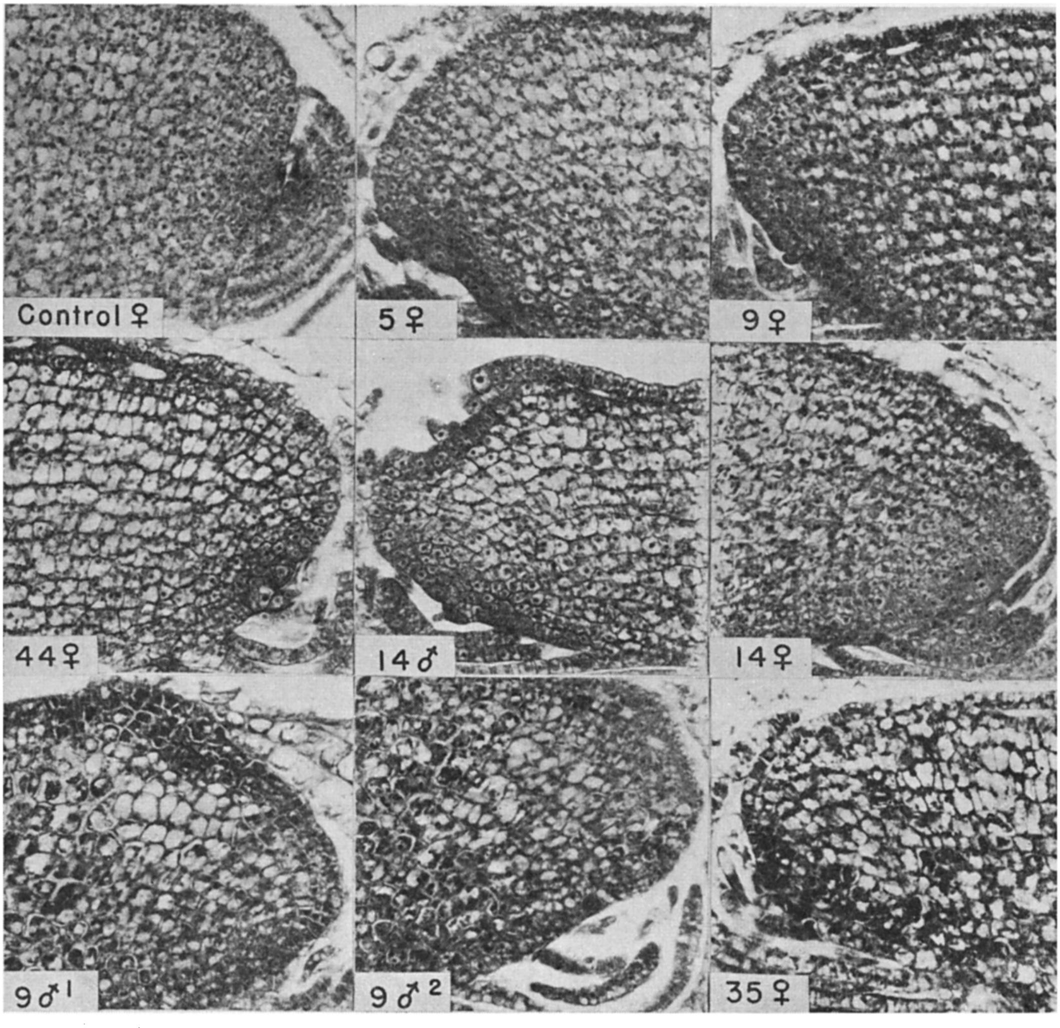


FIG. 5.—Vertical longitudinal sections through apical notch of *M. polymorpha*. Control is growing as stock plant on complete nutrient solution. Numbers indicate position in triangles. Apical cells show most clearly in 44 ♀, 14♂ 14 ♀, and 35 ♀.

ened tips showed only advanced stages of terminal degeneration, with no recognizable apical cell or even apical notch area. All sections shown in figure 5 were from tips which possessed no external evidence of necrosis, such as discoloration, and no collapse.

First evidences of unhealthy conditions found in longitudinal sections through the tip of a calcium-deficient plant are the larger vacuoles in the cells of the maturing region, the darker-staining nuclear zones, and the smaller number of chloroplasts. These conditions are shown in figure 5, position 9♀ (and to a lesser degree position 5♀), even though plants in the former position possessed no outward evidences of unhealthy development, while many plants in the latter culture had blackened tips. REED (10) describes the premature vacuolization and polarization of meristematic cells of the stem tips of apricot and peach trees and the accumulation of phenolic materials in maturing cells when zinc is deficient, but subsequent necrosis when this element is lacking or deficient is not reported.

Associated with the internal symptoms just noted, the meristematic zone is limited to a few concentric layers of cells derived from the apical cell, all containing a relatively large proportion of protoplasm and small vacuoles (fig. 5, 9♀ and 5♀). When such abrupt zonation becomes especially evident, the apical cell is easily distinguishable (fig. 5, 44♀ and 14♂). Meristematic activity continues in the dorsal region, where air chambers develop (fig. 5, 44♀). Gemmae-cup differentiation also continues (fig. 5, 14♂), even when cells below the single floor layer of the cup are mature—as shown by large cell size and extreme vacuolization. The ventral segment of the apical cell in position 44♀ (fig. 5) is in the process of transverse division, but the spindle is not clear because of the reduction in size of this figure.

Following the period of limited meristematic activity with concomitant preponderance of mature cells, necrosis in the dorsal areas (near the tip of the plant) occurs (fig. 5, 14♀). At times this zone of dead cells involves an embryonic gemmae cup (fig. 5, 9♂^{1}). The breakdown of dorsal tissues is in contrast to the longevity and persistence of the small ventral cells which surround the bases of smooth rhizoids and are the source of regenerated thalli (15).

Most tips in which necrosis is beginning possess apical cells which apparently are still functioning. Sections of a single tip in position 9 (fig. 5, 9♂^{1} and 9♂^{2}) show that dichotomy is still in progress, since different apical cells are present in these sections, which are located on one microscope slide. One section apparently has a living apical cell, while a few microns farther on (fig. 5, 9♂^{2}) the other half of the dichotomy possesses a necrotic apical cell region and a zone of living cells forming a V just posterior to the apex. As in position 35♀ (fig. 5), all cells of the tip eventually die.

The next event usually is collapse of the cells of the tip, accentuated or even initiated by decay. Cells in the interior of the older portions of the thallus collapse (15, figs. 11, 12). Regeneration of plants from the small cells surrounding the base of smooth rhizoids located a few millimeters posterior to the tip has been described previously (15).

The rank of male and female plants in the upper two triangles of figure 3 indicates that, despite the unhealthy internal condition of most of the tips in the $\frac{1}{9}$ Ca row, accumulation of dry weight was better than average and in some instances nearly maximum for this experiment. Until the tips began to blacken, the gross appearance of these plants was among the best in the cation triangle.

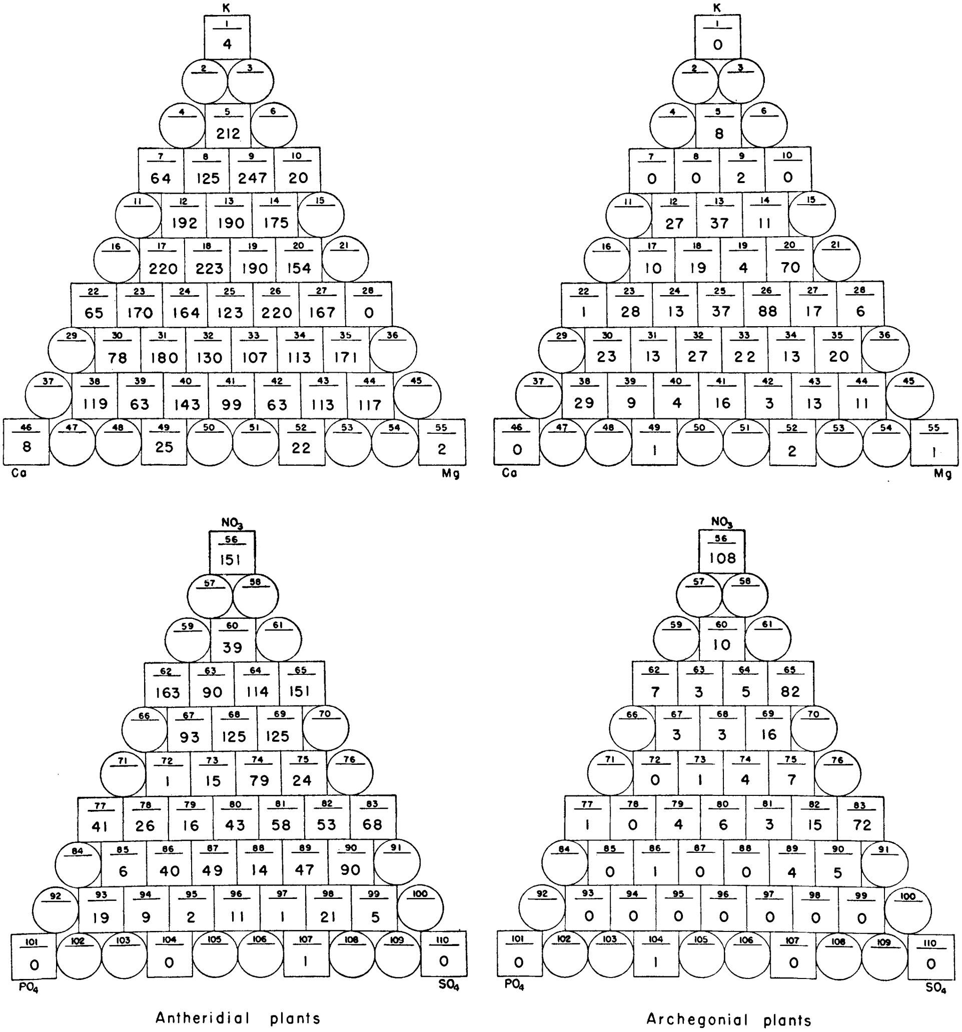
VEGETATIVE CHARACTERS CORRELATED WITH SEX

In experiments 8 and 9 (15), where antheridial and archegonial plants were not grown simultaneously, the number of gemmae cups on the female plants was considerably less than on the male plants (568 on ♀, 2300 on ♂; a ratio of 1:4), but no significance was expressed since the data were insufficient to warrant a conclusion. By growing both sexes simultaneously under comparable conditions in the experiment under discussion, the earlier results were confirmed (946 gemmae cups on female, 6268 on male plants; a ratio of 1:6.6) and a rough correlation between sex and gemmae-cup production was established. Total gemmae-cup counts for each culture, sex, and triangle are given in figure 6.

Other factors being equal, decrease of the phosphate supply tends to favor greater gemmae-cup production. When all phosphates are absent, however, the number of cups on male plants tends to decrease while on female plants the number increases. Cup ratios are average when phosphates are present in as little as $\frac{1}{9}$ proportion (0.47 millimols per liter); but when phosphates are omitted the ratios are much lower, so that a male plant cannot be distinguished from a female on this basis. As shown in figure 6, decreased nitrate supply results in the production of fewer gemmae cups. Maximum number of cups seems to depend, therefore, largely upon a high nitrate level and a very low phosphate supply. When nitrate supply is high and all common ions are present in the nutrient solution, the sex of vigorously growing cuttings of *M. polymorpha* may be determined reasonably accurately by observing the thallus width approximately 1 cm. back of the apical notch and of the wings. Male plants are narrow when compared with female plants.

The upper surface of vigorously growing female plants is smoother and reflects light more readily than similarly cultured male plants. Whether this appearance is dependent upon differences in cuticle, size of air chambers, curvature of air-chamber roof, relative protrusion of chimney cells of the pore, or upon a combination of these factors is not yet determined.

In general the margins of the male plant are more undulating and tend to curve downward abruptly, especially if infested with blue-green algae or growing in a medium with a high pH. Often the marginal row of ventral scales tends to curve out and up, forming a wavy white margin in the male plants. Under these conditions, the smaller number of gemmae cups, the broader thallus tip, and the plane



Antheridial plants Archegonial plants

FIG. 6.—Number of gemmae cups on six plants of each culture. Counts on male plants in two triangles at left, female at right. Counts include cups present on original cutting. Ratio of cups on all male plants to those on female is 6.6 : 1. In anion triangle greatest cup number is associated with decreasing amounts of PO_4 in nutrient solution. Omission of PO_4 results in very slight differences in number of cups on male and female plants. Larger numbers of gemmae cups and higher nitrate supply correlated positively.

surface of the female plants, known as culture B, serve to distinguish it from culture A, the male plants. These distinctions have definite taxonomic value in the clones just mentioned. Even with a low calcium supply (fig. 4), the distinction between culture A (bottom) and culture B (top) is possible on the basis of gemmae-cup number and gross appearance. Cultures of *M. polymorpha* plants from widely scattered localities in the United States are now being studied to determine the validity of these observations on other clones and to determine a possible correlation between sex and the relative number of gemmae cups produced by any particular culture. If a positive correlation is found, indicating possible sex linkage, further inquiry into the mechanisms of gemmae-cup initiation and factors influencing their development would be highly desirable. If maleness and the presence of large numbers of gemmae cups are not associated in other clones, an extensive field of genetic investigation would be opened. Similarity in number of gemmae cups on both male and female gametophytes when phosphates are absent indicates a need for study of the possible relation of this ion to the factors concerned in the expression of sexual characters.

Gametangiophore production

On the 888 plants grown during the present study, only eleven antheridiophores and three archegoniophores differentiated. This response is to be expected, since light intensity decreased during the term of the experiment (September–October).

Summary

1. As in previous experiments with *Marchantia polymorpha*, the omission of K, Ca, NO_3, or PO_4 ions results in characteristic differences in the gross appearances of the plants. The lack of K produces plants with tan-colored bases and slightly narrower tips. Absence of Ca results in almost immediate death of the growing tips. Deficiency of NO_3 and PO_4 is indicated by reddening of scales, of rhizoids, and of lower epidermis. Plants lacking the former ion become light green, possess few gemmae cups, and fork infrequently; but plants growing on solutions lacking PO_4 soon become dark green, have abundant gemmae cups, and because of frequent dichotomy are rosettes. Deficiencies of Mg and SO_4 are not indicated by any characteristic symptoms.

2. With a concentration of 0.3 millimols of calcium per liter of solution, death and degeneration of the plant apices result. Microscopically an early and rapid vacuolization occurs in enlarged cells near the apical cell. Necrosis first begins in the more nearly mature cells and continues until the entire tip is dead.

3. When grown on glass cloth and supplied with abundant nitrates, plants with many gemmae cups, narrower thalli, and incurved margins are male, belonging to culture A. Plants of culture B (female) have fewer cups, broader thalli, and plane surfaces.

4. On the basis of dry-weight accumulation and total area of plants, optimum vegetative growth is evident in certain areas of each triangle. On this basis, and with consideration of the gross and microscopic aspects of the plants, molar concentrations of the six ions may be suggested as meeting the requirements of *M. polymorpha:* K 0.0012, Ca 0.0007, Mg 0.0014, NO_3 0.0034, PO_4 0.0004, and SO_4 0.0008 mols per liter. In practice, a solution may be made up using the following quantities of a 0.5*M* solution of each of the following salts: KNO_3 1.6 cc., $Ca(NO_3)_2$ 1.4 cc., $Mg(NO_3)_2$ 1.2 cc., KH_2PO_4 0.8 cc., and $MgSO_4$ 1.6 cc. per liter of solution. Slight variations in the proportions of these salts do not affect the mineral requirements of this plant.

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LITERATURE CITED

1. BAMFORD, RONALD, Changes in root tips of wheat and corn grown in nutrient solutions deficient in calcium. Bull. Torrey Bot. Club **58**:149–178. 1931.
2. DAY, DOROTHY, Some effects of calcium deficiency on *Pisum sativum.* Plant Physiol. **4**:493–506. 1929.
3. DE TURK, E. E., Plant nutrient deficiency symptoms. Physiological basis. Indust. and Eng. Chem. **33**:648–653. 1941.
4. LUTMAN, B. F., Cell size and structure in plants as affected by various inorganic elements. Vermont Agr. Exp. Sta. Bull. 383. 1934.
5. MCMURTREY, J. E., JR., Relation of calcium and magnesium to the growth and quality of tobacco. Jour. Amer. Soc. Agron. **24**:707–716. 1932.
6. ———, Distinctive plant symptoms caused by deficiency of any one of the chemical elements essential for normal development. Bot. Rev. **4**:183–203. 1938.
7. NIGHTINGALE, G. T., Potassium and calcium in relation to nitrogen metabolism. BOT. GAZ. **98**:725–734. 1937.
8. NIGHTINGALE, G. T., ADDOMS, RUTH M., ROBBINS, W. R., and SCHERMERHORN, L. G., Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. **6**:605–630. 1931.
9. REED, H. S., The value of certain nutritive elements to the plant cell. Ann. Bot. **21**:501–543. 1907.
10. ———, Effects of zinc deficiency on cells of vegetative buds. Amer. Jour. Bot. **28**:10–17. 1941.
11. SCHREINER, OSWALD, and SKINNER, J. J., The triangle system for fertilizer experiments. Jour. Amer. Soc. Agron. **10**:225–246. 1918.
12. SOROKIN, HELEN, and SOMMER, ANNA L., Changes in the cells and tissues of root tips induced by the absence of calcium. Amer. Jour. Bot. **16**:23–39. 1929.
13. ———, Effects of calcium deficiency upon the roots of *Pisum sativum.* Amer. Jour. Bot. **27**:308–318. 1940.
14. TRUE, R. H., The function of calcium in the nutrition of seedlings. Jour. Amer. Soc. Agron. **13**:91–107. 1921.
15. VOTH, P. D., and HAMNER, K. C., Responses of *Marchantia polymorpha* to nutrient supply and photoperiod. BOT. GAZ. **102**:169–205. 1940.