

## NOTE ON THE HISTOLOGY OF GRAIN ROOTS

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In the winter of 1920, Dr. Sophia Eckerson and the writer were engaged in a microchemical study of the physiology of the absorption of different salts by plants grown in water cultures, especially *Zea mays* (white dent) and *Triticum vulgare*. The experimentation was carried out in Dr. Rodney H. True's laboratories in the U. S. Bureau of Plant Industry, at Washington. In this work it was observed that roots of Indian corn and wheat grown in solution cultures showed an interesting structural feature that has apparently not yet received attention in the literature. Openings appeared in the root cortex, the time and place of their appearance varying with the plant and with the solution used. No serious study of the causes involved in the formation of these openings was attempted, but some histological observations were made as to the general manner of their occurrence. Hand sections of the roots were used. Since it was necessary to discontinue these studies for the time being, this note has been prepared in order to present the information which has thus far been gained.

The seeds were germinated between sheets of filter paper moistened with distilled water, and the seedlings were placed in the solutions when the roots were 1-3 cm. long. Some cultures were maintained in darkness at a temperature of about 18° C., while others were under laboratory conditions at a temperature between 17° and 22° C. Tests showed that the solution concentration varied considerably during an experiment; the solution was not changed during the culture period, 7-14 days. Single-salt solutions of  $\text{Ca}(\text{NO}_3)_2$ ,  $\text{Mg}(\text{NO}_3)_2$ ,  $\text{KNO}_3$ ,  $\text{CaSO}_4$ ,  $\text{MgSO}_4$ , and  $\text{K}_2\text{SO}_4$  were employed, each with 0.00024 normal concentration. Also, both plant forms were grown in 3-salt solutions containing  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$ , with a trace of  $\text{FePO}_4$ . Openings were found in plants of both species grown in each of these solutions, these openings appearing in the corn roots after 3 or 4 days, much sooner than in the case of wheat. They appeared in wheat roots, in all the single-salt solutions, after 8 days, developing more rapidly in solutions with the calcium salts than in those with either the magnesium or the potassium salts.

As to the openings themselves, when they are first observed they are of about the size of the adjoining cortical cells, as shown in figure 1, and appear like large intercellular spaces formed by a separation of adjacent cells in the cortical tissue. In later stages the openings appear larger, and there is evidence (at least in the case of corn) that some cells have broken



down, as if the openings had been enlarged through the disintegration of adjoining cells. In corn roots with well developed openings, fragments of cell walls are often seen projecting into the openings, as shown in figure 3. In a wheat root that has been growing fairly rapidly for a period of eight days, the openings are 1 or 2 cortical cells in diameter and are nearly spherical. At a later stage about nine openings are seen in a cross section of the root, separated by septa composed of one or two layers of unmodified cortical cells.

It was thought that this occurrence might be due to lack of proper physiological balance in the nutrient medium. The single-salt solutions each contained only two of the six essential elements, and there was no evidence that the 3-salt solutions employed were well balanced; moreover they had not been renewed as frequently as is customary in water-culture work. This suggestion was tested by employing Shive's solutions R<sub>1</sub>C<sub>2</sub> and R<sub>2</sub>C<sub>5</sub> (optimal series), which are to be regarded as at least fairly well balanced for wheat. The solutions were renewed on the fourth day and the cultures were continued for 10 days, beginning March 1. Openings appeared just as before, being visible on the fifth day and well developed on the seventh.

Beginning about the middle of June a few experiments were carried out for the sake of further observations on the openings in question. Cultures were grown in Shive's solution R<sub>5</sub>C<sub>2</sub> (sub-optimal series), in a good garden soil and in sand. Marquis wheat, from the lot of seed used in the cooperative project of the Division of Biology and Agriculture, of the National Research Council<sup>1</sup> was used, and the water-culture seedlings were supported by perforated cork stoppers in pint "Mason" jars.

The solution cultures were in the greenhouse at temperatures varying between 25°-45° C. Some of the solutions were renewed daily for the first three days, but this treatment seemed to exert no influence on the formation of the openings, which appeared at about the same time and in the same way in all cultures.

Rather large intercellular spaces were observed on the fifth day. Two days later a cross section of the region 4-6 cm. below the seed appeared as is shown in figure 1. On the ninth day the openings were larger and presented the appearance shown in figure 2. The roots were then 18 cm. long and openings were present 2 to 14 cm. below the seed, those toward the tip of the root being smaller. The smaller openings certainly contained gas. All the plants of these solution cultures appeared to be healthy and vigorous throughout the experiment; on the thirteenth day, when the cultures were discontinued, the shoots were about 19 cm. high.

The sand and soil cultures mentioned above were continued for 13 days in the greenhouse, at temperatures between 25° and 45° C., the media

<sup>1</sup> Livingston, B. E. A plan for cooperative research on the salt requirements of representative agricultural plants. Baltimore, 1919.



being kept as moist as in ordinary greenhouse cultures. At the end of this time the shoots were about 15 cm. high, while the roots were about 15 cm. long. There was no indication in any of these roots of openings in the cortex. The plants grew somewhat less rapidly in the soil and sand cultures

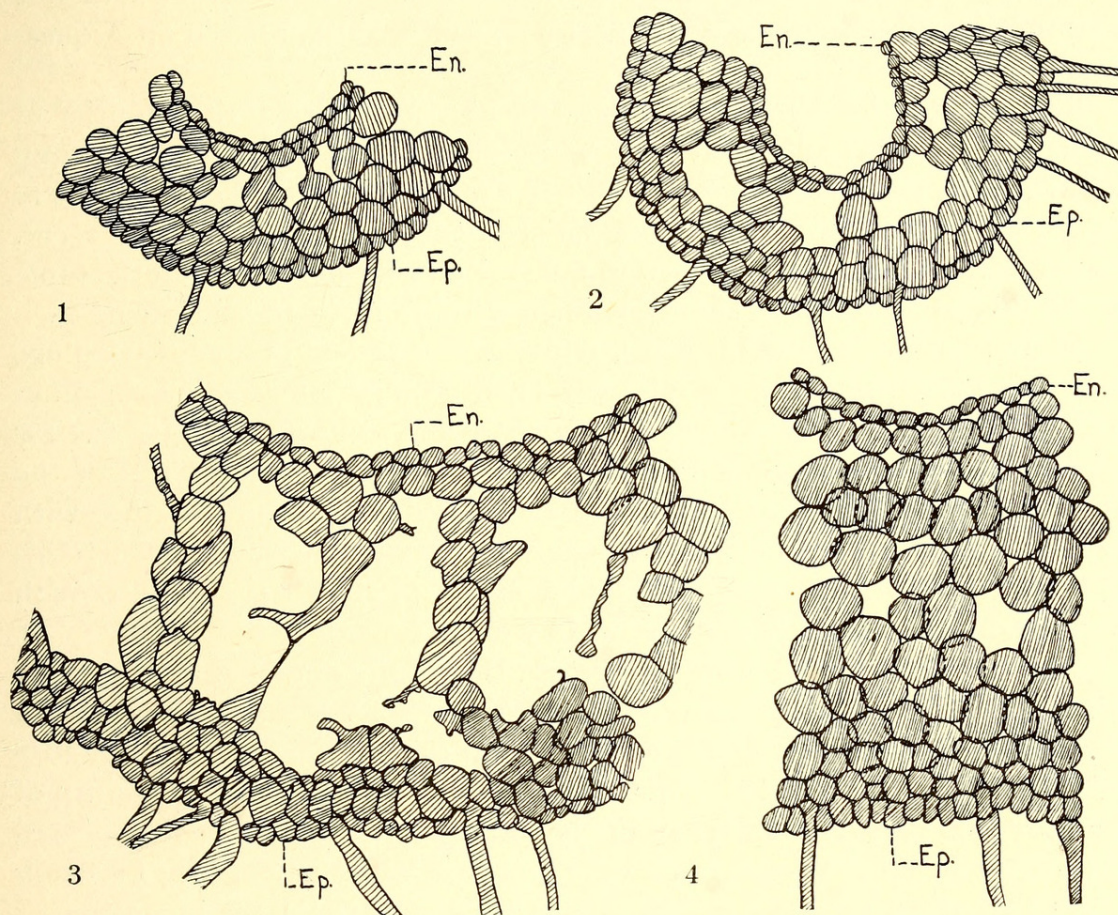


FIG. 1. Transverse section of wheat root, 4 cm. below seed. Plant grown 7 days in 3-salt solution.

FIG. 2. Transverse section of wheat root, 4 cm. below seed. Plant grown 9 days in 3-salt solution.

FIG. 3. Transverse section 3½ cm. below seed of corn root 26 cm. in length. Plant grown 8 days in garden soil.

FIG. 4. Transverse section 3 cm. below seed of corn root 18 cm. in length. Plant grown 8 days in garden soil.

*En.* endodermis; *Ep.* epidermis.

than in the solution cultures, but the roots in the sand and soil cultures were as long at the end of the experiment as were those in the solutions when pronounced openings were noticeable.

Summarizing all the results so far available for wheat, the openings here considered were regularly obtained in roots grown in solution, whether the latter were fairly well balanced or decidedly unbalanced and whether the plants were grown under winter or summer greenhouse conditions.



The openings do not appear to have been related to the presence or absence of any chemical element or salt in the solution. But wheat roots failed to exhibit these cortical openings when grown in sand or soil for 13 days at summer greenhouse temperature. Whether wheat may be expected to show these openings always when grown in liquid media, and whether it may sometimes show them when grown in soil or sand, remain open questions.

Turning to the observations on Indian corn, during the months of January, February, and March the white dent variety was grown in many different solutions (with from one to five salts), and cortical openings were regularly developed in roots that had reached a length of 10 or 12 cm. Plants of the same variety grown in moist, rich garden soil within the same period uniformly failed to show openings in roots 10 to 18 cm. in length.

In June, Shive's solution R<sub>4</sub>C<sub>5</sub> (sub-optimal series) produced openings like those shown in figure 3. The seeds had been germinated in sphagnum (15°–35° C.), and the seedlings had been in the solution (20°–35° C.) for only 70 hours. The openings were present to within about 3 cm. of the tip of the root. At about the same time similar results were obtained with distilled water and with single salt solutions (0.00048 normal) of Ca(NO<sub>3</sub>)<sub>2</sub> and Mg(NO<sub>3</sub>)<sub>2</sub>. Apparently the liquid media produced the same growth response in these roots in summer as in winter.

With soil and sand cultures, however, the results obtained in June were somewhat different from those recorded for the winter and early spring. Corn plants that had grown 12 days in moist, rich garden soil (greenhouse, 20°–40° C.) showed many cortical openings, even to within 3 cm. of the tip of the root (fig. 3). The openings in the older portion of the root appeared very large in cross section and were about 8 cells in length. Perfectly parallel results were obtained with moist sand cultures at the same time.

White dent corn seedlings that had been grown in sphagnum (in the greenhouse) until the roots were 10 cm. long showed pronounced openings in the older portions of the root (to 4 cm. below the seed) when the test was made in June, but showed no openings in the case of similar tests made in the winter months.

It appears that cortical openings were regularly formed in white dent roots more than 10 or 12 cm. in length, grown in liquid media, whether in winter or in summer, and they also appeared in roots 12, 14, or 16 cm. in length, grown in soil, sand, or sphagnum during a period of high temperatures in the summer. The rate of growth of the root rather than the age of the seedling seems to be the factor determining the time of appearance of these openings. It is at once suggested that temperature and oxygen supply may be among the environmental conditions influencing the phenomenon in question. It seems safe to suppose that roots grown in sand, soil, or sphagnum generally have better opportunity for absorbing oxygen than those grown in liquid media, and this consideration may possibly furnish



an explanation of the difference observed in winter, when the temperature was relatively low and when growth, respiration, etc., were not as rapid as in summer. It appears as if the higher temperatures of summer, with higher respiratory activity, etc., may have rendered the better aerated, solid-medium cultures more like those in liquid media.

Several other experiments, not here described, seemed to support the idea that temperatures that gave slow growth in soil cultures retarded or prevented the development of the cortical openings, while temperatures that gave rapid growth resulted in numerous well developed openings. This suggestion seems to be along somewhat the same line as that expressed by Laetitia M. Snow<sup>2</sup> regarding the formation of gas cavities in the stems of *Scirpus*.

It is to be noted, however, that the development of cortical openings in corn roots frequently occurs after the root tissue has ceased to elongate. These openings may form, apparently, either in the elongating region or in the older part of the root where elongation has stopped.

One other experiment is worthy of mention here. On June 12 two soil cultures of white dent corn were prepared, one being placed in the greenhouse (higher temperatures) and the other in the open (lower temperatures). At the end of 8 days the greenhouse culture had roots about 26 cm. long, with large cortical openings (as in fig. 3) throughout the older portion, to within about 5 cm. from the tip. The openings in the upper part of the root were larger than those nearer the tip. At the same time the out-door culture had roots only about 18 cm. long, with all the pronounced openings (as in fig. 3) confined to the 3 cm. just below the seed. In the remainder of the root occurred only very small openings, merely intercellular spaces such as those shown in figure 4.

*General Conclusion.* It appears that the cortical openings considered in this note generally appear in solution cultures of both Marquis wheat and white dent corn, under greenhouse conditions of both winter and summer. They were not observed in the sand or in the soil cultures of wheat in either winter or summer, but none of these cultures were continued for more than thirteen days. The time of appearance of the cortical openings in sand or soil cultures of the white dent corn seems to vary with the rate of growth of the roots.

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<sup>2</sup> Snow, Laetitia M. Diaphragms of water plants. II. Effect of certain factors upon development of air chambers and diaphragms. Bot. Gaz. 69: 297-317. 1920.



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