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PHYSIOLOGICAL STUDIES OF PLANT ROOTS

1. INFLUENCES OF SOME ENVIRONMENTAL CONDITIONS ON THE GROWTH OF ISOLATED ROOTS OF RICE PLANT AND WHEAT

By

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This paper concerns a fundamental study on the sterile cultures of the isolated monocotyledonous plant roots in liquid media. Seedlings of wheat and rice plant were used since their physiology is of special interest owing to the great agronomic importance of them in many parts of the world.

Isolated root tips from these seedlings were cultured under various culture conditions with respect to environments and medium constituents. The growth rates of the root tips were also observed to find the optimal conditions for their tissue cultures.

Materials and Methods

The experimental materials were the excised roots of rice plant (*Norin, No.* 16) and wheat (*Norin, No.* 55) they were prepared as follows. The seeds used were selected by hand with respect to size and general appearance, all apparently unusual ones being discarded. These seeds were pretreated by soaking for a moment in 70 per cent alcohol. Furthermore they were sterilized by the treatment with a solution of 0.1 per cent $HgCl_2$, for 30 minutes after being soaked in depressed pressure for five to ten minutes(5). After the treated seeds were throughly rinsed with heat sterilized distilled water, they were set aside in a dark room with the temperature of 25°C and germination was allowed to proceed for about four days untill the roots had attained a length of about 20 to 30 millimeters.

From the supply of apparently normal roots thus obtained, the terminal 10mm tips were removed by means of a sharp scalpel, each tip being promptly introduced into its culture flask, which had previously been charged with the

requisite solution. The culture vessels were Erlenmeyer flasks with a volume of 100 ml. Usually the flasks were inoculated with four root tips, and each experiment was repeated with five parallel cultures. The volume of the nutrient solution amounted to 15 ml and in some experiments to 20 ml. The cultures were placed in dark thermostats at 20°C and generally the experimental period was a week. At the end of this time the length of each root was recorded and used as a measure of its growth. Plate 1, shows the manner of cultures carrying the isolated roots of rice plants.

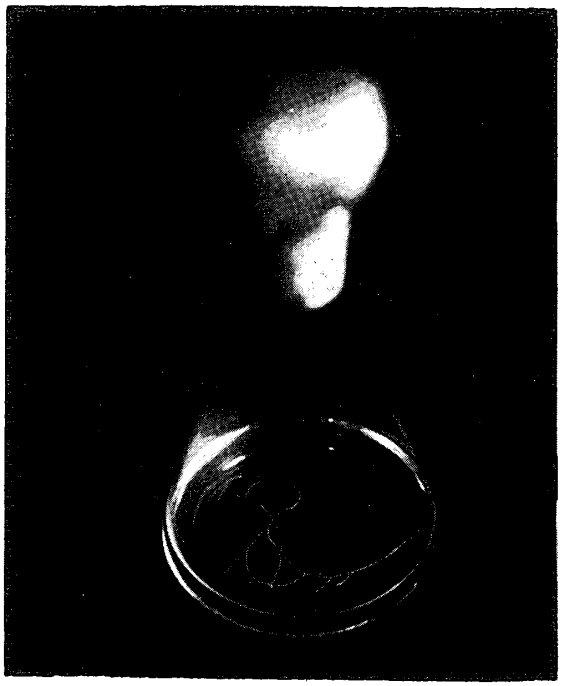

Plate 1. Two isolated roots of rice plants growing in the liquid medium for ten days.

The solution used as the basic medium contained the following composition ; KNO_3 0.2, KH_2PO_4 0.3, $Ca(NO_3)_2$ 0.4, and $MgSO_4$ 0.2 milli Mol per litre, $Fe_2(SO_4)_3$ 1, $MnCl_2$ 1, $ZnSO_4$ 0.02, KI 0.02, H_3BO_3 0.02 and NH_4-Molybdate 0.02 mg per litre respectively. Glucose was added as the source of carbohydrate at the rate of 0.05 Mol per litre. The standard addition of vitamins was ; nicotinic acid 0.5, thiamin 0.1 and pyridoxin 0.5 mg per litre. This formula was used as the basic medium by Burström and Almestrand (1) in their experiments. The salt concentrations of this solution are remarkably lower than those of the solutions generally used by other investigators (3) (4). All culture solutions were used after being autoclaved at 10 lbs. pressure for 10 minutes.

Results and Discussion

1. On a few problems of medium composions

A) *Vitamins as the growth substances*

The isolated roots of the rice plant and wheat were cultured in a complete nutrient medium and their lengths were measured every day. Fig. 1, represents the growth curves of these root tips. The average daily increases in length during the first ten days of culture amounted to 5.8 mm for the rice plant and 5.0 mm for the wheat.

An experiment was carried out to culture the isolated roots in the nutrient medium lacking vitamins *viz.* thiamin, pyridoxine, and nicotinic acid, which had been known as the growth substances for dicotyledonous plant. When the isolated roots of the rice plant and wheat were cultured in the medium without vitamins, their growth was retarded and their root lengths were about 70 per cent for rice plants and about 80 per cent for wheats compared respectively with those cultured in the complete media (Table 1). Table 1 shows that the supplement of thiamin or pyridoxine had a good effect on the growth of isolated roots of the rice plants at least during the culture period of seven days, while the growth of wheat roots was accelerated by the addition of pyridoxine. Almestrand(2) also reported good effects of pyridoxine to some kinds of wheat. The growth rate of these roots were gradually diminished with the elapse of

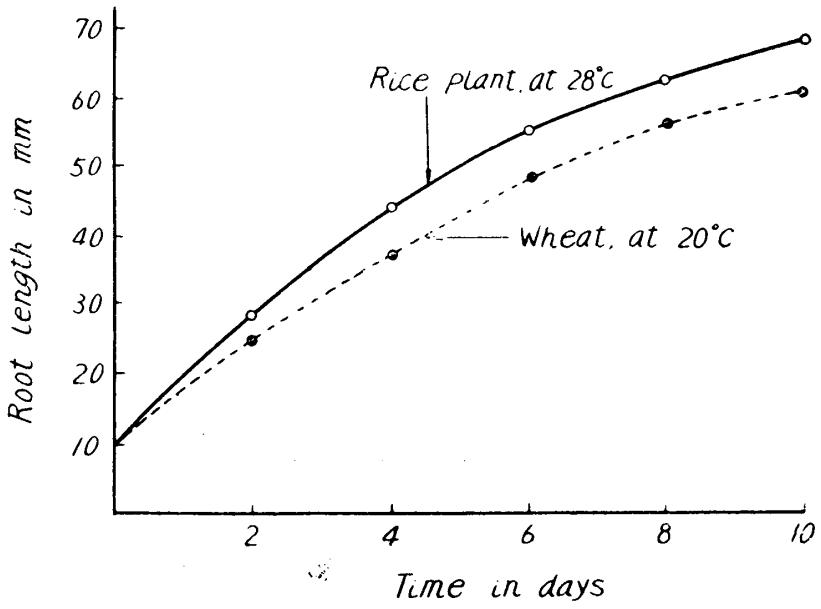

Fig. 1. Growth curves in length of isolated roots of rice plants and wheats cultured in darkness with 15 ml media.

time and their growth completely ceased in two weeks after inoculation.

To examine how the initial length of the excised root tips affected the growth succeeding them, three series of culture were started with root tips of the initial length of 5, 10 and 20 mm respectively. After a week the differences in growth between the roots elongated from tips with different initial lengths were not

Table 1. The lengths in mm of isolated roots grown in a media containing different vitamins for a week in darkness at 25°C. Average of 16 to 20 roots.

Medium	Rice plant	Wheat
	mm	mm
Without vitamins	35.6	35.6
Supplied with thiamin	50.0	38.8
〃 〃 〃 pyridoxine	53.1	42.8
〃 〃 〃 nicotinic acid	40.7	37.3
〃 〃 〃 three vitamins	53.5	44.4

notable for both rice plant and wheat. Thus, it seems that the substances originally present in the root tips have little affect on the later developement of isolated roots.

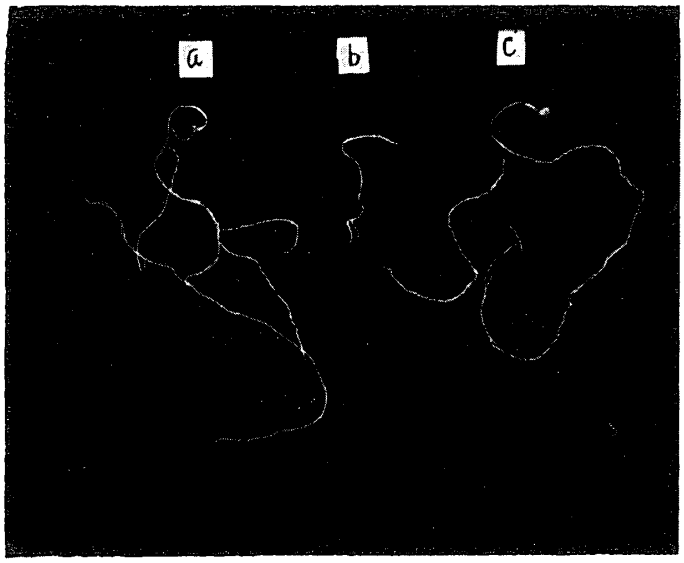

Plate. 2 Representatives of the isolated rice plant roots cultured for ten days at 25°C. (a). The root attached with the scutellum and grown in a nutrient solution lacking vitamins. (b). The root detached from the scutellum and grown in complete medium. (c). The root attached with the scutellum and grown in the medium the same as for (b).

Plate 2, shows a comparison of the growth between the roots with the scutellum and those without it. Although vitamins were contained in the medium, the rice plant roots having their suctellum (a) elongated much more and showed a more increased number of lateral roots than those without scutellum (b). When the roots were attached with their scutellum, the supplement of vitamins in the medium appeared to have no effect on their growth (a and c). Though the portion of scutellum was covered with paraffin, the

growth of the roots were not affected. Therefore, the better growth of the roots with the scutellum was not due to the absorption of the nutrient by the scutellum, but probably to an unknown growth substance contained or synthesized there. This seemed to be true also for wheat roots.

B) *Carbohydrate sources in the nutrient medium.*

Although in our experiments glucose was used in a concentration of 0.05 Mol, the kinds and the concentrations of sugars was also examined as the source of carbohydrate. The isolated roots of rice plant and wheat were cultured in solutions of different concentrations of glucose and sucrose. Fig. 2, demonstrates

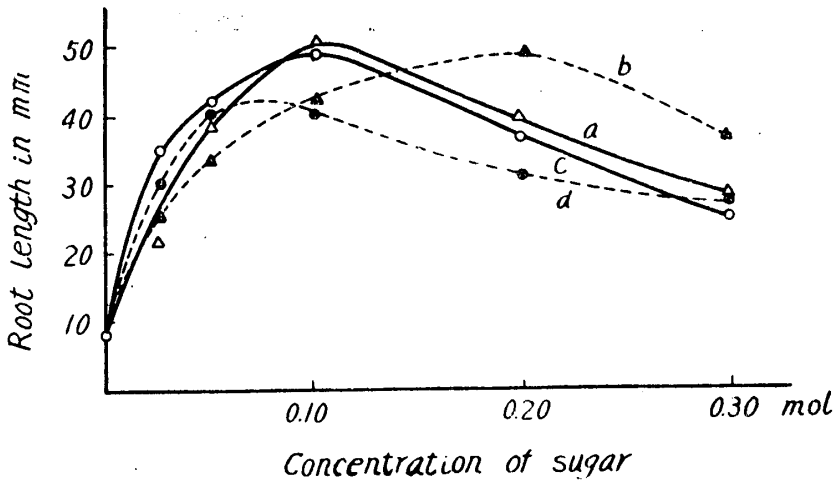

Fig. 2. Growth responses of isolated roots to sugar concentration cultured at 20°C for a week. (a). Rice plant roots in glucose. (b). Rice plant in sucrose. (c). Wheat roots in glucose. (d). Wheat roots in sucrose.

the relations between the relative root growth and concentrations of sugar, namely glucose and sucrose. It seemed that glucose was obviously the better source of carbohydrate than sucrose for the growth of isolated wheat roots and the best result was obtained by the 0.1 Mol glucose medium. In the case of rice plants the growth responses of their isolated roots to sugars were somewhat different from those of wheat roots. The maximum growth of rice plant roots was attained in 0.1 Mol concentration with glucose and 0.2 Mol with sucrose. These results were at variance with the observations by Almestrand(1), who found the best effect to be 0.05 Mol glucose in his cultures of isolated oat and barley roots. Increases in the sugar concentration produced shorter roots and they colored brown. On the contrary, in a solution lacking sugar the roots were hardly possible to grow.

2. Relations between the growth of isolated roots and the H-ion concentration of the medium.

The following experiments were carried out to determine the optimal pH value for root elongation. The H-ion concentration of the media were previously adjusted with 0.1 N HCl or 0.1 N NaOH to get seven series of cultures varied in the medium pH. After autoclaving, the initial pH values of each series, measured by use of a glass electrode, were as follows ; 3.1, 4.2, 5.1, 5.5, 6.2, 6.9, and 7.5. No attempt was made to buffer the solution, since phosphate or borate buffers would have altered the concentrations of the constituent inorganic ions. The graphs in Fig. 3, show the root lengths in relation to the initial pH of the medium. The optimum hydrogen ion concentration of the medium for

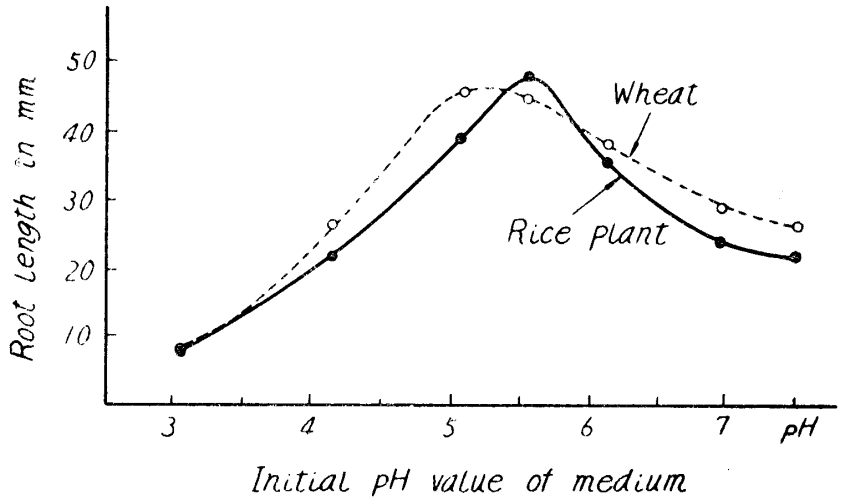

Fig. 3. The lengths of isolated roots of rice plant and wheat grown in media with different initial pH values for a week at 25°C.

the rice plant root was found at pH 5.5, while for wheat roots it was between 5.0 and 5.5. Thus the optimum pH range for wheat roots seems to be wider than that for rice plant roots. Roots of both plants cultured at higher pH were usually brownish coloured.

3. The influence of temperature and light on the growth of isolated roots.

Three different, maintained temperatures, (20°, 25° and 28°C) were tested. At the end of the one week period a marked influence of temperatures was indicated, as is shown by the representative roots given in Plate 3. In the wheat roots the best results were obtained at 20°C whence the root length was 35.0 mm and for 28°C it was only 20.5 mm. In the rice plant roots, on the otherhand, there were no great differences for differnt temperatures ; for 25°C the condition

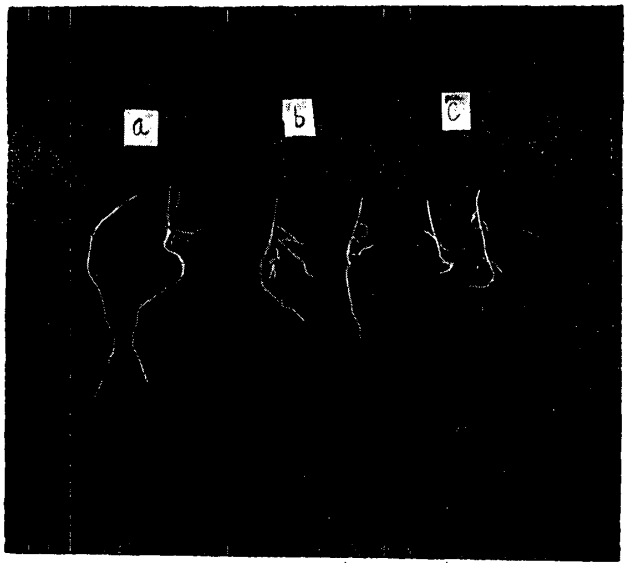

Plate 3. Representatives of isolated root tips of rice plant grown one week in full nutrient solution at three different temperatures.
(a). At 20° C. (b). At 25° C. (c). At 28° C.

of the roots was excellent and the length was 45 mm. On the whole, the optimal temperature appears to have been between 25°C and 28°C for rice plant roots.

When the culures were run for a week under full illumination with ten 20 W. day light fluorescent lamps, the growth of the isolated roots was worse than in the dark. The same result had been obtained by Alemestrand(1) and he suggested negative effects of the light on the root growth. In darkness the roots became straight and had relatively few branches, but under illumination they became shorter and increased the number of lateral roots as shown in Plate 4.

4. Relations between the growth of isolated roots and aeration of the medium.

The culture vessels were Erlenmeyer flasks with a volume of 100 ml and the volume of the nutrient solution amounted to 15 or 20 ml usually. In the early culture experiments, it was found that the growth of the root tips was retarded when a large volume of medium was used. Thus some observations were made on this subject. The flasks used were all of the same size, and contained 15, 20, 30 and 50 ml of the solution respectively. Each flask was inoculated with four excised root tips of rice plants. Elongation of these roots was retarded when they were cultured in flasks containing a volume of 50 ml or 30 ml medium. In the cultures with 20 ml or 15 ml medium, on the other hand, the growth was excellent. For the wheat roots the same results were obtained. When the volume of the medium is large, it is supposed that the air supply entering into

Plate 4. Representatives of isolated wheat roots grown one week with continuous artificial illumination at 20°C.

the flask through the cotton-plug may not give satisfaction to oxgen requirements of the roots in the bottom of the vessel. On the contrary, cultures with too small volumes of media would lead to accumulation of injurious materials resulting from the metabolism of the root tips. Therefore in our experiments the volume of the medium was chosen at 15 or 20 ml medium. Doubtless the relations between aeration and medium volume mentioned above seems to be influenced by the growth rates of the excised roots and by other experimental conditions.

Summary

Fundamental experiments for the tissue culture of excised root tips of wheat and rice plant in liquid medium were made with concern to the environmental conditions and some medium constituents. The results obtained may be summarized as follows:

1. Among the three vitamins tested as growth substances, thiamin and pyridoxine accelerated the growth of excised rice plant roots and in the case of wheat roots pyridoxin was most effective. Isolated plant roots attached with their scutellum elongated much longer than these without the scutellum, and to the growth of the former, vitamin supplements in the media seemed to have no influence.

2. As the carbohydrate source in the medium, both glucose and sucrose had almost the same effects on the growth of the isolated rice plant roots, and by the concentration of 0.1 Mol and 0.2 Mol respectively the best results were obtained. On the other hand, for wheat roots glucose was more effective and

the most suitable concentration was 0.1 Mol.

3. The optima of H-ion concentration of the medium were at about pH 5.0 for wheat and about pH 5.5 for rice plant roots.

4. The optimum temperature for growth of the isolated rice plant roots was found at 25°C, while for wheat roots at 20°C. Continuous illumination by fluoresecent lamps gave injurious effects on the growth of roots and these roots became shorter and more branched than in the dark.

5. Relatively large volumes of medium in culture vessels such as 50 ml or 30 ml in 100-ml flasks, depressed the elongation of the root tips probably due to the lack of aeration, and 15 or 20 ml was chosen as the medium volume in our cultures.

References

1) Almestrand, A. (1949). Physiol. Plant., **2**, 372.
2) Almestrand, A. (1951). Physiol. Plant., **4**, 224.
3) Takeuchi, K. (1951). Biol. Sci. (Tokyo) (in Japanese)., **3**, 122.
4) White, P. R. (1932). Plant. Physiol., **7**, 613.
5) White, P. R. (1943). A Handbook of Plant tissue Culture. P. 131, The Ronald Press Company. (New York).