Influence of Certain Organic Substances Upon the Growth Behavior of Excised Root Tips

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INTRODUCTION

The idea that certain organic substances may exercise an influence upon the amount and type of plant growth has been a controversial question for many years. Some work of this nature has been done with excised root tips as the subject. Kotte found that root tips were affected beneficially by the addition of meat extract to Knopf's solution. Robbins found beneficial effects from the addition of peptone and autolyzed yeast to a modified Pfeffer's solution. Other organic substances used by both men had no beneficial effect. In this study legumin-trypagar, brain heart infusion, yeast-dextrose agar, and different concentrations of dextrose and peptone were tried on different species of excised root tips with varying degrees of positive results.

MATERIALS AND GENERAL METHODS

The procedure to secure sterile root tips involved several steps and in the main followed that of Robbins (2). The seeds were soaked for 4 hours in a solution of Zonite diluted 1-30. They were then transferred by means of steel forceps to Petri dishes containing a thin layer of 0.75 per cent sterile agar agar, where they were allowed to germinate. The ends of the forceps were passed through a Bunsen flame and cooled in sterile water each time before touching the seeds. When the roots had grown approximately one inch, the tips were cut off in uniform lengths of 1 cm. by a steel scalpel, which was flamed and cooled before each operation. The excised root fragments were transferred by means of a sterilized wire loop to Pyrex Erlenmeyer flasks of 125 ml. capacity carrying 50 ml. of sterile nutrient media. The inoculated flasks were kept in a laboratory with curtains drawn, where they received very weak diffuse light and where the temperature varied no more than 3° F. (68-71° F.) during the experiment.

The nutrient solution was a modification of Pfeffer's formula to which was added dextrose and peptone.* Immediately after being made, the solution was measured into the flasks, which were closed with cotton plugs and autoclaved at 15 pounds pressure for 20 minutes.

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* $Ca(No_3)_2$ 2.0 gm; KH_2PO_4 0.5 gm; KNO_3 0.5 gm; KCl 0.25 gm.; M_gSO_4 0.5 gm.; $FeCl_3$ 0.005 gm.; Dextrose 2.0%; Peptone .04%; Distilled H_2O 6000 C.C.

TABLE I.—A SUMMARY OF DATA ON 100 ROOT TIPS OF CORN CUT AT ORIGINAL LENGTHS OF TEN MILLIMETERS AND GROWN IN THE MODIFIED PFEFFER'S SOLUTION WITH VARYING CONCENTRATION OF PEPTONE

Peptone per cent	Average daily increment in mm. of length of 10 root tips for 1st 10 days										Average total length in mm. at end of 28 days	Range in lengths in mm. of individuals	Average number lateral roots	Range in number of lateral roots among individuals	Average dry weight in gms. per 10 roots
	1	2	3	4	5	6	7	8	9	10					
0.04	4.6	3.4	3.4	0.2	5.6	2.6	2.3	3.6	6.6	2.5	145	95–290	89	34–103	.223
0.1	1.2	2.6	3.0	2.0	4.8	1.4	2.4	2.5	1.3	1.6	115	55–210	59	24–103	.212
0.2	2.0	1.0	1.5	1.3	2.2	1.4	1.8	0.2	2.8	1.2	62	38–160	9	0–27	.200
0.3	1.6	0.6	1.5	1.6	1.7	1.8	1.6	2.4	1.6	1.2	54	31–70	5	0–21	.215
0.4	1.4	1.6	1.0	0.8	0.7	1.0	1.2	1.5	1.0	1.0	44	30–65	4	0–13	.180

Daily increments in length were measured by placing a mm. rule under the flasks. Final data on dry weight, total length and number of laterals were tabulated and summarized for reference.

Deviations from the general method are stated in the discussion of results for each separate treatment.

DISCUSSION OF RESULTS

(1) Influence of increasing the amount of peptone in the nutrient solution upon the character of growth of root tips of dent corn.

In a preliminary experiment a mistake was made by the addition of too much peptone to a quantity of the basic solution. This solution was used as made up to determine its influence upon the growth behavior of excised root tips of corn. Their growth differed so markedly from that of the same kind of root tips grown with the normal amount of peptone in the basic solution that a series of experiments was performed to determine what concentration of peptone initiated the effect. The root tips became quite thickened in diameter, grew less in length and produced fewer branches, which were also quite thickened.

The basic solution was made up with five concentrations of peptone as follows: 0.04 per cent as in the normal solution, 0.1 per cent, 0.2 per cent, 0.3 per cent, and 0.4 per cent. Root tips of corn were allowed to grow in these solutions for four weeks.

Figure 1 shows clearly a noticeable change in growth habit as soon as the concentration of peptone was increased to 0.2 per cent. There was a preponderance of shorter and thicker roots with fewer laterals. This tendency became more marked as the concentration was increased to 0.4 per cent of peptone, where all the roots were short and thick with very few to no laterals at all.

Table I, however, shows no noticeable difference in average dry weight until the concentration of peptone reached 0.4 per cent. There was a distinct inhibition of elongation within the first ten days, when the concentration reached 0.2 per cent.

It might be said that the effect is due to increased osmotic concentration of the solution. To test this possible factor, the osmotic concentration of the basic solution was increased by adding more dextrose and maintaining the normal concentration of peptone. The phenomena of osmosis is dependent upon numbers of particles in solution and not upon weight. The empirical formula of dextrose is known, while that of peptone is not known definitely. Peptone, whatever its empirical formula may be, is composed of molecules which are much larger than those of dextrose. It follows that a given weight of peptone

TABLE II.—A SUMMARY OF THE DATA ON 50 ROOT TIPS OF CORN CUT AT ORIGINAL LENGTHS OF 10 MILLIMETERS AND GROWN IN THE MODIFIED PFEFFER'S SOLUTION WITH VARYING CONCENTRATIONS OF DEXTROSE

Dextrose per cent	Average daily increment in mm. of length of 10 root tips for 1st 10 days										Average total length in mm. at end of 28 days	Range in lengths in mm. of individuals	Average number lateral roots	Range in number of lateral roots among individuals	Average dry weight in gms. per 10 roots
	1	2	3	4	5	6	7	8	9	10					
2	2.6	4.5	4.7	3.2	2.0	3.2	2.3	6.0	2.0	6.5	138	54–234	94	0–118	.213
2.5	3.0	2.0	4.5	1.4	3.3	2.3	3.7	4.2	4.3	3.0	141	41–251	87	10–121	.208
3	3.2	4.0	2.3	4.7	5.9	2.5	3.5	2.1	1.0	4.0	136	45–268	97	0–128	.224
3.5	3.70	2.15	4.92	3.02	5.01	4.00	5.35	3.77	5.89	4.27	129	38–189	72	3–97	.170
4.0	2.71	3.20	3.23	2.94	1.20	2.74	3.93	1.33	2.63	2.91	78	32–91	20	0–54	.185

would have a smaller number of osmotically active particles than the same weight of dextrose. Neither substance ionizes in solution. The concentrations of glucose used, namely, 2 per cent, 2.5 per cent, 3 per cent, 3.5 per cent and 4 per cent, produced an increase of osmotically active particles per unit volume far beyond the range produced by the increased concentrations of peptone.

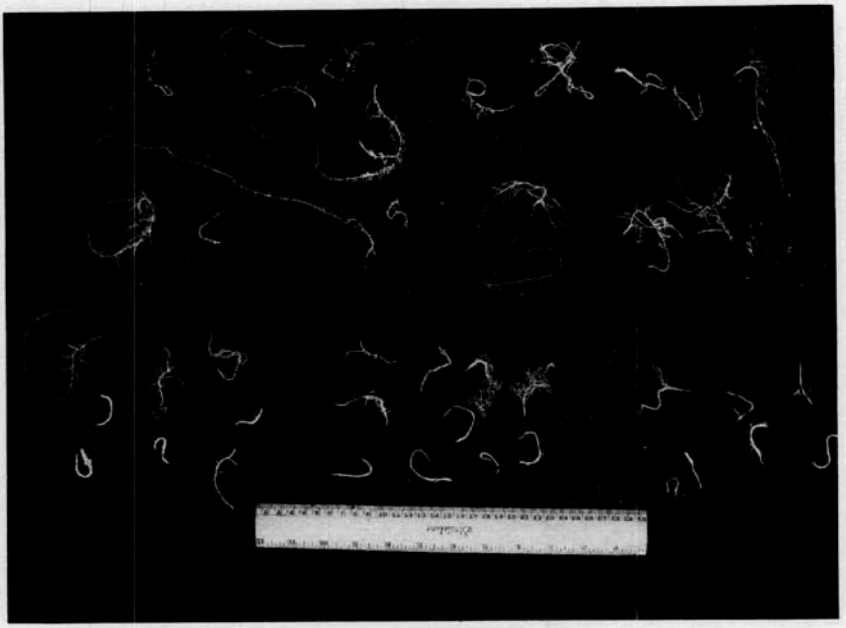

Fig. 1.—GROUPS OF CORN ROOTS, WHICH HAVE BEEN GROWN IN THE BASIC SOLUTION WITH VARYING CONCENTRATION OF PEPTONE. Upper left: normal concentration of peptone (0.04 per cent). Upper right: 0.1 per cent peptone. Lower left: 0.2 per cent peptone. Lower center: 0.3 per cent peptone. Lower right: 0.4 per cent peptone.

Figure 2 shows that there was no particularly great difference in character of growth until a concentration of 4 per cent dextrose was reached. There was the usual wide variation in each group of roots; thickened roots with few or no laterals occurred throughout the series, and not until a concentration of 4 per cent dextrose was reached was there a marked reduction in average total length, in average number of lateral roots, and in average dry weight (Table II.) This is hardly in accord with Robbins' (3) findings that corn root tips grew to greater lengths and produced more laterals in the dark in twenty-four days in 4 per cent dextrose than in 0.2, 0.5, 1, 2, or 6 per cent dextrose. However, a comparison of results cannot be made directly with those of Robbins, because Robbins used a modified Pfeffer's solution six times

TABLE III.—SUMMARY OF DATA ON 35 ROOT TIPS OF CORN GROWN IN THE MODIFIED PFEFFER'S SOLUTION WITH 2 PER CENT DEXTROSE, 0.04 PER CENT PEPTONE, AND 0.1 PER CENT LEGUMIN TRYPAGAR

Species	Average daily increase in mm. of length of 30 root tips for first 10 days										Average total length in mm. at end of 10 days	Average final total length in mm.	Range in final lengths in mm. among individuals	Final average number lateral roots	Range in final number of lateral roots among individuals	Average dry weight in gms. per 10 roots
	1	2	3	4	5	6	7	8	9	10						
Corn	3.65	2.93	2.04	3.86	4.75	5.23	3.05	2.17	2.00	3.25	32.93	179.3	55–210	221	78–330	.312

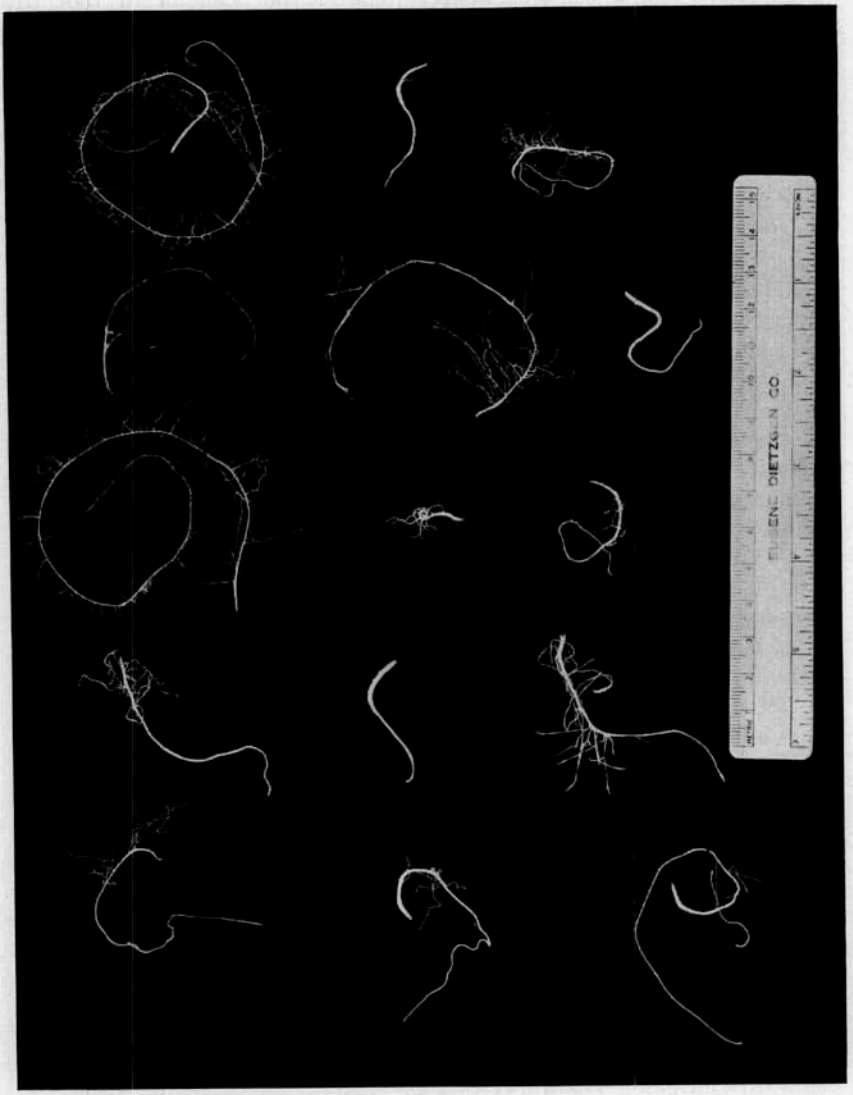

Fig. 2.—REPRESENTATIVE ROOT TIPS OF CORN GROWN 28 DAYS IN PFEFFER'S SOLUTION AND PEPTONE WITH DIFFERENT CONCENTRATIONS OF GLUCOSE. Top row, 2 per cent glucose; second row, 2.5 per cent glucose; third row, 3 per cent glucose; fourth row 3.5 per cent glucose; fifth row, 4 per cent glucose.

the original strength and added no peptone. The presence of peptone used in the present experiments and the weaker concentration of salts might have had a combined effect to alter the behavior of root tips grown in such a solution, to which increasing amounts of dextrose were added.

(2) Influence of legumin-trypagar, brain-heart infusion, and yeast-dextrose agar upon the growth of excised root tips of dent corn, pea and cotton.

To the basic solution was added as much of the yeast-dextrose agar as would keep the concentration of peptone in the final solution at 0.04 per cent, in order to avoid the influence of additional amounts of peptone, which would have seriously complicated the results, as has already been pointed out earlier in this paper. In the second series of experiments brain-heart infusion was added to the basic solution with the same precaution. In the third series of experiments 0.1 per cent of legumin-trypagar was added to the basic solution. In the latter case no peptone was present in the compound added; therefore, no calculations were necessary to keep within bounds in regard to peptone, since it was added separately in the normal amount. The three compounds were obtained from the Digestive Ferments Company, Detroit, Michigan.

Thirty ten-millimeter root tips, in three series of ten individuals each of cotton, corn, and pea were grown for eight weeks in each of the three solutions. Daily observations of increment in length were made for a period of about two weeks. At the end of the experiments the usual final data were recorded.

Since no difference in behavior were secured from the use of brain-heart infusion and of yeast-dextrose agar in the three species of root tips used the results are not reported here in tabular form. They were too nearly like those obtained in the first experiments reported in this paper with the use of the basic solution alone to warrant space for recording.

However, the legumin-trypagar acted as a marked stimulant to the growth of corn root tips and in a few isolated cases to the growth of pea root tips, but no effect was noticeable in cotton.

Figure 3 shows one corn root, which was obtained entire and which shows very well the uniformly thickened root with its well-developed laterals thickened in the same manner. This one root was typical of about sixty-five per cent of the root tips that were grown in the legumin trypagar. There was in every case a comparatively short root with an abundance of long and very well developed laterals. The entire root system was quite uniformly thickened. This robust appearance was never observed in well developed roots of corn grown in the original basic solution. In the latter case the roots were more slender and the

laterals were usually smaller in diameter than the main axis of the root. One individual corn root tip grew to astounding proportions, attaining a dry weight of 0.254 grams with a length of 143 millimeters, having 332 laterals and subbranches ranging from 9 to 97 millimeters, making a grand total of approximately 9920 millimeters of root, representing an increase of 992 times the length of the original fragment. This root could not be photographed because in washing it out for examination it broke apart due to the bad entanglement within a 125 millimeter flask.

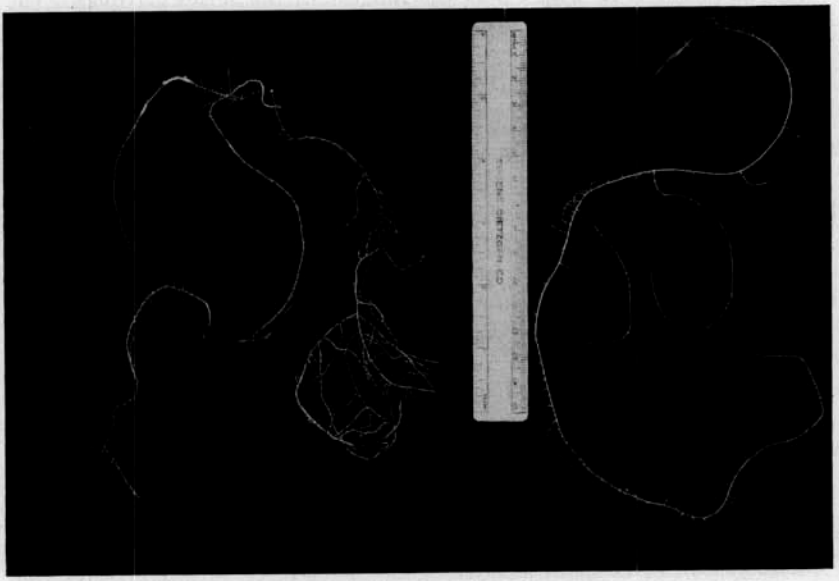

Fig. 3.—UPPER LEFT, PEA ROOT GROWN IN LEGUMIN TRYPAGAR; LEFT, CORN ROOT GROWN IN LEGUMIN TRYPAGAR; RIGHT, CORN ROOT GROWN IN DEXTROSE SOLUTION.

The root tips of pea, which seldom developed lateral roots under any circumstances and then only very short stubby ones of one or two millimeters length, grew to greater length about forty per cent of the time and produced from one to five laterals, ranging from three to twenty-nine millimeters (Fig. 3).

Although no differences in general behavior were noted in cotton, three of the roots deviated far from their ordinary habits. In these three roots the epidermis split longitudinally in one place and through this slit there emerged small outgrowths of the parenchymal tissue of the cortex, which reached the size of a cabbage seed. The cells were very large and loosely arranged as in certain peculiar proliferations secured in a few cases from one-millimeter tips of sunflower. These outgrowths were easily discerned because of their contrasting tan color to the black epidermis.

(3) The effect of dextrose and distilled water on the growth of root tips of dent corn.

In this experiment ten root tips each ten millimeters in length were grown eight weeks in a 2 per cent solution of dextrose to determine their behavior in the absence of mineral salts. These roots exhibited the same uniformly thickened appearance as that caused by legumin trypagar, but there were few laterals (Fig. 3). In several cases there was liberal pigmentation due to anthocyanin, although anthocyanin never developed in root tips of corn grown in the original basic solution. The root pictured in Fig. 2 was the longest in the series. None of the others in the series were half as long.

SUMMARY

(1) Peptone in concentrations of 0.2 per cent or more inhibited the growth and formation of laterals in root tips of corn.

(2) A concentration of 4 per cent of dextrose was reached before noticeable changes were observable in corn root tips.

(3) No noticeable harmful or beneficial effects were secured from brain-heart infusion nor from yeast-dextrose agar on the growth of root tips of dent corn, pea, and cotton.

(4) Legumin-trypagar acted as a stimulus to the growth of excised root tips of dent corn and pea, but had no effect on cotton. The stimulus caused an increased development of lateral roots.

(5) Corn root tips grown in the presence of 2 per cent dextrose without mineral salts developed into short thick roots with few laterals.

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