STUDIES ON THE GROWTH PROMOTING SUBSTANCE OF THE EXCISED WHEAT ROOTS I. EFFECTS OF PEPTONE ON THE GROWTH

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STUDIES ON THE GROWTH PROMOTING SUBSTANCE OF THE EXCISED WHEAT ROOTS

I. EFFECTS OF PEPTONE ON THE GROWTH

by

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It has been stated by many investigators that to obtain continuous growth with the excised roots of the monocotyledonous or woody plants is very difficult because presumably of the unknown growth factor, such as B-vitamins for the excised roots of some dicotyledonous plants. More extensive reviews on the tissue culture of the plant roots have been described by Robbins (14), White (22), Burström (3) and Street (17) *et al.* Robbins (14) stated that potentially unlimited growth has not been obtained for the roots of any monocot or cucurbit, and the roots of many woody plants have proved to be refractory. Burström (3) also emphasized that there are reasons to look upon the successfull growth of monocotyledonous plant roots more sceptically. The failures to achieve the continuous culture of these excised roots may be due to the satisfactory nutritional requirement for the growth.

This problem recently has been studied by Almestrand (1, 2), who clarified the effects of large numbers of organic materials on the excised root growth of the grasses, and he reported that all of the tested roots declined rapidly in the meristem size and failed to continue growth under excised conditions, particularly on the subculture, notwithstanding the supply of many kinds of organic substances. Roberts and Street (16) recently found that the rye roots required a new growth factor, which appeared to be tryptophane activated by autoclaving. The culture technique described by these workers, however, only made the establishment of clones derived from 10 per cent of the grains of the rye sample selected among the same species, and it also failed to establish excised root clones from the other cereals tested.

As described above, the relation between the excised root growth of monocot or grass and its growth factor seems to be not yet fully solved, and further studies on this problem, therefore, should be the purpose of the present or the future research. We tried to complete the culture of the excisd wheat roots under some different nitrogen sources supplied in the medium to find the progressive promoting effect of peptone on the growth, although this may depend on the unknown growth factor instead of the nitrogen source contained in peptone. In the present paper, the physiological effects of peptone on the growth of excised wheat roots are described, together with some remarks on the environmental conditions under supplying peptone.

Materials and Methods

Our experimental materials were the roots of wheat (*Norin No. 55*). Culture methods of the excised roots used in this study were fully described in the previous reports (5, 6) and brief outlines are given here.

In 100 cc Erlenmeyer flasks containing 20 cc of the liquid-medium, the excised roots were cultured under the sterile condition. The inoculation, unless otherwise noted, was carried out with one root tip for each flask and the average length of six to 12 main axis was taken for one of the measurements of the growth rate at the end of the culture period. The dry weight of the cultured roots was weighed after kept in the desiccator during several days. The basal medium for these expriments was Almestrand's solution, which has the following composition; 0.0002 M KNO_3, 0.0003 M KH_2PO_4, 0.0004 M $Ca(NO_3)_2$, 0.0002 M $MgSO_4$, 1.0 ppm $Fe_2(SO_4)_3$, 1.0 ppm $MnCl_2$, 0.02 ppm Zn SO_4, 0.02ppm KI, 0.02 ppm H_3BO_3, 0.02 ppm Ammonium-molybdate, 1 per cent glucose, 0.5 ppm of nicotinic acid, 0.5 ppm of pyridoxine hydrochloride, 0.1 ppm of thiamin hydrochloride.

Nitrogen contents of the harvested root samples were analyzed as follows. Total nitrogen was estimated according to Kjeldahl-Gunning method and nitrogen in the residue after extracted with boiling water for 15 min was also estimated in the same way.

For the estimation of the nutritional uptake by the excised roots, the following methods were applied to the nutrient solution before and after the culture period. Glucose; Somogyi's titration method. Phosphorous; Allen's colorimetric method. Potassium; flamephotometory. Calcium; titration by permanganate. Magnesium; colorimetory by titan yellow.

The respiratory intensity of the excised roots was measured with Warburg manometer to determine the oxygen consumption at the temperature of 25°C. One ml of aliquot nutrient solution with 200 mg of the cultured roots was inserted in each conical Warburg vessel and reading of the manometers was performed every ten min for three hours.

Experimental Results

I. Peculiar effects of peptone as the nitrogen source on the growth of excised wheat roots.

The problem of the nitrogen requirement of the tissue or organ *in vitro* has been the subject of many researches on the tissue culture since the early days of its history. White (20) reported that it had no significant difference whether NO_3 or NH_4 was purposely present or absent for the excised wheat root growth within a given period. According to Robbins and Schmidt (15), excised tomato roots apparently required no other of nitrogen than nitrate for the unlimited growth. It was reported also that both nitrate and urea were excellent sources of nitrogen for the sunflower tissue (4, 11). Robbins *et al.* (13, 15) found the beneficial effects of peptone or yeast extract on corn and tomato root growth and Ziebur (23), that of casein hydrolysate on the embryos.

To investigate the influence of nitrogen nutrients on the growth, excised wheat roots were grown in the nutrient solution containing various nitrogen sources. For removing nitrate ion from the basal medium, KNO_3 and $Ca(NO_3)_2$ were respectively replaced with KCl and $CaCl_2$. Besides peptone, six different species of nitrogen sources, namely sodium nitrate, sodium nitrite, ammonium sulfate, urea and casein hydrolysate were tested in the range of 5 ppm to 500 ppm as nitrogen. Only urea was sterilized apart from the basal medium to avoid its heat-decomposition, being added aseptically to the medium without nitrogen.

The results are presented in Figs 1, and 2. Peptone seems to exert the

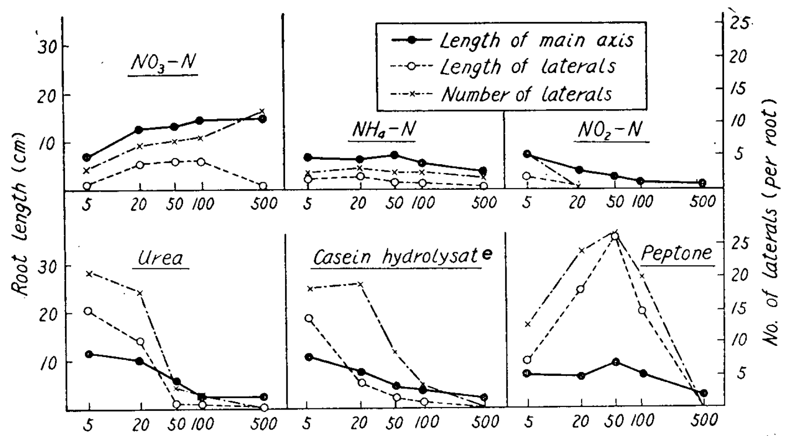


Fig. 1. Influence of the various sources of nitrogen on the excised root growth (I). Concentrations of each nitrogen compound rise progressively from left to right. Glucose concentration ; 1 per cent. Culture time ; two weeks.

conspicuously beneficial effects in the production and development of the secondary roots between 20 ppm and 100 ppm of the nitrogen concentrations. Effects of urea and casein hydrolysate also were apparently somewhat similar to that of peptone in their lower concentrations, but being inferior to it in the higher concentration. All of those organic nitrogen sources exerted no excellent influence for the growth of the main aixs tips. Nitrate, however, led the length of the main root tips to increase gradually in proportion to

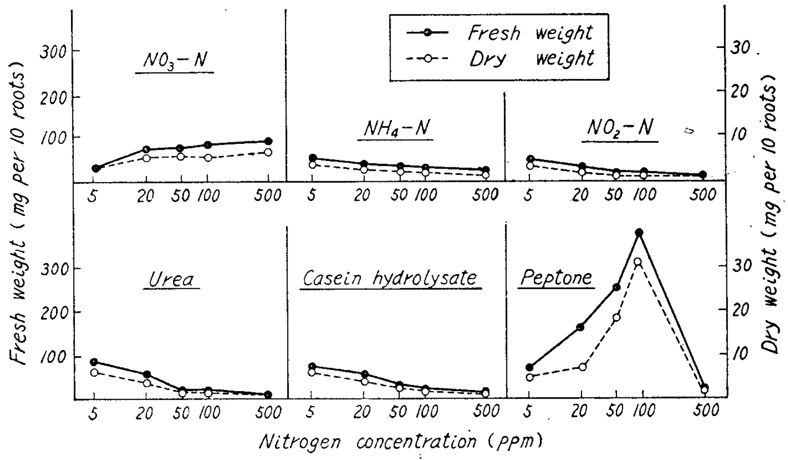


Fig. 2. Influence of the various sources of nitrogen on the excised roots (II). Weight of the excised roots of each sample in Fig. 1.

its concentration, although there were no great differences within the concentrations as low as 30 ppm and as high as 500 ppm. Ammonium sulfate exerted no influence in the lower concentrations and was harmfull in the higher. Nitrite was strongly toxic even in such lower concentration as 20 ppm.

As described above, peptone was distinctly beneficial for the growth of the excised wheat roots in the medium without any other nitrogen sources.

Table 1. Influence of different concentrations of peptone in the medium with or without nitrate. Glucose concentration ; 1 per cent. Culture time ; two weeks.

Peptone conc. (ppm)	Length of main axis (cm)	Length of laterals per root (cm)	No. of laterals per root	Fresh weight per 10 roots (mg)	Dry weight per 10 roots (mg)	Total-N per 10 roots (mg)	N absorbed per 10 roots (mg)	N-content (%)
(a) without nitrate								
0	10.7	10.1	13.8	150	10.8	0.17	0.00	1.6
10	9.7	30.5	30.6	228	21.8	0.42	0.25	1.9
30	10.1	37.3	33.7	297	26.3	0.65	0.48	2.5
50	9.1	29.4	24.7	300	27.4	0.95	0.78	3.5
70	8.4	25.7	25.6	302	33.8	1.18	1.01	3.5
90	8.2	28.7	24.0	270	43.8	1.59	1.42	3.6
(b) with nitrate								
0	16.9	15.5	13.5	189	13.7	0.44	0.27	3.2
10	13.1	27.0	22.7	228	17.7	0.71	0.54	4.0
30	10.9	34.1	24.6	267	19.4	0.89	0.72	4.6
50	9.8	29.4	27.3	264	19.6	1.91	1.74	4.7
70	10.4	30.6	24.3	348	26.4	1.14	0.97	4.3
90	7.2	23.1	16.0	520	43.9	2.01	1.84	4.6

Then it should be considered whether the effects of peptone due to a stimulant action of some organic compounds, such as vitamins included in it, or to the supply of such organic nitrogen sources as amino acid or amide for synthesizing protein. It seems necessary, at first, to investigate the effects of peptone on the growth of the excised roots in the medium containing sufficient nitrate as the nitrogen source for the protein synthesis.

The influences of the different concentrations of peptone in the medium with or without nitrate are shown in Table 1, and the typical photograph of the excised roots in Fig. 3. From the results of this experiment, we may

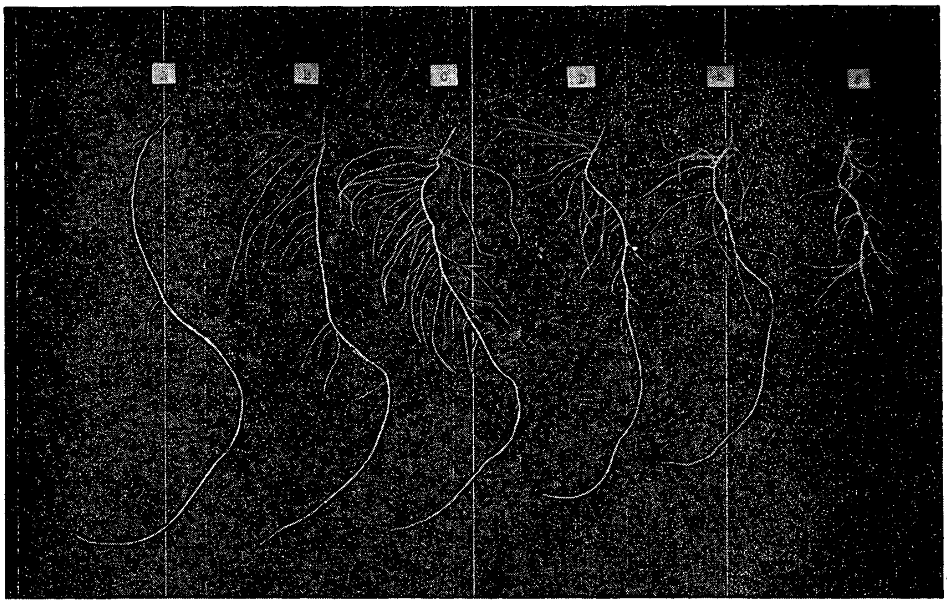


Fig. 3. Excised wheat roots grown in the medium containing different amounts of peptone. Peptone concentration as nitrogen (ppm): from left to right, (A) 0, (B) 10, (C) 30, (D) 50, (E) 70, (F) 90.

draw some points as follows: (a) The optimum concentration of peptone appears to be in 30 ppm as nitrogen or approximately 300 ppm as dry weight. When the concentration of peptone was kept lower than this amount, the total nitrogen contents of the excised roots were respectively depended upon the amount of peptone supplied in the medium. At 70 ppm as nitrogen, the amount of applied peptone seems to be excess notwithstanding the presence or the absence of nitrate, because the browning, fragile nature of the roots and abnormal swelling of their tips were observed. (b) Peptone promoted markedly the production and development of the secondary roots or laterals, although rather conversely to the growth of the main axis tips. These beneficial effects could be found in lateral growth whether nitrate, as nitrogen source, in the basal medium was present. (C) The fresh or dry weight of the excised roots increased parallel to the amount of peptone in the medium, independently of the growth of the main axis tips. The conspicuous increase of the root weight may be due to the large numbers of thickened or swelling roots. (d) From comparison of the growth in the presence of nitrate with that in the absence, especially lacking peptone, nitrate appears to be favorable for the growth of the main axis. The same facts were also emphasized from the data given in Fig. 1.

II. Investigation of some environmental conditions under applying peptone medium

To obtain more sufficient peptone effects for the growth of the main axis tips as well as for that of the laterals, we tried the culture of the excised roots in the nutrient solution with peptone, considering some environmental conditions for more satisfactory growth. Peptone was applied in the amount of 300 ppm, and the excised root tips were grown for ten days under some various conditions for this purpose.

(*1*) *The volume of the liquid and number of root tip inoculated*

As the general methods of the excised root culture, White (21) recommended to use 125 cc Erlenmeyer flask containing 50 cc of liquid in his publication. According to his indication, many investigators have carried out the culture of the excised roots. As stated in the earier report, however the growth of the excised wheat roots retarded under such a condition and was progressively improved under less amount of liquid, presumably through better aeration. Glasstone (7) showed in his preliminary expriment that the most satisfactory growth of the excised tomato roots was obtained with 25 cc of solution in 125 cc Erlenmeyer flasks, and deeper solution and tighter plug gave less satisfactory results.

Fig. 4 shows the relation between the excised root growth and the amount of liquid contained in 100 cc Erlenmeyer flasks. It is clear from the result that the less amount of the liquid is kept in the flasks, the better the main axis tips grow. If the amount of the liquid was kept over 30 cc in this vessel, the growth was notably retarded. The growth of the laterals also showed the same tendency as the main axis for the amount of the medium or the thickness of liquid layer. This accords with the results in the basal medium without peptone obtained previously.

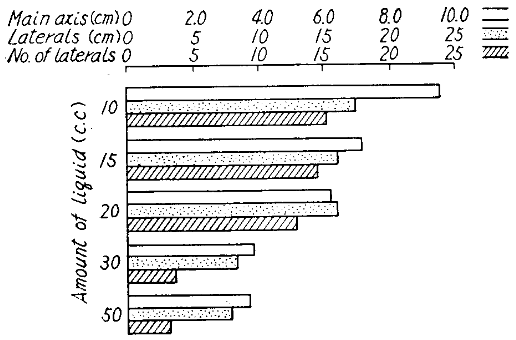


Fig. 4. Relation between the amount of the liquid and the root growth. The excised roots are respectively grown in 100 cc Erlenmeyer flasks containing different amounts of the liquid for ten days.

The inoculation of the excised root in the tissue culture technique, generally have been carried out with one root tip for each flask. In our previous experiments, four root tips were grown together in a vessel. As is represented in Fig. 5, the habit and the rate of the growth of the excised roots greatly depend upon the numbers of growing roots in a vessel. If four root tips were inoculated and grown together in a vessel, the beneficial effects of peptone were found more clearly in the length of the main axis than the production and the elongation of the laterals, as compared with the case of one root tip for one vessel. It may be remarked in this experiment that the growth habits of the excised roots are influenced by the numbers of root tips inoculated in a vessel. Practically inoculation should be carried out with one tip per flask containing 20 cc liquid, since the component of the medium would be easily changed by the excretion of some substances and the uptake of nutrients by the growing roots.

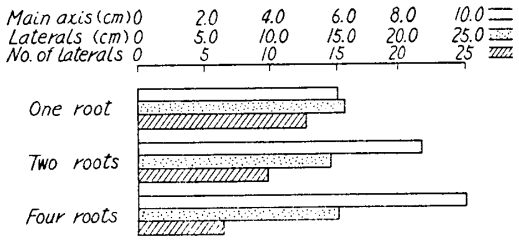
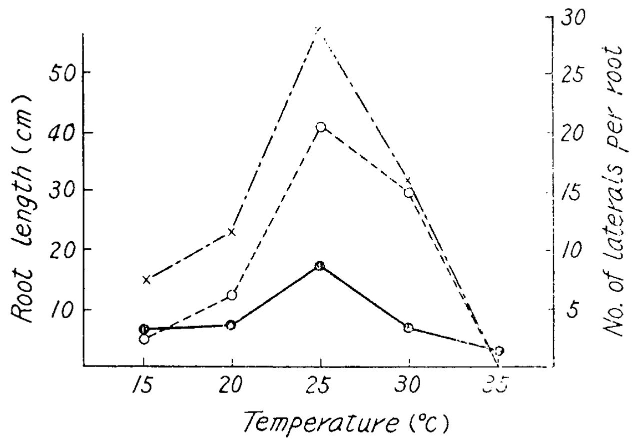


Fig. 5. Relation between the number of the excised root tips grown in a vessel and their growth. In 100 cc Erlenmeyer flasks containing 20 cc of the liqiud, inoculation was carried out with different numbers of the root tips.

(2) Light and temperature conditions

To clarify whether the light is beneficial for the growth, the cultures in the presence or the absence of peptone, were run under the illumination of about 1000 Lux by day light fluorescent lamps. The majority of the excised root tips in the peptone solution became swollen and translucent, and the



—●— Length of main axis
- - ○ - - Length of laterals per root
—·—×—·— No. of laterals per root

Fig. 6. Influence of the different temperatures on the excised root growth. In this experiment, glucose concentration in the medium was increased to 2 per cent. Culture time ; ten days.

growth stopped within several days, although undergoing little or no effects in the medium without peptone. This injurious effect was especially observed highly in the earlier days of culture, but only a little or none for the same growing roots after the culture duration of about one week. Under the addition of peptone, at least, it had better to avoid illumination of the strong light for initial growth during the several days.

Fig. 6 shows that the optimal temperature of the excised root growth is around 25°C. It appears to be supraoptimal at 30°C. The injurious effects with higher temperature were also similar to the case of light, being notably observed in the earlier period.

The phenomena mentioned above suggest that the excised root tips, just after the initiation of culture, are most sensible for such external environmental factors as the temperature or the light, and seem to grow up gradually to be adaptable for them as the time passes.

(*3*) ***Influence of the sugar concentration and pH of the nutrient solution.***

As the carbohydrate source in the plant tissue culture, glucose, fructose and sucrose have been usually employed. We already reported on the relation between the variety of their sugar sources and the growth of the excised roots in the previous paper (5).

Glasstone (7) demonstrated that the growth of the excised tomato roots in the 1 per cent and 2 per cent sucrose solution was equally good enough and he adopted actually the solution of 2 per cent sucrose. According to Neylor and Rappaport (10), the amount of sucrose in the nutrient solution influenced markedly on the growth of excised pea roots, and one variety of pea roots grew most vigorously with 6 and 8 per cent sucrose in the medium, while the other two varieties grew well with 4 per cent.

From the results shown in Fig. 7, it is clear that the growth of the excised wheat roots is excellent with 2 per cent glucose solution under supplying peptone. The growth of the main axis tips in 1 per cent of glucose amount was less than that in 2 per cent of it. With increasing the sugar amount by 2 per cent of two times, the beneficial effects of peptone were found not only in the laterals, but also in the main axis length (Fig. 8). This result suggests that the concentration of the carbohydrate source is a limiting factor through the culture adopting the medi-

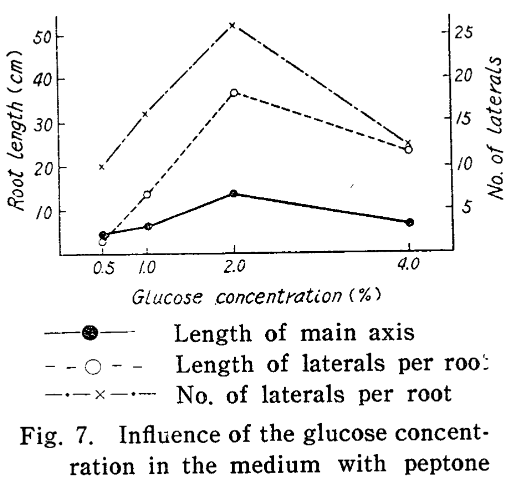


Fig. 7. Influence of the glucose concentration in the medium with peptone on the growth. Culture time ; ten days.

um containing peptone. The abnormal swelling of the root tips in the medium containing 4 per cent of glucose indicates that this amount is already injurious.

Toble 2 shows the influence of hydrogen-ion concentration of the medium on the growth. The excised roots were grown in various pH, namely in

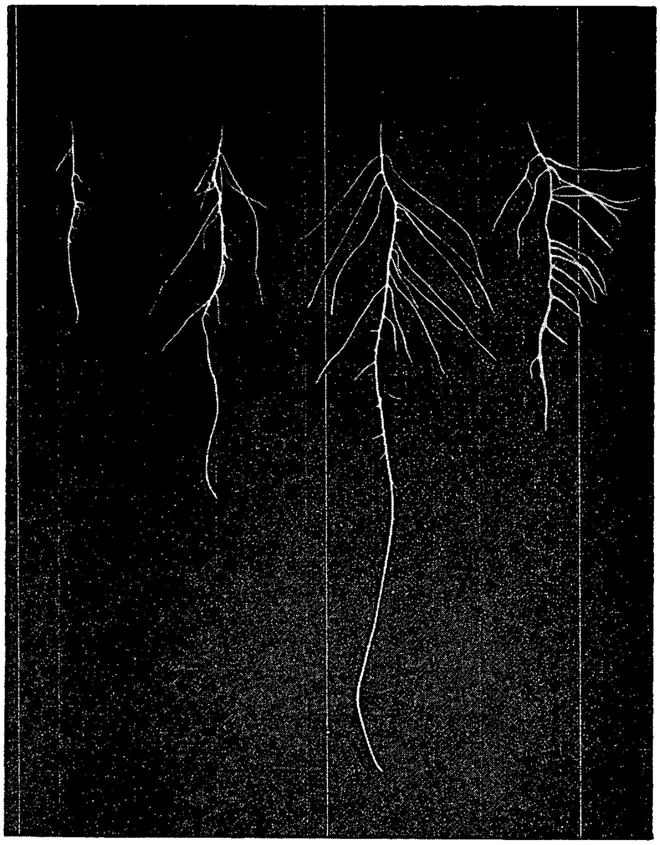


Fig. 8. Growth of the excised roots in the medium containing different concentrations of glucose. Culture time; ten days. Glucose concentration (%) ; from left to right, 0.5, 1.0, 2.0 and 4.0.

Table 2. Influence of hydrogen-ion concentrations of the medium on the growth. pH values were adjusted with 0.1 N HCl and 0.1 N NaOH. Glucose concentration ; 1 per cent. Culture time ; ten days

Hydrogen-ion conc. (pH)			Length of main axis (cm)	Length of laterals par root (cm)	No. of laterals per root	Fresh weight par 10 roots (mg)	Dry weight per 10 roots (mg)
after autoclaving	before autoclaving	after culture					
3.4	3.4	3.2	1.2	—	—	—	—
3.8	3.8	3.7	4.4	8.7	13.8	106	4.5
4.3	4.3	3.7	7.2	20.7	25.5	160	6.0
4.6	4.9	3.8	6.9	15.9	20.0	151	7.7
5.1	5.9	4.0	8.3	17.9	18.5	186	7.7
5.3	6.4	4.2	7.8	15.4	18.5	137	7.0
5.7	7.1	4.4	6.9	8.4	14.0	117	5.7
5.9	7.5	4.7	5.2	4.6	11.2	96	4.8

eight grades from 3.4 to 5.9. As shown in the table, pH value of the medium diminished abruptly with the autoclaving of 10 lb, 10 min and went down gradually during the culture period, especially in the alkaline side. The best growth was obtained in pH 5.1 of the solution, which was adjusted to initial pH 5.9 before autoclaving. The optimum hydrogen-ion concentration of the solution supplied with peptone may be around pH 5.

III. Beneficial effects of peptone under the improved environmental conditions

According to the experimental results obtained above, cultures of the excised roots were performed during the longer period under the improved conditions as follows, and the peptone effects were observed in more detail with the naked eye or by the microscope.

materials; The root tips of 10 mm length were aseptically excised from the seedling roots of 30 to 40 mm in length and were transferred to the nutrient solution. The excised root tips were grown in 100 cc Erlenmeyer flasks containing 20 cc of the nutrient solution.

environmental conditions; Kept at 25°C of temperature, in the dark, the excised roots were cultured during a given period. The aeration through shaking of the flasks was carried out once a day by hand.

medium; Only the amount of glucose as the carbohydrate source was increased by 2 per cent, although the constituents of the other nutrients in the medium were the same as those in the basal medium. Neopeptone was added in the amount of 300 ppm. The initial hydrogen-ion concentration of

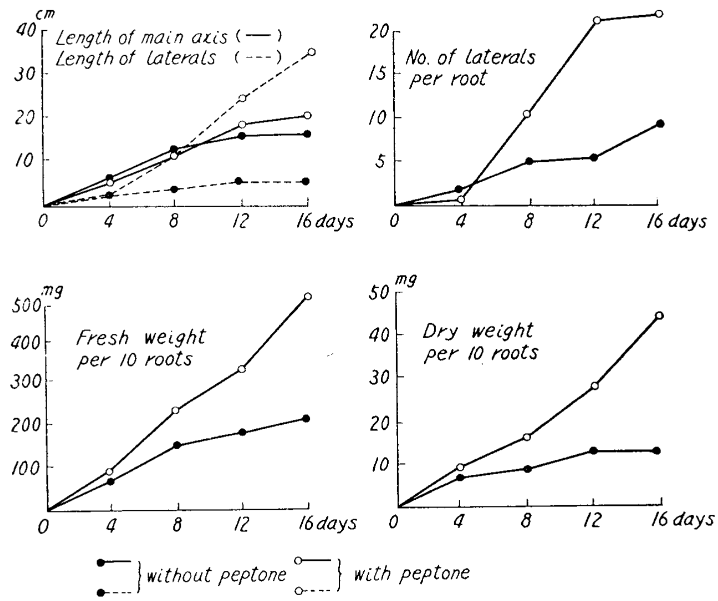


Fig. 9. Changes in the growth of the excised roots in the medium with or without peptone with time. Glucose concentration ; 2 per cent.

the medium was regulated with 0.1 N NaOH to keep in pH 5.1 after autoclaving of 10 lb, 10 min.

Fig. 9 represents the growth curves of the excised root under the improved environmental conditions. The length of main axis in the absence of peptone only increased gradually with time, but the increase ceased almost after 16 days. In the medium with peptone, on the other hand, the growth of the main axis tips proceeded more progressively between the eighth and 12th day and surpassed that of the excised roots without peptone during this period, although rather less until the initial several days. The beneficial effects of peptone were also observed in the production of laterals, which was represented by the numbers of the laterals as shown in Fig. 9. The production of laterals was found from approximately the fourth day, and its development was conspicuous in the medium with peptone and then increased straightly with time. The root weights, namely fresh or dry, always were better in the presence of peptone than in the absence of it.

The excised root tips were examined under the microscope at the end of two week culture period. As seen in the photomicrograph, the main axis tips in the absence of peptone gave taper shape and the root cap cells were hardly observed on account of the falling off (Fig. 10). This conversion of

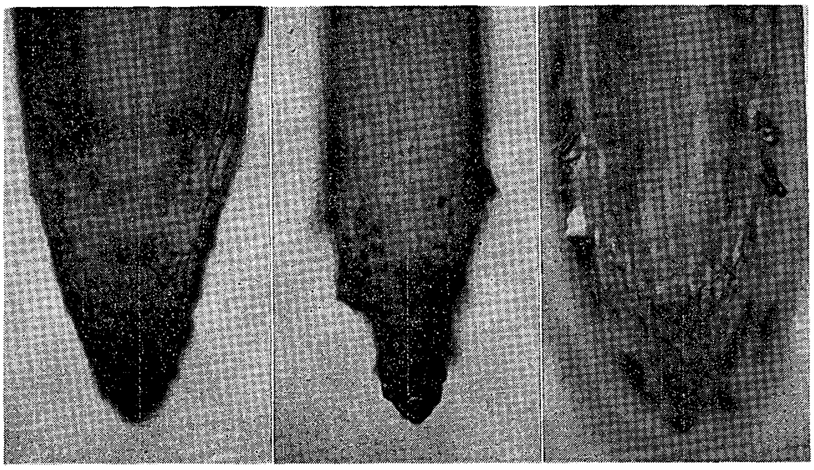


Fig. 10. Excised wheat root tips cultured in the medium with or without peptone for 16 days. Left ; before culture. Middle ; without peptone. Right ; with peptone.

the shape in the tips with time was so severe that its original structure of meristem apparently could not be discriminated, presumably being deprived of their meristematic activity. On the other hand, the root tips in the presence of peptone were thick and white in color and gave no great difference in their shapes, as compared with those before the culture. This phenomenon implies that peptone lead the tips of the excised root to retain their meristematic activity.

Moreover, the beneficial effects of peptone were confirmed by the root culture during such a longer period as three months. The nutrient solution was renewed twice every month. The growing state of the excised roots was demonstrated in Fig. 11. In the absence of peptone, the growth of the main axis tips declined gradually and ceased almost after three or four weeks,

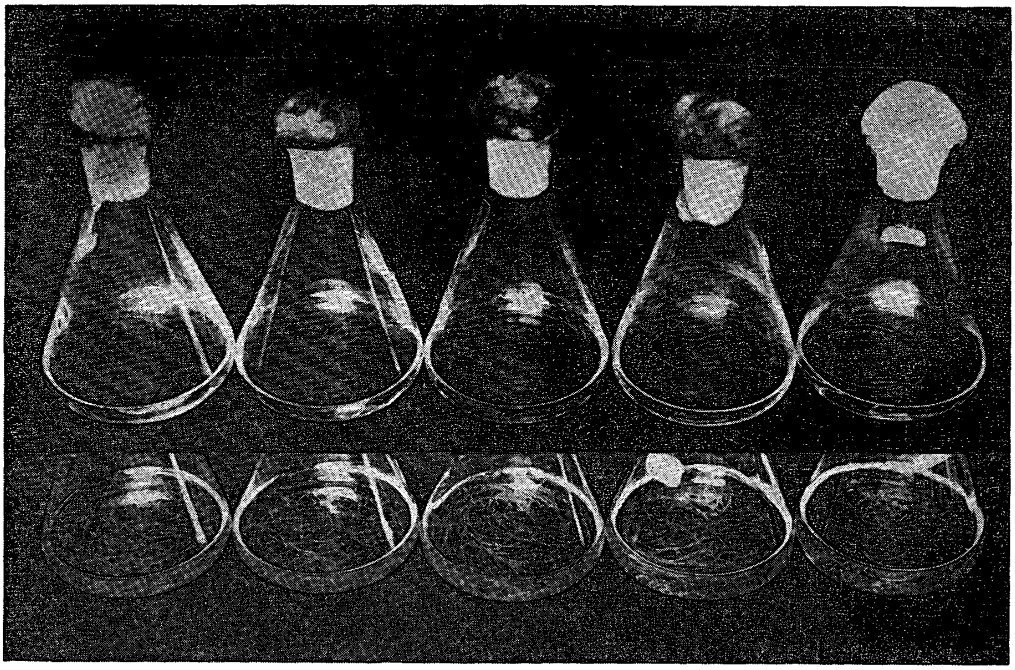


Fig. 11. Beneficial effects of peptone on the growth of the excised roots under the improved conditions. Upper ; without peptone. Under ; with peptone of 300 ppm. Culture time ; three months.

attaining only to 20 or 30 cm in length. The roots, both main axis and laterals, were entirely slender and brown in color. In the presence of peptone, the main axis tips continued to grow with the rate of about 10 mm per day and attained to about 100 cm at the end of the culture period. The apparent state of the root tips under the microscope was obviously different from that in the absence of peptone, permitting the normal form to be capable to grow still more.

However, a few main axis tips ceased to grow by the way of the process in spite of the presence of peptone. In such a root, the peptone effect was found in abnormal development of the laterals rather than in the increase of the main axis length, and the root weight was somewhat superior to that of the normal roots. This fact seems to indicate that peptone can not exert equally both to the main axis and laterals, but inclines only to one of them and gives more conspicuous influence on the laterals rather than on the main axis.

According to Street and Roberts (18), the main axis meristem exerted a depressing effect on the growth rate of young lateral roots and the duration of meristematic activity of the main axis could be prolonged by the excision. These experimental results seem to be similar with ours as mentioned above.

It was evident as shown above that excised wheat roots were able to continue normal growth during the longer period under favorable or carefully controlled conditions, if peptone was added to the basal medium. However, attempt to subculture weekly the tips from main axis or laterals have not yet proved successfully, although the beneficial effects of peptone were clearly observed in the subcultured root tips as well as in the original root tips. If the root tips excised from seedling roots were grown for a week, and then 10 mm of their main root tips were cut off and transferred to the fresh nutrient solution containing peptone, the growth rate decreased more in the second period than in the first and ceased in the third or the fourth. Subculture of the tips from the laterals also brought about the same results as the case of the main axis tips noted above. The root tips in the first passage may contain any favorable materials for growth, which will not be supplied yet adequately with peptone.

IV. Influence of peptone on the nutrient uptake, nitrogen contents and respiratory rate of the excised roots.

The beneficial effects of peptone on the apparent growth of excised wheat roots were metabolically confirmed through the investigation on the nutrient uptake, nitrogen contents and respiratory rate of the excised roots. Fig. 12 shows the effects of peptone on the uptake of nutrients, *i. e.* phosphorous, potassium, calcium and glucose, with the excised roots. Typical curve of the nutrient uptake with time was found most distinctly in the case of glucose, which is one of the most important nutrient and controls for the growth. In the absence of peptone, the amount of glucose uptake increased gradually with time, and then reached the maximum after the 12th. On the other hand, the glucose amount absorbed in the medium with peptone increased straightly until the 16th with time, and surpassed that in the

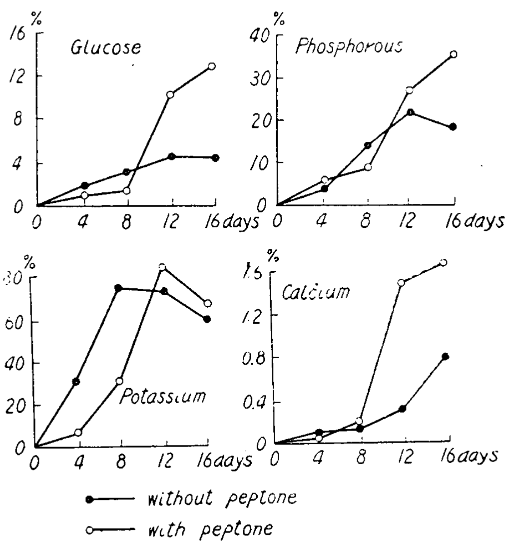


Fig. 12. Changes in the uptake amount of the nutrients of the excised roots with time. In this experiment, four root tips were grown together in a flask. The amount of uptake of each nutrient was expressed as per cent, i. e. ratio of the amount in the medium after the culture for that of before culture.

medium without peptone between the eighth and the 12th, although it was less until the initial eight days. The similar facts were also observed in the case of some inorganic major ions, namely phosphorous, potassium and calcium except magnesium. Only magnesium was an exception and its uptake in the solution without peptone was better than that with peptone through all the time, though the result is not shown here. It may be necessary to further investigate on this point. Potassium, absorbed ahead with the excised roots was taken off again to the solution and appeared to be a movable-ion in the living material. It may be markedly emphasized from the experimental results that the uptake of glucose by the excised roots in the presence of peptone was slightly controled in the earlier stage, and increased abruptly in the next stage. The similar pattern of the nutrient uptake with time was obtained in the uptake of some inorganic nutrients.

Fig. 13 shows the changes in nitrogen contents of the excised roots with time. Between the presence and the absence of peptone in the medium, no grave differences were noted in the total or the insoluble nitrogen contents per root until the fourth or the eighth day. In the presence of peptone, however, a notable increase in the total nitrogen content of the excised roots was marked from the eighth to the 12th or 16th.

This increment of total nitrogen content in the presence of peptone is surely due to the rise of insoluble nitrogen, which is mostly regarded as protein nitrogen. This implies that peptone plays, either directly or indirectly, an important part on the synthesis of protoplasmic protein in the root cell.

Observation on the respiratory change of the excised roots further confirmed that peptone was beneficial for the growth. Influences of peptone on the respiratory rate of the excised roots are given in Fig. 14. The respiratory rate per unit root increased notably with peptone, although was very weak without peptone. These curves of oxygen uptake with time were very similar to those of nitrogen compounds of roots in Fig. 13. Thus, it was apparent for the excised roots in the presence of peptone to respire vigorously in parallel to protein synthesis.

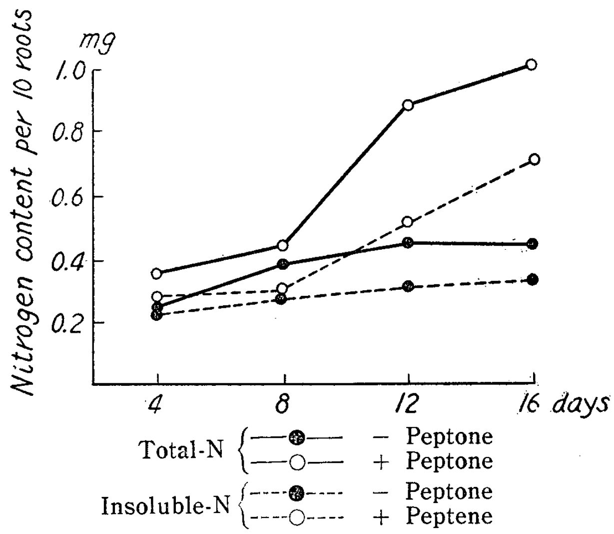
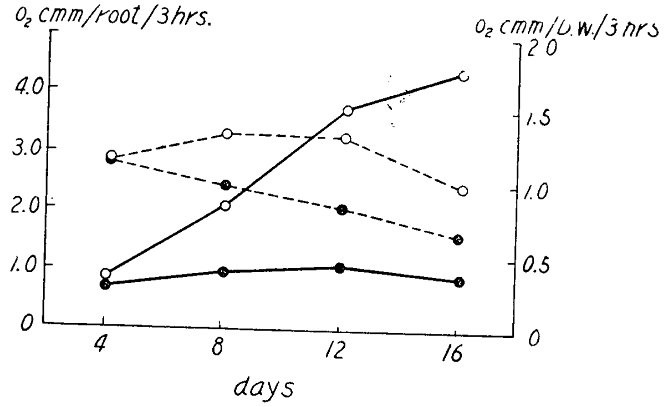


Fig. 13. Changes in the nitrogen contents of the excised roots grown in the absence or the presence of peptone with time.

Based on the amount of dry weight, the respiratory rate of the roots fell off more rapidly in the absence of peptone than in the presence of it. The decrease of respiratory rate of the excised roots in the presence of peptone with time is probably due to the increase of the dry



—●— Per root, − Peptone ----●---- Per mg dry weight, − Peptone
—○— ,, ,, , + Peptone ----○---- ,, ,, ,, , + Peptone

Fig. 14. Changes in the respiratory rates of the excised roots grown in the absence or the presence of peptone with time.

weight, which depends upon the accumulation of inert nonrespitratory materials, and the decrease marked in the absence of peptone seems to have different contents, signifying the loss of respiratory activity with the aging of the roots.

Rabideau and Whaley (12) reported that both the growth and the respiratory of the excised tomato roots increased by the supplement of B-vitamins, as if by peptone in our experiments. According to Machlis (9), the respiratory intensity of the roots consisted in that of the different segment of the root and its rate decreased according to the distance from the apex. Kandler (8) also reported that a new stimulation was found, when the laterals developed. These reports suggest that the respiratory intensity of the root depends on the numbers of laterals or of root tips having meristematic activity, and the increase of the respiratory intensity of the excised wheat roots with peptone, therefore, seems partially to be due to the indirect effects through the stimulant action of production of the laterals roots.

Discussion

It was formally pointed out from the observations under the microscope that the excised main tips in the presence of peptone were capable of keeping on original meristem size to grow continuously through the given culture period. The absence of peptone from the nutrient solution in which the excised roots were allowed to grow, resulted in the advent of taper shape of the main axis tips.

This appears that the cell division in the root meristem region becomes increasingly sluggish and ceases essentially in the advancing stage, unless peptone was supplemented. From the histological point of view, some substance contained in peptone may be referred to as a hormonal substance for cell division in the roots.

It was further shown in this paper that excised wheat roots in the presence of peptone were excellent in the elongation of the main axis length, production and development of the lateral roots and the increaes of fresh or dry weight of the roots. Robbins (13) demonstrated with the excised corn root tips that peptone was beneficial in the elongation of the length, production of secondary roots and dry weight. The results obtained by Robbins seem nearly to accord with ours.

By what mechanism peptone exerts those effects on the excised roots, we are not able to answer now because of the inadequate knowledge on any promoting substance contained in it. From the results observed metabolically, however, we allow peptone to be beneficial for growth throughout some physiological phenomena. The growth of the main roots in the presence of peptone was less than in the absence during the first several days, but proceeded greatly in the later half of the culture period. The shifts from the first phase to the next in the presence of peptone appear to imply adaptation of the root itself to circumstances *in vitro*. In correspondance with a pattern of the growth, the similar changes in the physiology of growth process may naturally be expected. The nutrient uptake curves of the excised roots with time actually accompanied with this growth pattern along the longitudinal axis.

Together with uptake of sugar, which plays a principal role both in the metabolism and the structure of the organ, the increase of oxygen uptake of the roots in the presence of peptone suggests that the energy is liberated and make available to energy using the reaction of the roots through the process of the respiration. In the excised roots with peptone, thus synthesis of the new chemical substances, the uptake of inorganic ions and the maintenance of living condition, which depended on energy derived from respiration, would be smoothly proceeded.

What constituent in peptone would immediately exert such a promoting effects? The possible role of peptone may be due to either a particular amino acid or peptide group which is not produced in the roots themselves though it is essential for protein synthesis, or any unknown substance to exert as a stimulant like B-vitamins for the growth of some dicotyledonous plant roots. Virtanen and Linkola (19) stated that the significance of organic nitrogen compounds could be great, since the uptake of those nitrogen compounds even in small amounts may effect the plants markedly. In addition to exertion as the building blocks for proteins, peptone may act as the growth promoting substance. The facts that peptone effects are found in such special symptom as the lateral production and the cell division may be referred to the stimulus action as the characteristic substance instead of as nitrogen source. This speculation seems to be supported by the reports that many excised roots have been able to synthesize protein in the root itself and to succeed the unlimited growth of some excised roots in only a nitrogen source of nitrate. By what constituent of peptone and through what mechanism the excised wheat roots bring about the beneficial effects mentioned above, are being carried out.

Summary

(1) Physioloical effects of peptone on the growth of the excised wheat roots were described, with some remarks about the environmental conditions under the supply of peptone.

(2) Neopeptone was beneficial for the growth of the excised roots in comparison with the other organic or inorganic nitrogen sources. The beneficial effects were notably found in the production and the development of the laterals or secondary roots.

(3) To obtain the more satisfactory growth in the mdeium containing peptone, the influence of the physical or chemical environmental conditions on the growth were investigated. By increasing the amount of glucose in the basal medium by 2 per cent of two times, the desired beneficial effects were found in the growth of the main axis tips, too.

(4) The optimum concentration of peptone was in approximately 300 ppm notwithstanding with or without nitrate as the nitrogen source of the basal medium, and the effects with peptone were unable to be replaced with nitrate.

(5) Under the improved environmental conditions, the main axis length in the presence of peptone attained to about 1 m in length after three months, whereas only to 20 cm in the absence of peptone. However it failed to establish the excised root clones in the presence of peptone on account of ceasing growth in three or four passages of subculture.

(6) It was a characteristic phenomenon in the growth curve of the main axis that the growth in the medium with peptone was always less rather than that without peptone in the earlier period and surpassed it in the advancing period. The similar trend was also seen in the uptake of the nutrients, inorganic ions and glucose.

(7) Peptone effects were metabolically observed, being found to result in the nutrient uptake, the synthesis of protein or root material and the respiration of the excised roots under the interaction between them.

(8) Since the changes in the microscopical structure of meristem region

with time are negligible in the presence of peptone, it is believed that substances contained in peptone are referred to as a stimulant substance for cell division in the roots.

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