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INCREASED GROWTH OF ROOTS OF VICIA FABA L. ON A HORIZONTAL CLINOSTAT

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Abstract

1. Roots of *Vicia faba*, on the horizontal clinostat revolving at velocities of 1 revolution per hour and 1 revolution per 2 hours, grew faster than vertical roots.

2. When roots were placed in a horizontal position at rest the upper side grew faster than vertical roots during the first hour. The growth rate of the lower side was not significantly different from the growth of vertical roots during the first hour. After that period the growth of the lower side decreased markedly.

3. It is suggested that the growth-increase of roots on the horizontal clinostat is caused by the fact that each root cell is at the upper side for a sufficiently long time to react with accelerated growth, but that this period is too short to inhibit the growth of the root cells when these are at the lower side.

Introduction

The effect of revolving roots on a horizontal clinostat has been investigated by Brain (1935) and by Larsen (1953). In Brain's experiments the growth of roots of *Helianthus*, *Lupinus* and *Avena* was less on the horizontal than on the vertical clinostat, but roots of *Zea mays* grew faster on the horizontal clinostat. Larsen, who worked with roots of *Artemisia* seedlings, found a growth reduction the size of which depended on the velocity of the horizontal axis. The reduction was maximal at 1 revolution in 16½ minutes. In the experiments of Audus and Brownbridge (1957), the growth of roots on the horizontal clinostat seemed to be faster than that of normally growing roots. The difference was, however, not statistically significant. In the present paper experiments are reported on the growth of roots of *Vicia faba* in a horizontal position both on a clinostat and at rest; roots were also grown in the vertical position at rest as controls.

Material and methods

Seeds of *Vicia faba*, cultivar "*Witkiem*" were germinated in vermiculite in a dark room at 26° C. 2½ days after sowing, seedlings which had formed straight roots of about 2 cm length were selected.

The roots were marked with printer's ink at a distance of 1 cm from the tip. They were allowed to grow for a period of 16½ hours either on a horizontal clinostat, or at rest in horizontal and vertical positions on a bench in the same room. The seeds were kept in place by means of pins which were stuck into wooden discs covered with foam-plastic. Each disc was fixed to an axis of the clinostat; the roots of the seedlings were parallel to the axis. Plastic wash-basins were placed over the seedlings to keep the atmosphere around the roots at 100 % relative humidity. The edges of the wash-basins were firmly pressed against the foam-plastic by means of rubber bands.

While the roots were being placed on the clinostat, they were kept in a vertical position. The seedlings were handled under weak light from a Tungsten filament lamp.

At the end of the experiment, the amount of growth which had occurred was recorded; the distance between the root tip and the ink-mark was measured to the nearest millimetre by means of a caliper gauge.

The clinostat used was of the Vendrig–de Bouter type (cf. Vendrig, 1960). It had six horizontal axes which turned at velocities of one revolution in 1, 2, 10, 30, 60 and 120 minutes.

To determine the growth of the upper and lower side of horizontal roots during geotropic curvature, the roots were attached to a similar disc as that on the clinostat. An inverted plastic wash-basin, the bottom of which had been replaced by two sheets of Plexiglas, (double, to prevent condensation of water on the window) served to maintain a high humidity.

Controls using roots in the vertical position were also set up. These vertical roots were fixed to wooden discs covered with foam plastic in the same way as the roots on the clinostat, with the exception that they remained in the vertical position throughout the experiment.

At intervals the roots were photographed through the Plexiglas window in the light of a Philips flash bulb. The photographs were projected on to a white wall at 6 times magnification. By means of a curvimeter the length of upper and lower side of the image were determined starting from a fixed point on the root.

Results

1. *Growth-rate of roots on the horizontal clinostat and of vertical roots at rest*

Table 1 shows the elongation of roots in 16½ hours. As the whole of the growing zone was included in the part between the ink-mark and the root tip, the figures in Table 1 represent the total growth of the roots.

Table 2 gives the differences between the average growth of the roots on the clinostat and that of the vertical control roots. The growth of individual roots varied considerably. In the *t*-test the differences listed in Table 2 were non-significant at the 5 % level. However, in 9 out of 11 experiments the average growth of the roots, which had made 1 revolution in 60 minutes, was more than that of the vertical roots. Similarly, in all 6 experiments in which the roots on the clinostat had made 1 revolution in 120 minutes, the average growth was higher than that of the controls. If roots revolving

TABLE 1

Elongation of roots in vertical position and in horizontal position on the clinostat. The figures represent the average increase in length in mm in 16½ hours of a zone of 10 mm from the root tip.[1])

Expt. no.	Vertical	Clinostat one revolution in:			
		10 min.	30 min.	60 min.	120 min.
1	9,2 (22)	9,5 (25)	9,9 (23)	11,5 (22)	
2	7,8 (14)	9,6 (14)	8,7 (15)	9,5 (13)	
3	8,8 (36)	8,6 (32)	8,8 (32)	9,7 (32)	
4	8,0 (28)	8,0 (27)		8,0 (30)	8,4 (27)
5	8,3 (26)			8,9 (28)	8,4 (24)
6	15,7 (16)		15,6 (10)	15,8 (17)	16,7 (18)
7	15,7 (26)		15,6 (24)	18,1 (18)	17,0 (19)
8	14,0 (36)			14,2 (32)	
9	17,6 (9)			19,6 (9)	19,1 (9)
10	10,7 (30)			10,0 (30)	11,3 (30)
11	16,8 (23)			18,0 (21)	

[1]) The figures between brackets indicate the number of measured roots.

TABLE 2

Growth of the vertical roots of table 1 and the differences in growth between these and the roots on the clinostat (mm/16½ hours).

Expt. no.	Vertical	1 rev./ 10 min.	1 rev./ 30 min.	1 rev./ 60 min.	1 rev./ 120 min.
1	9,2	+ 0,3	+ 0,7	+ 2,3	
2	7,8	+ 1,8	+ 0,9	+ 1,7	
3	8,8	− 0,2	± 0,0	+ 0,9	
4	8,0	± 0,0		± 0,0	+ 0,4
5	8,3			+ 0,6	+ 0,1
6	15,7		− 0,1	+ 0,1	+ 1,0
7	15,7		− 0,1	+ 2,4	+ 1,3
8	14,0			+ 0,2	
9	17,6			+ 2,0	+ 1,5
10	10,7			− 0,7	+ 0,6
11	16,8			+ 1,2	

on a horizontal clinostat remain unaffected by this treatment, then it would be expected that only 50 % of the readings would be higher than the vertical controls, whilst the other 50 % would be lower.

The probability that the deviations from a fifty-fifty distribution were due to chance variations was tested by means of the signed rank test of Wilcoxon (DIXON and MASSEY, 1957). It proved to be 1.4 % for roots which had made 1 revolution in 60 minutes and 1.6 % for roots which had made 1 revolution in 120 minutes. These figures show that at these velocities, roots on a horizontal clinostat grow faster than vertical roots, with a significance well above the 5 % level.

Only the two lowest revolving velocities of the clinostat caused a significant increase of growth. LARSEN (1953) also found that roots grew faster on the clinostat at low velocities (though in his experiments never faster than vertical roots). LARSEN pointed out that at low revolving velocities the preparatory process in the root for the geotropic reaction will be completed before it is counteracted by a stimulus in the opposite direction. If this results in a change of the growth-rate (as LARSEN assumed) a study of the first stages of the geotropic reaction might yield a clue to the explanation of the increased growth of roots on the clinostat.

2. *Growth of upper and lower side of horizontal roots at rest*

Horizontal roots were photographed at intervals and the length of the lower and the upper side was determined. The growth of vertical roots from the same batch under identical conditions was measured simultaneously. The results were plotted on paper and the growth during the first hour was determined by interpolation.

Table 3 shows that the average growth of the upper side is faster than that of the corresponding vertical roots in all six experiments. The lower side had a higher average growth than the vertical roots in two experiments; it grew less than the vertical roots in three experiments, and growth was equal in one experiment. As in the foregoing section each figure in Table 3 is statistically non-significant, although it is the average of a number of measurements.

TABLE 3

Average growth of the lower side (left column) and of the upper side (right column) of horizontal roots during the first hour, and average growth of vertical control.

Expt. no.	Horizontal Lower side	Difference	Vertical	Difference	Horizontal Upper side
	mm	mm	mm	mm	mm
1	5.0	+ 1.7	3.3	+ 3.8	7.1
2	3.9	0	3.9	+ 2.1	6.0
3	1.9	− 1.2	3.1	+ 0.3	3.4
4	3.9	+ 0.7	3.2	+ 1.6	4.8
5	3.4	− 0.5	3.9	+ 0.4	4.3
6	2.9	− 0.5	3.4	+ 0.4	3.8

(Total number of roots measured in the 6 experiments: horizontal 58, vertical 56).

Application of Wilcoxon's signed rank test to the differences listed in column 4 of Table 3 shows that there is a probability of 1,6 % that the upper side of the horizontal roots was growing at the same or at a lower rate than the vertical roots. In other words, the growth of the upper side was increased during the first hour after placing the roots in a horizontal position. The growth of the lower side was not significantly altered during the first hour.

Discussion

The increase in the growth of roots on the clinostat at low revolving velocities may be explained in the following way. In a horizontal root at rest the growth of the upper side increases during the first hour, whereas the growth of the lower side remains unaltered. On the clinostat, however, each longitudinal row of root cells will be above the median horizontal plane for ½ or 1 hour, and in that period its growth will increase. After it has passed the median horizontal plane it will be part of the lower side of the root for an equal length of time. Since this period, in the present experiments, never exeeded one hour, it was too short for an inhibition of the lower side to begin. The result will be that the growth of the root cells is increased during one half of the rotation time and that it is not correspondingly inhibited during the second half. In other words, these roots grow faster than vertical roots.

As was mentioned above, increased growth on the horizontal clinostat has been found by Brain (1935) with roots of *Zea mais*. Brain evidently considered this reaction as exceptional, since in three other species the growth of roots of seedlings on the horizontal clinostat was reduced, and since the main root of *Zea mais* has been found to be slightly plagiogeotropic. (Porodko, 1924). Larsen (1962) suspects that the absence of a significant effect of the clinostat on the growth of roots in the experiments of Audus and Brownbridge may be due to the "waterdrop effect", i.e. a retardation of the growth of a vertical root by the accumulation of a waterdrop around the tip (Cholodny, 1932; Navez, 1933).

The same criticism might be applied to the present results, but it seems rather unlikely to be valid for the following reasons. In the first place waterdrops were never observed around the tip of the vertical roots. In the second place, in order to use the waterdrop hypothesis to explain the fact that in horizontal roots the growth of the lower side equals the growth of vertical roots, one must assume that the lower side is covered by a layer of water along its whole length. This is, of course, possible, but it is not likely to have occurred under the conditions of the experiments. Thirdly, it is unlikely that, during the first hour, the difference in growth of the upper and lower side in horizontal roots is caused solely by an unequal distribution of adhering water. Internal factors, e.g. shifting of auxin to the lower side, are probably active during the same period. If these internal factors are causing a retardation of the lower side, and if the adhering water also is inhibiting, the growth of the lower side should be less than the growth of a vertical root, which is inhibited by adhering-water only. Since a significant difference in growth of the lower side and of vertical roots could not been detected, it is not very probable that any "waterdrop effect" was active in the present experiments.

One may ask if the present results fit the theory of Cholodny–Went. The fact that the retardation of the lower side lags behind the acceleration of the upper side may be accounted for by this theory if it is assumed that, at the concentration prevailing in the root, the relationship between concentration of auxin and growth is non-linear. In fact, the results of AUDUS and GARRARD (1953) and AUDUS and DAS (1955) show this relationship to be logarithmic. Although the present results are compatible with the theory of Cholodny–Went, they furnish no proof for this theory as long as the unequal distribution of IAA (or some other growth hormone) has not been demonstrated in horizontal roots.

The present results are at variance with those of AUDUS and BROWNBRIDGE (1957). In the experiments of these authors the growth of *Pisum* roots was accelerated at the upper side and retarded to a greater extent on the lower side during the first hour. Rather than trying to explain this and other discrepancies, on a methodological basis, it seems safer to assume that different roots react to the horizontal clinostat in different ways.

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REFERENCES

AUDUS, L. J. and M. E. BROWNBRIDGE. 1957. Journ. of Expt. Botany **8**: 105–124.
——— and N. DAS. 1955. Journ. of Expt. Botany **6**: 328–347.
——— and A. GARRARD. 1953. Journ. of Expt. Botany **4**: 330–348.
BRAIN, E. D. 1935. New Phytologist **34**: 97–112.
CHOLODNY, N. 1932. Planta **17**: 794–800.
DIXON, W. J. and F. J. MASSEY JR. 1957. Introduction to statistical analysis. (New York-London-Toronto. 2nd Ed.)
LARSEN, P. 1953. Physiol. Plantarum **6**: 735–774.
———. 1962. Handbuch der Pflanzenphysiologie **17/2**: 34–73.
NAVEZ, A. E. 1933. Botan. Gazette **94**: 616–618.
PORODKO, TH. M. 1924. Ber. Deutschen Bot. Gesell. **42**: 405–419.
VENDRIG, J. C. 1960. Wentia **3**: 1–96.