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A COMPARISON OF THE MORPHOLOGICAL DEVELOPMENT OF AERATED AND NON-AERATED PRIMARY ROOT SYSTEMS OF PHASEOLUS VULGARIS L.

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SUMMARY

A comparison was made of the morphological development of the primary root systems of aerated and non-aerated two weeks old bean plants (*Phaseolus vulgaris* L.). Elongation of the tap root axis was analysed with regard to cell division and cell extension. The development of non-aerated root systems proved to be directly, locally inhibited in the tap root. Moreover, indirectly, the development of the secondary roots was stimulated. It is shown that branching density in itself is no valid criterion of branching activity.

1. INTRODUCTION

Several authors have studied aspects of the morphological development of roots in relation of different aeration conditions (ERCKSON 1946; BROUWER 1977; GEISLER 1965). An inhibition of the growth in length of non-aerated roots is generally agreed upon. An analysis of the root elongation in cell extension and cell division, necessary as Burström has pointed out for understanding the effect of a factor on root length, was not found in literature (BURSTRÖM 1942). The effect of poor aeration, or none at all, on the branching of the root is subject to a difference of opinion. Some authors report a reduction in branching (GEISLER 1965), others a stimulation (BROUWER 1977).

The difference in development of aerated and non-aerated roots has been attributed to a number of possible causes. Several factors may be operative simultaneously. For their disentanglement a detailed description of the effect of absence of aeration on root morphology is necessary.

The aim of the present paper is to describe in some detail the morphological differences between root systems that have been grown on aerated and non-aerated nutrient solutions.

2. METHODS

Seeds of *Phaseolus vulgaris L.*, var. *Berna*, were germinated on moist filter paper or sand. When the tap root (radicle) was 5–6 cm long, the seedlings were placed in a growth cabinet on containers with a, slightly modified, half strength Hoagland solution according to Steiner (STEINER 1968; STEINER & VAN WINDEN 1970).

In some of the containers the solution was aerated, in the others not. All solutions were renewed twice a week. A 16 hours' light period was used. Four

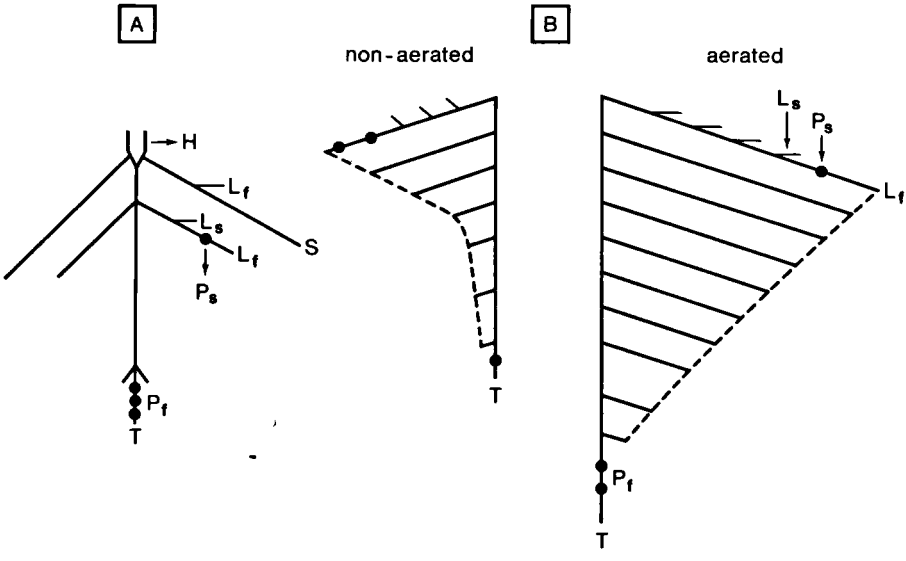

Fig. 1. A schematic graph of A: the primary root system of a two weeks old plant of *Phaseolus vulgaris* L., B: the tap root, grown with and without aeration.
Key: H = hypocotyl, T = tap root, S = secondary root, L_f, P_f, L_s, P_s = laterals and primordia of the first resp. the second order.

Philips HPL lamps provided a light intensity at the height of the primary leaves of about 45 Watt. m^{-2}. The temperature was 23°C during the light period and 15°C during the dark period and the relative humidity was 60 %. The primary root systems were examined when the plants were two weeks old.

The oxygen content of the nutrient solutions was measured by platinum membrane electrode and expressed as the percentage of the oxygen content of oxygen-saturated water of the same temperature. The aerated solutions of the two weeks old plants had an oxygen content of 85–98 %, the non-aerated ones of 40–50 %.

In the time course experiments, where the roots were small at the beginning, the oxygen content near the tap roots was 90–98 % in aerated solutions. In non-aerated solutions it decreased gradually to about 60 % in four days and to 20 % in nine days.

The quantification of morphological aspects: Root systems were fixed and the cortex made transparant in FAA. Thus the primordia could be counted. The length of the axes was measured on a ruler, that of the first order laterals on graph paper (*fig. 1A*). In most experiments the tap root systems were chosen for comparison. In order to obtain an overall picture of the primary root system in some experiments the secondary roots were also examined.

Estimation of average cell length and number of cell divisions: Cell extension and cell division were determined as follows. At the beginning and at the end of a growth period of three days, during the period of rapid growth of the tap root

axis, the distance from a marked point at the axis to the root tip was measured.

On the newly grown root part the length of 50 mature epidermal cells was measured microscopically, 12–13 cells in four different areas. The number of cells formed in longitudinal rows was calculated by dividing the increase in length of the axis by the average length of the epidermal cells. The meristem and the zone of stretching cells, which contain small, immature cells, are included in the total length of the root. In our calculations it was assumed, that the number of cells in a longitudinal row in the zones of cell division and cell elongation were approximately the same at the beginning and at the end of the growth period.

The development recorded by photography: Plants were grown on the same nutrient solution as mentioned above and at approximately the same light conditions in glass boxes of 30 × 10 × 60 cm. The roots were photographed every day at the same time, after which the boxes were covered again to exclude the light.

In each of seven experiments the development of two aerated and two non aerated tap roots was recorded. The number of first order laterals was counted every day in situ. The growth of the tap root axis was measured on the photographs. A comparison of the elongation of the first order laterals was made by estimating the total length of the first order laterals on the photographs. Towards the end of the experiments the root members got too much entangled to permit the measurement of particular laterals.

The total length of the first order laterals was estimated by the following procedure. On transparent graph paper, placed over the photograph, the tap root axis was drawn and also an outline of the system by sketching a line along the tips of the first order laterals. At several points along the axis a clearly distinguishable lateral was traced. A schematic graph of the system, based on the measurement of these laterals and the general outline, permitted an estimate of the average length of the laterals (*fig. 1B*). Sometimes it was necessary to divide the axis in two or three regions. From the average length and the number of the laterals the total length was estimated.

3. RESULTS

If there was no aeration in the root medium, the elongation of the axis, the emergence and elongation of the first order laterals were reduced (*figs. 2a* and *2b*). The outlines of aerated and non-aereated tap roots were rather different (*fig. 1B*). The length of the non-aerated first order laterals decreased with a steep gradient along the upper 6 cm of the axis. On the aerated axis the length of the laterals decreased more gradually (*fig. 1*). In the first days after branching of the axis started, the oldest non-aerated first order laterals, closest to the surface, were sometimes longer than their aerated counterparts.

In regions of the tap root axis, where laterals had already developed, new ones might emerge a few days later.

A comparison of an aerated and a non-aerated tap root at the age of two weeks (*table 1*) shows a considerable reduction in total length as well as in number of

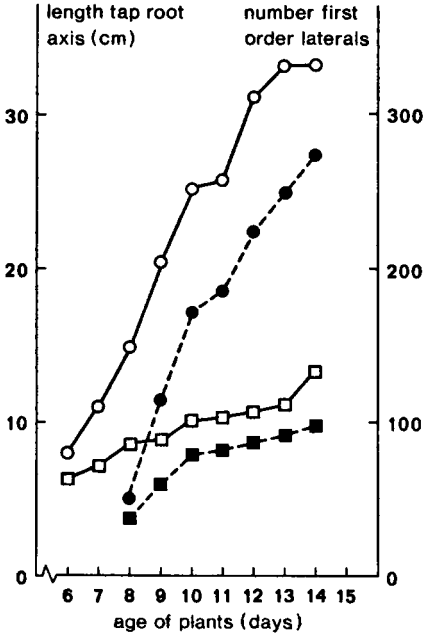

Fig. 2a. Development in time of the length of the axis and the number of first order laterals of an aerated and a non aerated tap root.
○ and □ = length of axis, aerated, and non aerated, respectively.
● and ■ = number of laterals, do.

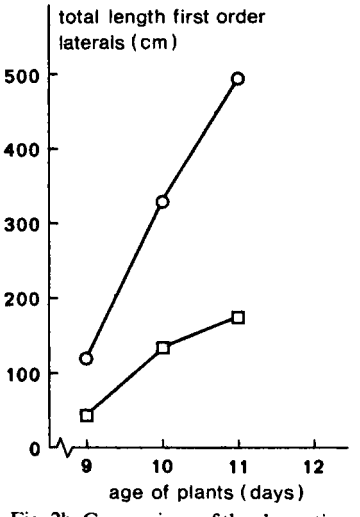

Fig. 2b. Comparison of the elongation of aerated and non aerated first order laterals during two days in the growth period.
○ = aerated, □ = non aerated.

Table 1a. The number of root members and primordia in the two weeks old primary root systems of an aerated and a non-aerated plant.

Treatment	Root system	Laterals, first order	Primordia, Laterals, first order	Primordia, Laterals, second order	Primordia, Total second order	Total of root system	Total of the primary root system
aerated	tap root	207	148	1355	1180	2890	
	sec. root	554	309	66	254	1183	4073
non-aerated	tap root	132	20	393	225	770	
	sec. root	773	252	369	874	2268	3038

Table 1b. The total length of root members of two weeks old primary root systems of an aerated and a non-aerated plant in cm.

Treatment	Root system	Axes	Laterals, first order	Laterals, second order	Total, root system	Total, primary root system
aerated	tap root	43.7	1028.9	646.5	1719.1	
	sec. root	130.0	819.5	15.2	964.7	2683.8
non-aerated	tap root	37.0	359.3	214.1	610.4	
	sec. root	125.3	1131.3	122.9	1379.5	1989.9

Table 2. The number and average length of epidermal cells, formed during a growth period of three days, on the axis of the top roots of aerated and non-aerated plants.

Root medium	Plant no.	Tap root, growth in cm	Epidermal cells, average length in μm	Number of newly formed cells	Confidence interval of length of epidermal cells (μm)
aerated	1	16	131.73	1215	123.37–140.09
	2	11	101.63	1082	95.64–107.62
	3	16	112.68	1420	103.84–121.50
	4	$10\frac{1}{2}$	110.21	953	103.31–117.11
non-aerated	1	6	97.99	617	90.26–105.72
	2	$3\frac{1}{2}$	85.78	408	81.52– 90.04
	3	3	96.69	310	90.04–103.64

root members of a non-aerated tap root system.

An analysis of the elongation of the axis shows, that the difference in length between aerated and non-aerated tap roots is mainly caused by the reduction of cell division (*table 2*). A positive relation existed between the length and the branching of first order laterals, both in aerated and non-aerated plants. The branching density (branches per cm) was higher in the non-aerated laterals (*fig. 3*).

Contrary to the tap root system, the secondary roots were stimulated in their development in non-aerated plants, even to a point that the reduction in growth of the tap root was partly masked if the whole root system was considered (*table 1*).

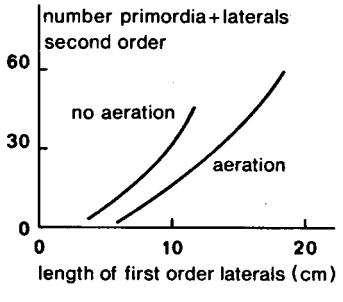

Fig. 3. The relation between the length and the number of branches of aerated and non aerated first order laterals in two weeks old bean plants.

4. DISCUSSION

We may distinguish a direct and at least one indirect effect of the absence of aeration on the whole primary root system. As far as the studied aspects are concerned, we regard as a direct, local effect the inhibition of the development of the tap root system. A more indirect, not specific, effect is the compensatory growth of the secondary roots and possibly as a result of this growth an additional inhibition of the development of the tap root system.

As secondary roots are near to the surface of the solution, it is difficult to decide whether their stimulated growth was due to oxygen diffusion through the solution or to an internal oxygen transport (GREENWOOD 1967 a and b; PAPENHUIJZEN 1978; VARTAPETIAN et al. 1978).

The higher branching density in first order laterals of two weeks old non-aerated tap roots might lead to the conclusion that branching is stimulated under non-aerated conditions. The time course study of development, however, shows that the number of branches formed per unit of time is decreased in non-aerated roots. To reach a certain length a non-aerated lateral needs a longer growth period in which more branches are formed than on an aerated one. So a higher branching density does not mean that branching has been stimulated. The non-aerated tap roots in our experiments resemble in all aspects the pea root systems of the decapitated plants of MC.DAVID et al. (1972).

In their experiments the removal of the shoot could be largely compensated for by the administration of indole acetic acid. Our plants were grown under different conditions, without such obvious reason for an inhibition of root development by a deficiency of auxin or assimilates from the shoot. If one assumes that in the present experiments the availability of oxygen is a major limiting factor of the metabolism connected with the development of root, the availability of compounds from the shoot will be of secondary importance. Further experiments on the influence of oxygen content of the nutrient solution on the morphological development of the root system will be published in a second paper.

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REFERENCES

BROUWER, R. (1977): Root functioning. In: *Environmental effects on crop physiology* (J. J. LANDSBERG & C. V. CUTTING, eds.). Ac. Press, 229–245.

BURSTRÖM, H. (1942): The influence of heteroauxin on cell growth and root development. *Ann. Agr. Col. Sweden* **10**: 209–240.

ERICKSON, L. C. (1946): Growth of tomato plants as influenced by oxygen in the nutrient solutions. *Am. J. Bot.* **33**: 551–561.

GEISLER, G. (1965): The morphogenetic effect of oxygen on roots. *Plant Physiol.* **40**: 85–88.

GREENWOOD, D. J. (1967a): Studies on the transport of oxygen through the stems and roots of vegetable seedlings. *New Phytol.* **66**: 337–347.

— (1967b): Studies on oxygen transport through mustard seedlings (Sinapis alba). *New Phytol.* **66**: 597–606.

MC.DAVID, C. R., G. R. SAGAR & C. MARSHALL (1972): The effect of auxin from the shoot on root development in Pisum sativum L. *New Phytol.* **71**: 1027–1032.

PAPENHUIJZEN, C. (1978): Preliminary observations on the effect of blocking the gas transport through the hypocotyl of Phaseolus vulgaris. *Acta Bot. Neerl.* **27**: 87–90.

STEINER, A. A. (1968): Soilless culture. *Proc. 6th Coll. Int. Potash Inst. Florence:* 324–341.

— & H. VAN WINDEN (1970): Recipe for ferric salts of ethylenediaminetetraacetic acid. *Plant Physiol.* **46**: 862–863.

VARTAPETIAN, B. B., I. N. ANDREEVA & N. NURITDINOV (1978): Plant cells under oxygen stress. In: *Plant life in anaerobic environments.* (D. D. HOOK & R. M. M. CRAWFORD, eds.).