Sterile Culture of Excised Tomato Roots in Sands of Different Grain Size

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WITH THREE FIGURES IN THE TEXT

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ABSTRACT

A simple technique to grow excised tomato roots in sand in sterile culture is described. The apparatus consists of a culture tube filled with sand linked to a small flask containing the nutrient solution. By raising the lower-placed flask the sand is flushed with solution, thereby replenishing nutrients at the root surface. Lowering the flask again allows air to enter the sand, thus effecting sufficient aeration.

It is further demonstrated that growth of the roots is related to aeration and not to mechanical impedance in sands of different grain size.

INTRODUCTION

SINCE the possibility of establishing excised root cultures was demonstrated by White (1934), these cultures have been used for numerous purposes. The usual substrate has been a fluid nutrient solution or the same solidified by means of agar addition. This method, which has the advantage of good visibility during growth, does not, however, permit investigations concerned with the influence of physical factors such as are encountered by a root growing in the soil.

An attempt has therefore been made to grow excised roots in a sandy substrate, in which at least some physical soil factors could be simulated. A successful technique developed in the laboratory of Street (personal communication) seemed rather intricate and laborious.

CULTURE TECHNIQUE

The culture technique is based on the principle that the sand substrate can periodically be flooded with the culture solution. After flooding, the solution is allowed to drain off and adequate aeration is achieved. This regular flooding is of importance in replenishing the supply of nutrients at the root surface. The necessity for this replenishment was borne out by the poor results in preliminary attempts, where roots were grown in ordinary tubes with moistened sand, without the possibility of flushing the substrate.

The apparatus consists of an ordinary glass culture tube, to which a small tube has been connected at the bottom. The culture tube is linked to an Erlenmeyer flask of 100 ml by plastic tubing (Fig. 1). By raising the flask

the sand is flushed with the solution, which is again allowed to drain back after 1–2 min. Flushing the substrate is carried out once or twice each 24 hrs.

The experiments are performed as follows: The bottom of the culture tube in front of the lateral opening is plugged with glass wool. The tube is filled with acid-washed sand of a specific particle size, which must be larger than 500 μ to obtain good results. The 100 ml Erlenmeyer flask is connected by

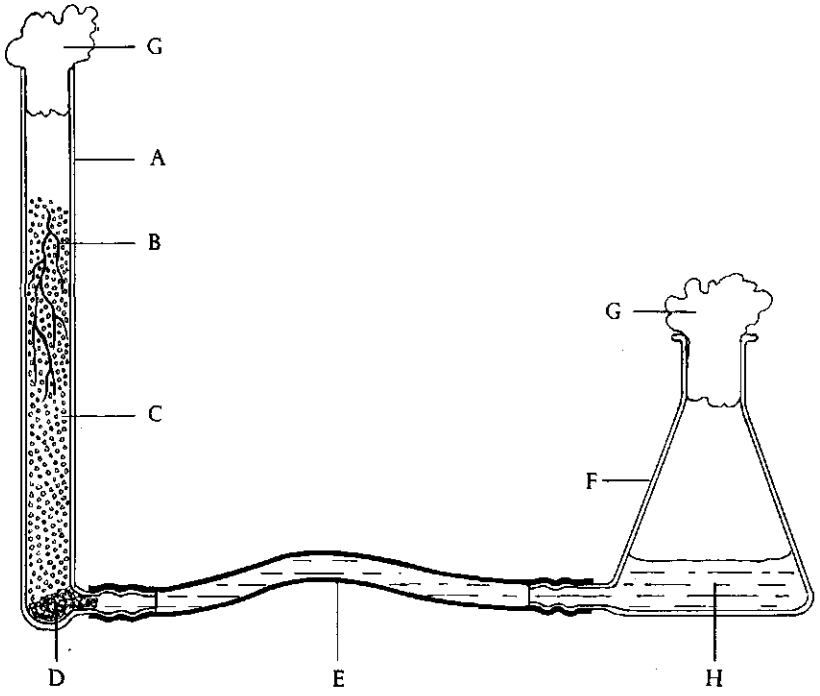

FIG. 1. Principle of the culture technique.

(*a*) glass culture tube.
(*b*) embedded root system.
(*c*) sand substrate.
(*d*) glass wool plug.
(*e*) plastic tube.
(*f*) Erlenmeyer flask.
(*g*) cotton wool plug.
(*h*) nutrient solution.

a piece of plastic tube and filled with 50 ml of a solution containing, per litre: $Ca(NO_3)_2.4H_2O$, 236 mg; $MgSO_4.7H_2O$, 36 mg; KNO_3, 81 mg; KCl, 65 mg; K_2HPO_4, 20 mg; Fe, 2 mg (as FeEDTA); sucrose, 17·5 mg; thiamine, 0·1 mg; nicotinic acid, 0·5 mg; pyridoxine, 0·1 mg; $ZnSO_4.7H_2O$, 1·5 mg; $MnSO_4.4H_2O$, 4·5 mg; $NaMoO_4.2H_2O$, 0·25 mg; H_3BO_3, 1·5 mg; $CuSO_4.5H_2O$, 0·04 mg; and KI, 0·75 mg.

Tube and flask are plugged with cotton wool, mounted in a stand and sterilized in an autoclave at 110° C (1·4 atm) for 10 min. Two days in advance a number of root tips of 1–1½ cm long are cut off from an established clone of tomato-root cultures growing in the same nutrient solution and transferred to a fresh flask of this solution. After 2 days the best-growing tips are trans-

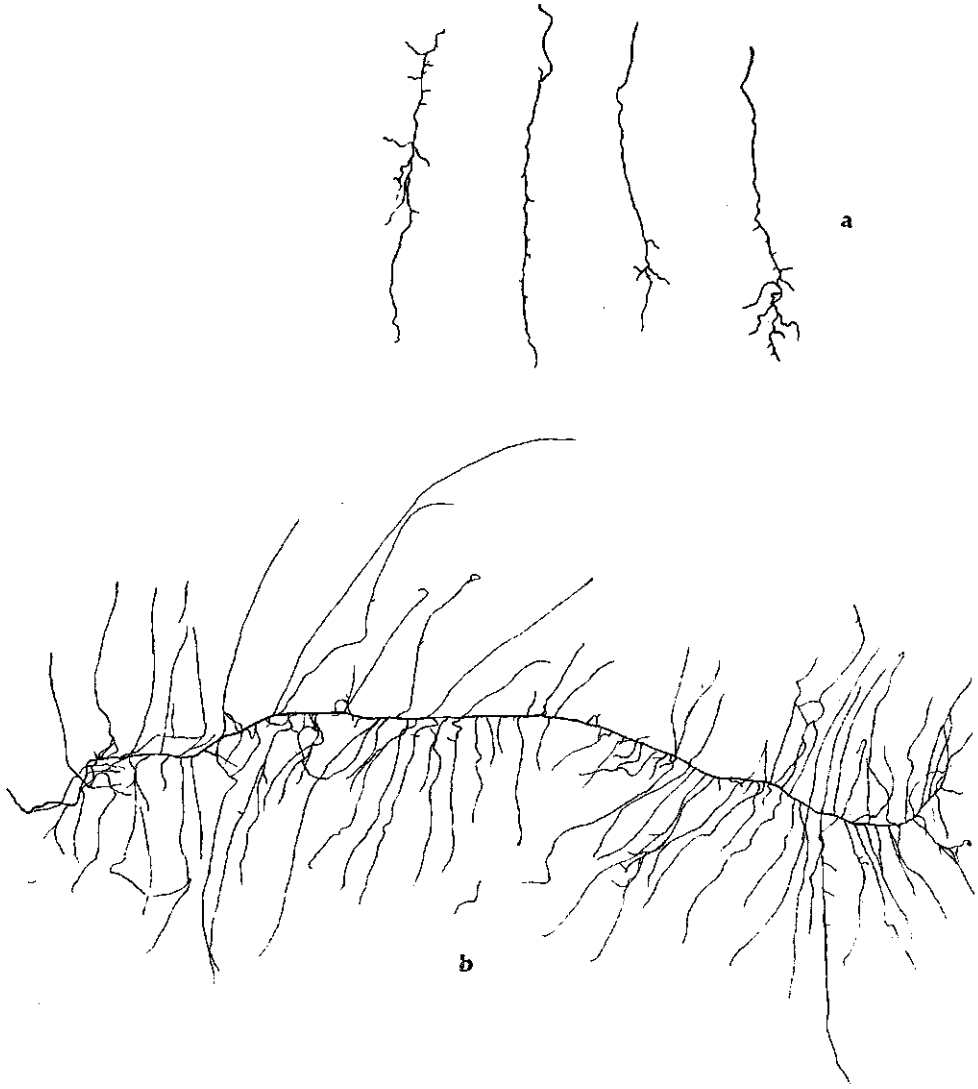

FIG. 2. Comparison of root growth in solution culture and in sand.
(*a*) growth after one month in sand of 850–1,200 μ grain size.
(*b*) growth over the same period in solution.

ferred aseptically to the culture tubes with sand. By tilting the tubes and gentle shaking, the moist sand can be brought to fill the tube with a slanting surface. The piece of root inoculum is then carefully placed on the lower part of the surface. With one or two raps the uppermost sand of the slanting surface can be made to fall on top of the inoculum. The root piece is then superficially buried in the sand without being damaged and is now protected against desiccation.

The stand, containing a number of tubes with their connected flasks, is now transferred to a cabinet, where the roots are allowed to grow in the dark at a temperature between 25–30° C. The sand is flushed with nutrient solution once or twice each 24 hrs by raising the Erlenmeyer flasks. Small evaporation losses at the top of the sand will be replenished by capillary rise of the solution as the level in the flask is above the bottom of the tube.

RESULTS

With the technique described tomato (*Lycopersicum*) roots can be satisfactorily cultured in a sand substrate. The growth of the roots, however, is very slow when compared with those in solution culture (Fig. 2). As the limiting supply of nutrient in the small amount of solution in the pores is the

TABLE 1

Length of root system and rate of oxygen diffusion in sand of different grain size

(*Root length*: mean values of base-to-tip length in mm. *Diffusion rate*: $g \times 10^{-8}$ O_2/cm^2/min, measured with a Pt electrode at 7 cm depth)

	Grain size, μ						
	150–210	210–300	300–420	420–600	600–850	850–1,200	1,200–1,700
Root length . .	—	—	28	31	46	81	99
O_2 diffusion rate .	6·3	8·4	10·6	18·8	—	29·4	—

main reason for the slow growth, this might partly be overcome by more frequent flushing.

The influence of a physical factor was studied by running a series of experiments in which the tubes were filled with sand fractions of different particle size. The results after culturing the roots for one month are given in Table 1. No growth occurred in the finest fraction and the pieces of inoculum died after some time. The coarser the sand the better the growth obtained (Fig. 3). Growth comparable with that in the coarser sand fraction also occurred using Vermiculite as a substrate.

The question arises as to what factor regulates the difference in growth as determined by grain size of the sand used. The two most obvious factors which might impede root growth are mechanical impedance or insufficient aeration.

By means of a simple needle penetrometer it could be demonstrated that depth of penetration under a load of 100 g was about the same regardless of the grain size of the sand. It therefore seems very unlikely that growth is restricted by mechanical resistance of the medium.

Rate of oxygen supply at about 7 cm depth in the sand was measured with the platinum micro-electrode (Lemon and Erickson, 1952). The data (Table 1) show a definite increase in rate of oxygen diffusion with increasing grain size. The low rates observed in the finer sand fractions at this depth are much lower than the value of 30–40 $g \times 10^{-8}$ $O_2/cm^2/min$, which is considered

as a minimum by Lemon and Erickson (1952). In the coarsest sand fraction where the roots grow downward to about 8 cm, a value of 29·4 is measure at 7 cm depth. This value is comparable to the above-mentioned critica value.

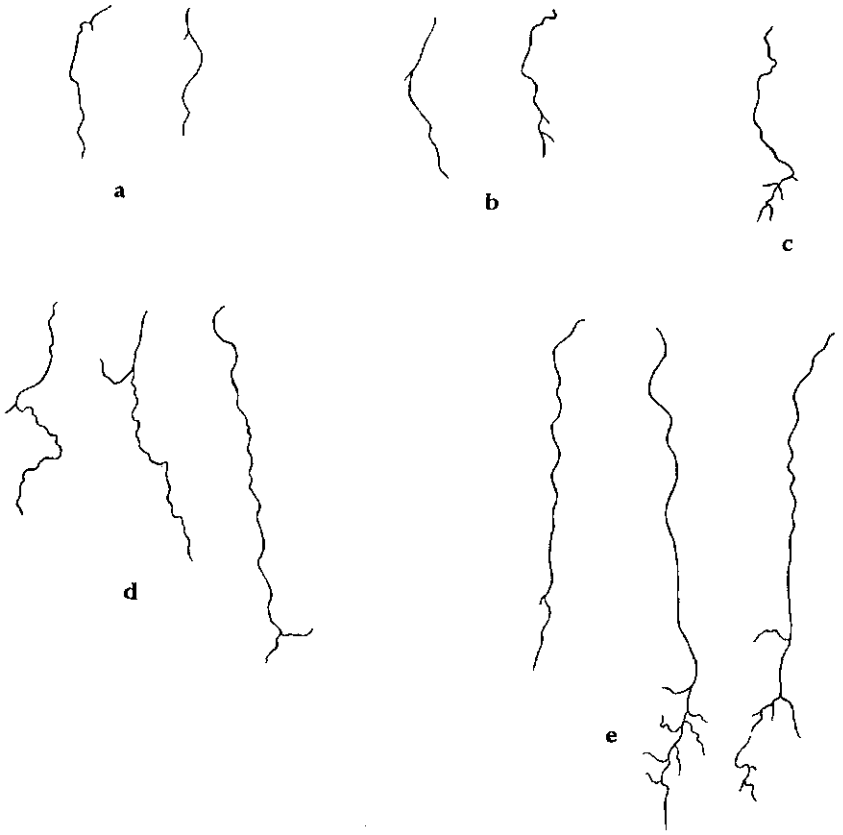

FIG. 3. Prints of root systems obtained in sands of different grain size.

(*a*) 300–420 μ
(*b*) 420–600 μ
(*c*) 600–850 μ
(*d*) 850–1,200 μ
(*e*) 1,200–1,700 μ

It may be concluded that oxygen supply is the factor regulating root length (depth of penetration) in the sand-filled tubes. The roots of intact seedlings of tomato and rye showed an identical relationship between depth of rooting and grain size of the sand.

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