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SOME CHANGES IN THE SUBCELLULAR STRUCTURE OF ROOT CELLS OF PHASEOLUS VULGARIS AS A RESULT OF CESSATION OF AERATION IN THE ROOT MEDIUM

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SUMMARY

Following cessation of aeration of the nutrient solution several effects could be observed in cells of the root tips of bean plants: Starch grains disappeared from the amyloplasts; small electron dense bodies, probably lipids, were formed and the rough endoplasmatic reticulum showed concentric arrangements. The internal structure of the mitochondria seemed unaffected.

1. INTRODUCTION

A recurring question in the study of the effects of insufficient aeration of nutrient solutions on root development is whether the low oxygen concentration may be considered as a primary inhibiting factor. Often a stronger inhibition of root growth is found than would be expected on the basis of the oxygen pressure measured in the medium. The oxygen concentration at the root surface, however, may be much lower because of the slow rate of diffusion of oxygen in water.

There are several reports on typical changes in the subcellular structure, induced by anaerobic circumstances (nitrogen atmosfere). A change in mitochondrial structure was observed in rice seedlings (KURSANOV et al. 1973, VARTAPETIAN et al. 1978) and in excised tomato roots (MORISSET 1978). Some instances of proliferation and concentric arrangements of the rough endoplasmatic reticulum are cited by FREY-WYSSLING & MÜHLETHALER (1965) and were observed more recently by PODBIELKOVSKA & BORYS (1975) in root tips of *Allium* and *Tradescantia.*

In connection with our studies on the effect of aeration on root morphology (PAPENHUIJZEN 1979) we considered it worthwhile to investigate whether cessation of aeration in the nutrient solution would induce similar subcellular changes in bean roots.

2. METHODS

Seeds of *Phaseolus vulgaris*, CV Berna, were germinated and the seedlings placed on containers with a slightly modified Hoagland solution, according to STEINER (1968) and STEINER & VAN WINDEN (1970) and micro-elements according to

CHALLA (1976). A 16 hours' light period was used. TLF 65/33 lamps provided about 45 Watt.m^{-2} at the level of the primary leaves. Temperature was 23 °C. Air was bubbled through the nutrient solution. When the plants were two weeks old, aeration was turned off in some of the containers.

The oxygen content of the nutrient solutions was measured with a platinum membrane electrode and expressed as the percentage of the oxygen content of oxygen-saturated water of the same temperature. In aerated containers the oxygen content was 90–98%. If the aeration had been turned off, an oxygen

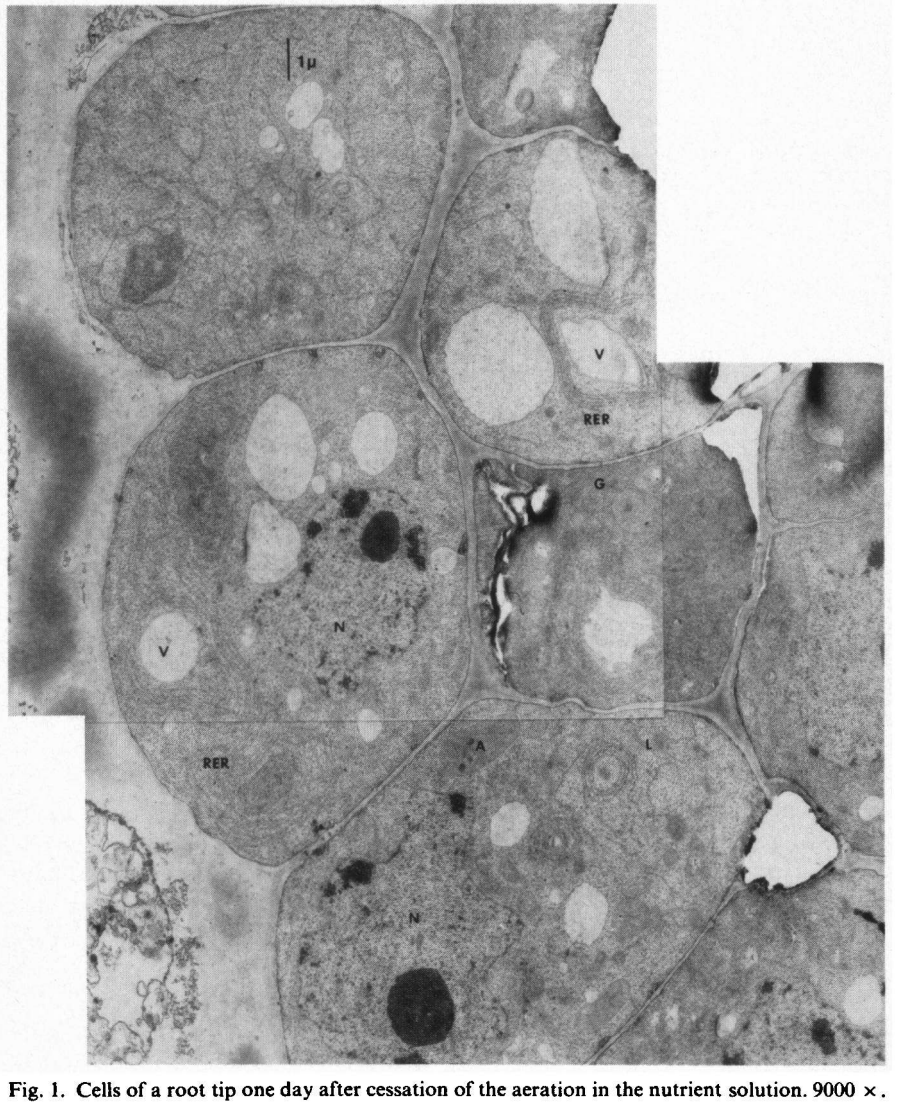

Fig. 1. Cells of a root tip one day after cessation of the aeration in the nutrient solution. 9000 ×.

content of about 40% was measured after one, two and three days. Root tips of aerated and non aerated plants were fixed immediately after the cessation of aeration and after one, two and three days.

A dialdehyde fixation was used (glutaraldehyde 1,25%, formaldehyde 1% in 0.1 M phophate buffer ph 7.4). Postfixation took place in 2% osmiumtetroxide in the same buffer. After dehydration the tips were embedded in Ladd plastic. Sections were stained with leadcitrate and uranylacetate. A Zeiss EM 9 electronmicroscope was used.

3. RESULTS

One day after cessation of the aeration of the nutrient solution cells of the extreme tip of the root axes had died. About 100 μm from the tip typical concentric and parallel arrangements of the rough endoplasmatic reticulum (RER) of cortical cells were observed (*fig. 1*). Sometimes the RER was arranged around an amyloplast. The amyloplasts of these young cells did not contain starch grains in contrast to those of the aerated control, where also such RER arrangements were absent (*fig. 2*).

Two and three days after the cessation of aeration these cells also had died. Older cells, at about 2–3 mm from the root tip, did not show any structural change of the RER, but their amyloplasts were free from starch.

The mitochondria did not show any obvious structural changes in the non aerated roots. The cells of the meristems contained electron dense bodies resembling lipid drops.

4. DISCUSSION

The disappearance of starch grains from the amyloplasts in the apical region, after the cessation of the aeration is an indication that sugar had been consumed while new synthesis of starch did not occur. This may be due to a reduced transport of assimilates from the shoot to the root, but also to an increased use of sugar for energy production, both of which may be caused by oxygen deficiency. As far as the lipid drops are concerned, several authors have reported their appearance as a response to anaerobic circumstances (PODBIELKOVSKA & BORYS 1975, COULOMB & COULOMB 1972, VARTAPETIAN et al. 1978).

Increase of RER is in itself not specific for anaerobiosis, but a general indication of stimulated metabolism. The concentric arrangements of RER, however, have often been attributed to anaerobic circumstances (FREY-WYSSLING & MÜHLETHALER, 1965, PERNER, 1966, PODBIELKOVSKA & BORYS, 1975). They have, however, also been connected with autophagy in cells (BAL & PAYNE, 1972). In our experiments, cells showing such arrangements were heavily stressed and (partly) in a premortal state, which means that these structures may indicate autophagy as well as anaerobic metabolism. In subsequent experiments an attempt is being made to determine more precisely the causal factor(s) of these changes in subcellular structure.

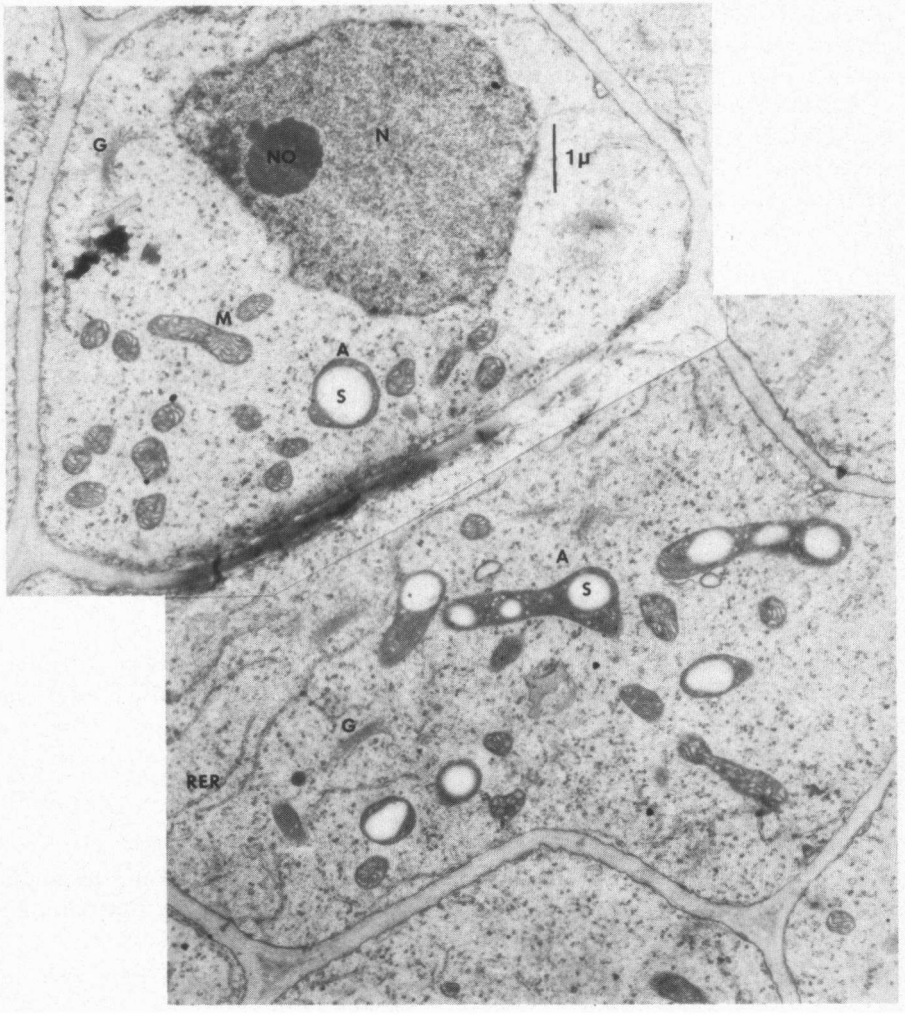

Fig. 2. Control. Cells of a root tip from an aerated nutrient solution. 11.400×.

Key:				
	A	amyloplast	No	nucleolus
	G	Golgi apparatus	RER	rough endoplasmatic reticulum
	L	lipid		
	M	mitochondrion	S	starch grain
	N	nucleus	V	vacuole

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