BIOLOGIA PLANTARUM 35 (4): 529-536, 1993

Gradient of electrical characteristics along roots of wheat seedlings under oxygen deprivation

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Abstract

The current-response method was used to characterize effect of oxygen deficiency on functional state of membranes along the roots of wheat seedlings. The results show apical parts of older roots being most affected by hypoxia, while the youngest roots behaved as effectively adapted.

Introduction

Responses of the roots of wheat seedlings to oxygen deficiency in the rhizosphere have already been described in anatomical and morphological terms (Barrett-Lenard *et al.* 1988, Thomson *et al.* 1990, Trought and Drew 1980a, c, Wiedenroth and Erdmann 1985, 1989) as well as in relation to gas exchange (Albrecht and Wiedenroth, unpubl., Erdmann *et al.* 1986, Thomson *et al.* 1989) and to the balance of adenylates and ion uptake (Buwalda *et al.* 1988, Novák *et al.* 1989, Trought and Drew 1980b). A suitable tool to analyze the overall state of root tissue is the method of current-response to an applied saw-tooth voltage to characterize the actual functional state of the membrane systems of a tissue (Dvořák *et al.* 1981). Previously we discussed the current-response (C-R) curves obtained from the metabolically most active medial part of the roots grown under different oxygen partial pressure or kept under strict anoxia up to 17 hours, respectively (Černohorská *et al.* 1989).

In the present paper, the partially distinct behaviour of roots of different order (Fitter 1991, Waisel and Eshel 1991, Wiedenroth and Erdmann 1985) as well as the 'ongitudinal gradient of each root (Lambers *et al.* 1991, Wiedenroth 1990) was nvestigated with the view on the adaptation capacity of the different roots of the root ystem.

eived 15 May 1992, *accepted* 18 November 1992.
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Material and methods

After swelling for 4 h, caryopses of *Triticum aestivum* L. cv. Hatri were germinated on moist filter paper in the dark at 25 °C. After 48 h, the seedlings were either planted in sand continuously flooded (variant F) or cultivated in full strength Knop's solution (iron added as Fe-EDTA). The root medium was aerated (control, variant C) or flushed with nitrogen continuously (variant N) so that the oxygen partial pressure in the solution was less then 1 kPa.

At the 12th day, plants were used for the experiments. Roots of different insertion level (number-code according to Wiedenroth and Erdmann 1985, Fig.1) were carefully selected and subjected to current-response measurement in the apical segment (A), in the medial (M) as well as in the proximal part (P), respectively.

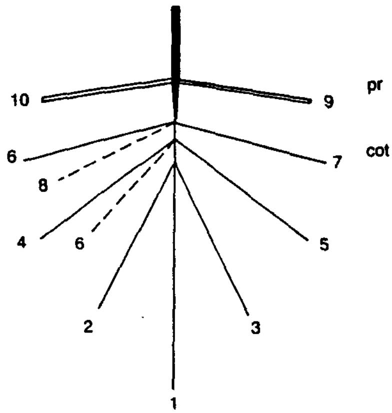

Fig.1. Scheme of root numbers of wheat seedlings according to Wiedenroth and Erdmann (1985). pr - node of the primary leaf, cot - node of the cotyledon.

To characterize the respective root segments under investigation we used the following code, *e.g.* C1M means "control plant - root no. 1 - medial segment", N9A means "plant cultivated on nitrogen-flushed solution - root no.9 - apical segment".

The values presented in this paper (Table 1, Figs. 2 to 4) are averages from 6 - 9 individual measurements.

The method of the current-response to the applied saw-tooth voltage was used for an analysis of physiological changes in wheat roots (Dvořák *et al.* 1981). Pulses 2 ms long and the input voltage, U_{max} = 10 V were used. The current-response is given by the relationship:

$$I = \frac{U_{max}}{T} \times \{C\,[(1 - e^{-a_1 t}) + (1 - e^{-a_2 t})] + R^{-1}\,t\}, \qquad I_{max} = I_{Rmax} + I_{Cmax}$$

The relative capacitance $C = I_{Cmax}/I_{max}$; a_1, a_2 = the velocity of capacitor charging (fast and slow, respectively) = $(R_i\,C)^{-1}$.

The radial direction of current passage was used for measurement of C-R curves, the diameter of area contact electrodes was 1 mm. Also longitudinal measurement of current passage was used for comparison, distance of area contact electrodes was 5 mm in that case. The parameters of C-R curves were calculated and grafically performed by means of computer programmes "CRREGRES" and "ZOBRAZ" (Dvořák *et al.* 1990).

Results and discussion

The calculated parameters of current-response curves measured in radial extension are summarized in Table 1. In the following text, the statements to the respective root segments in brackets refer to these figures.

Table 1. Characteristics parameters of current-response curves measured in radial extension of proximal (P), medial (M) and apical (A) segments of roots of different order. * indicates dead segments.

Cultivation variant	Root number	Segment	a_1	C_{rel}	I_{max} [μA]
Control	1	P	9.7	0.55	4.3
		M	9.5	0.58	6.7
		A	17.8	0.54	2.2
	2/3	P	9.6	0,52	4,3
		M	11.6	0.57	6.6
		A	25.6	0.48	2.0
	4/5	P	11.8	0.58	6.8
		M	11.1	0.57	5.7
		A	13.5	0.57	4.2
Flooding	1	P	18.0	0.50	2.7
		M	9.9	0.55	4.2
		A	*	*	*
	2/3	P	9.7	0.59	4.3
		M	9.7	0.55	5.4
		A	*	*	*
	9	P	12.0	0.51	5.0
		M	16.8	0.49	4.0
		A	14.1	0.51	2.7
N_2-flushing	1	P	15.5	0.43	2.3
		M	10.3	0.57	5.3
		A	6.8	0.59	5.9
	2/3	P	10.1	0.46	3.5
		M	10.3	0.48	4.5
		A	6.5	0.48	4.3

In apical segments of seminal roots of aerated plants the free space is not yet well developed and, therefore, conductivity (I_{max}) is low [C1A-2.2, C3A-2.0].

Under hypoxic conditions, the root tips are strongly damaged [F1A, F3A are dead] or et least ions are released into the free space affecting an increase in conductivity, [N1A - 5.9, N3A - 4.3] (Fig. 2, above).

The very young root no. 9 emerging under oxygen deficiency and anatomically well adapted to internal gas diffusion (Erdmann *et al.* 1986) was rather influenced by hypoxic cultivation and shows low conductivity [F9A - 2.7] as the seminal roots of control plants.

The high metabolic activity in the apical parts of seminal roots of aerated grown plants is reflected by very high a_1- values [C1A - 17.8, C3A - 25.6]. In contrast, the low values of seedlings cultivated on nitrogen-flushed solution [N1A -6.8, N3A - 6.5] indicate severe damage of the root tip even visible by vitrification of the tissue. Also with regard to this parameter, root no. 9 is little influenced by hypoxia [F9A - 14.1] and behaves similarly to roots of control plants (Fig. 2, below).

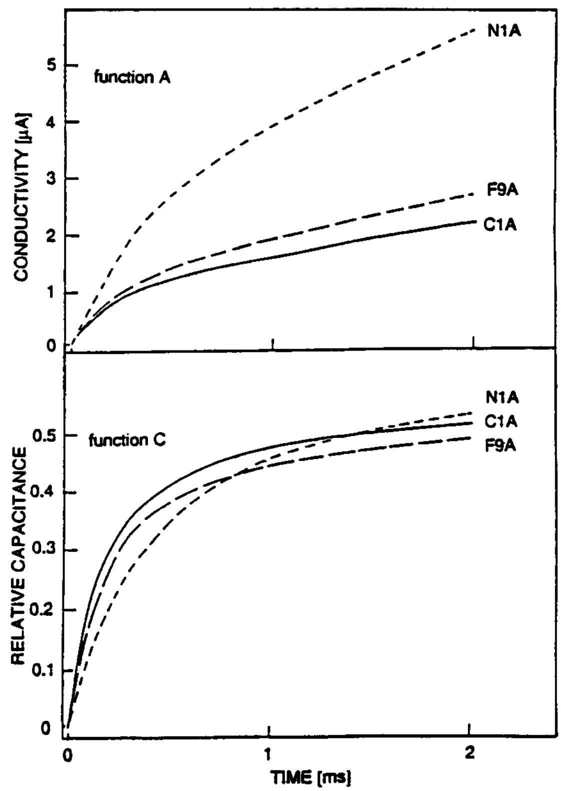

Fig.2. C-R curves of apical root segments following different oxygen regimes during growth.

Vacuoles are still small in cells of root tips of control plants and not yet able to accumulate greater amounts of ions. This is reflected by a more likely lower relative capacitance (C_{rel}) in combination with the very small conductivity [C1A - 0.54:2.2, C3A - 0.48:2.0] comparing with the corresponding values of the already aged or damaged apices of hypoxically grown plants [N1A - 0.59:5.9, N3A - 0.53:4.3] with an infinitive increase of capacitance level (N1A,Fig.3, below) meaning damage of membranes with unsufficiently working proton pumps. Once more, root no. 9 of flooded grown plants is more similar to the control [F9A - 0.15:2.7].

Independently from the oxygen regime during growth, the medial segments are the most effectively metabolizing parts of all roots. The free space is well developed causing highest conductivity (Fig. 3, above). Only in the younger roots no. 5 (in control plants) and no. 9 (in flooded grown plants) the conductivity is highest in the proximal parts corresponding physiologically to the medial segments of older roots (Table 1).

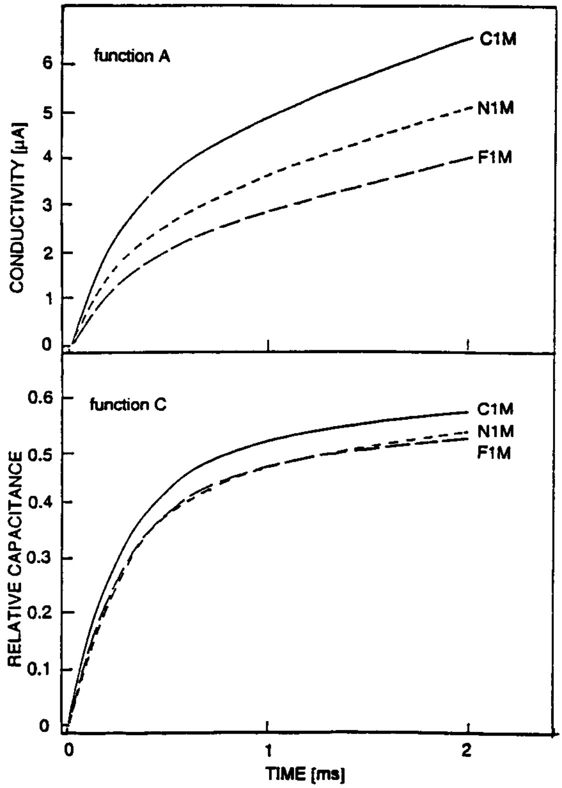

Fig.3. C-R curves of medial root segments following different oxygen regimes during growth.

Vacuoles and membrane systems are well developed in medial parts of seminal roots causing high values of relative capacitances in combination with the high conductivities mentioned above. The influence of oxygen availability remained comparatively low. In contrast, in apical segments the plateau level of capacitance is reached by hypoxically grown roots in the same time as by those of control plants (Fig. 3, below). Once more, only the younger roots no. 5 and 9 only show higher capacitance values in the proximal segments (Table 1).

The increase in the volume ratio of vacuole:cytoplasm does not influence the conductivity for it is compensated by the well developed free space, but causes an increase of the time to saturate the capacitors, particularly of a_1 comparing with the values of root tips [C1M - 9.5, C1A - 17.8; C3M - 11.6, C3A - 25.6]. The opposite is true following hypoxia [F1M - 10.3, F1A - 6.8; F3M - 10.3, F3A - 6.5] indicating that the capacitances are related mainly to cytoplasmatic colloids under these conditions.

In the proximal parts of seminal roots already a (small) decline in metabolic activities may occur. The further decrease of the volume of cytoplasm in favour of the vacuole as well as the partially lowered ion concentration diminish the conductivity comparing with medial segments. Following hypoxic cultivation sometimes extremely low I_{max} - values were observed [C1P - 4.3, F1P - 2.7, N1P - 2.3]. In younger roots no. 5 and 9, however, highest conductivities are found in proximal segments as mentioned above.

For membrane systems affected in proximal parts of seminal roots capacitances are lower then in medial segments independent from the oxygen regime during growth, but the time for saturating the capacitors is little influenced as the reduction of cytoplasm is at least partially compensated by the ion concentrations. The surprising high a_1-values of root no. 1 following hypoxia [F1P - 18.0, N1P - 15.5] may indicate a breakdown of the plasmalemma and, therefore, the tonoplast being the only membrane functioning as capacitor.

Comparing measurements of the current-response curve in longitudinal direction lead to the following conclusions. The conductivity is higher in axial extension because cells are stretched in this direction and not interrupted by intercellulars (Fig. 4, above). Capacitors are faster saturated in axial measurement because of the smaller amount of membranes compared with radial extension (Fig. 4, below).

The increase of relative capacitances and conductivity from apical (membranes not yet fully developed) over proximal (already aged) to medial segments (highest effectivity) was found by radial measurement only. Using axial measurements, these correlations are blurred for the distance of 4 mm between the electrodes used in these experiments does not allow the exact limitation of the very young part of the root. Therefore, the so-called "A-segment" is really the youngest part of the medial segment and shows, of course, the highest capacitance and a conductivity only little lower than the medial part *sensu stricto*.

To characterize the total root system as an entity the current response may be determined in axial as well as in radial direction. In this case, the axial measurement yielded higher conductivity and shorter time of capacitor charging. But, to distinguish the functional state of membrane systems *along* the root only radial measurements are usable.

The older roots no. 1 - 3 already existing at the onset of hypoxia are most affected particularly in their apical parts; sometimes root tips are killed or strongly damaged.

The youngest root no. 9 emerging up to day 12 under oxygen deprivation only is effectively adapted behaving, therefore, similar to seminal roots of not stressed control plants. This coincides with well developed aerenchyma, the location near the better oxygen provided surface of the medium, and the easier passable gas diffusion path from the cavity between coleoptile and primary leaf (Erdmann *et al.* 1988).

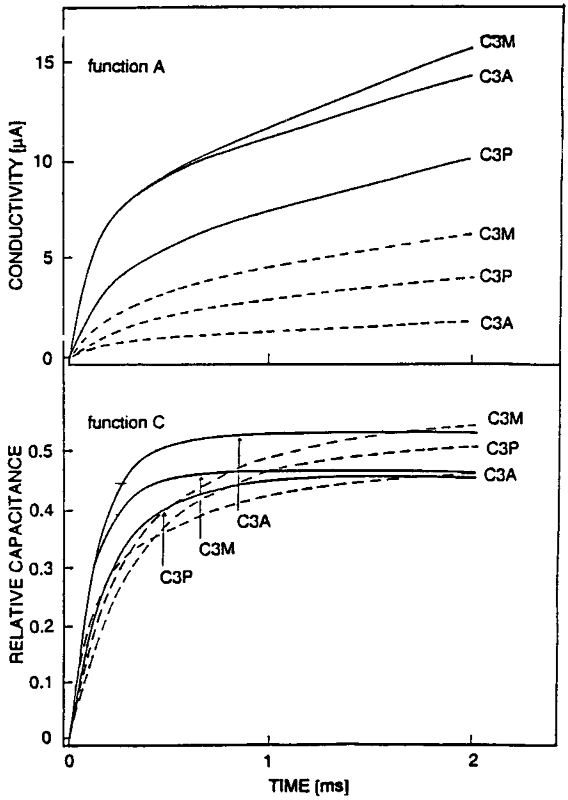

Fig.4. C-R curves recorded in radial (full line) and axial (dashed line) extension at root no.3 of aerated grown seedlings.

Since changes caused by hypoxia and by differentiation along the root as well as by different order of the roots interfere, only corresponding segments of roots of the same insertion level may be compared to characterize the effect of oxygen deficiency on functional state of membranes. For this purpose, the used method of current-response to an applied saw-tooth voltage is a very suitable and fast running one. The results, together with anatomical and physiological parameters, can help to characterize the adaptation capacity of the whole plant to stress.

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