Distribution of stomata on the second leaf of *Zea mays* following root hypoxia

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Abstract

The changes in leaf dimensions, transverse and longitudinal gradients in stomatal density and the total number of stomata under the influence of root hypoxia were followed. In spite of considerably reduced leaf area following hypoxia the total number of stomata per leaf was not changed significantly. The resulting increase in stomatal density was not uniform being the most prominent in the basal part of the leaf where the distances between stomata and between rows of stomata became shorter.

Introduction

Oxygen deficiency in the root environment is a widely observed phenomenon in our soils due to the use of heavy machinery in agriculture. The hypoxia can be deteriorated by flooding caused for example by long lasting rains coming often during spring months when plants pass their early ontogenetic phases.

The root hypoxia evokes various biochemical as well as morphological changes which are detectable in roots first of all (for review see Hook and Crawford 1978). As root and shoot are mutually dependent the changes in the root system are followed by changes in shoot very quickly. Under hypoxia leaves of maize plants show reduced leaf area, fresh and dry mass and water content. They are yellowish instead of green and they senesce more quickly (Sudakov and Petrushenko 1982, Votrubová *et al.* 1990).

The aim of the present paper is to analyse the relation between the anatomical features of leaf epidermis and growth related to hypoxia.

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Material and Methods

Caryopses of *Zea mays* CE 240 were surface sterilized with hot water (80 °C) for one minute. Then they were sown into plastic vessels (diameter 0.24 m, height 0.25 m) containing soil (leached chernozem), into the depth of 40 mm. Each vessel contained 10 plants. Plants were cultivated in two variants - control (soil which was freely showered) and hypoxical (the same soil mechanically hardened and flooded after the coleoptiles had emerged under the soil surface). Mechanical hardening enhanced bulk density of the soil from 1.11 g cm^{-3} to 1.40 g cm^{-3} and reduced minimum air capacity from 37.62 vol. % to 10.65 vol. %. Vessels with plants were placed in a greenhouse at nature irradiance. The experiments were carried out during May and June. Temperature fluctuated between 20 and 26 °C.

The length of shoots of forty plants in each variant was measured every second or third day. On the 21^{st} day of cultivation the second leaves were harvested and their dimensions and leaf area were measured. Five leaves from each variant were used for making replicas. Replicas were made from three parts of the leaf (base, middle and apex) and both leaf sides (adaxial and abaxial). The following parameters were measured: stomatal density across the whole width of leaf, the distance between rows of stomata and between stomata in rows and the number of stomatal rows. From these measurements the total numbers of stomata per leaf were calculated. For statistical evaluation *t*-test and polydimensional statistical analysis were used.

Results and Discussion

Hypoxia in the rhizosphere influenced the growth of shoots severely (Fig. 1).

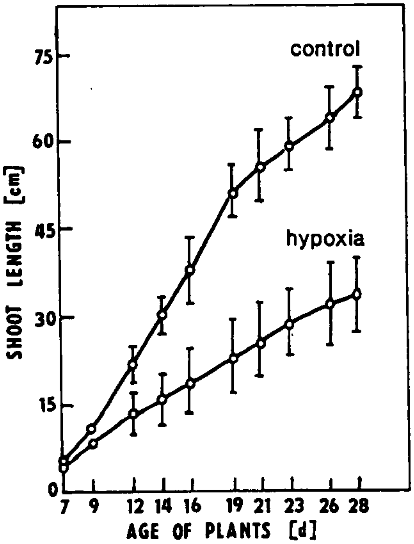

Fig. 1. The growth of shoots under the influence of root hypoxia. The differences between control and hypoxical variant were significant at $P = 0.01$ since the twelfth day, $n = 40$.

The reduced height of shoots was combined with reduced leaf area and length (Table 1). Stomatal density in the apical region was not influenced by root hypoxia, neither on the adaxial side nor on the abaxial one (Fig. 2). Stomatal density in the middle and above all in the basal part was much higher in hypoxically grown plants (Fig. 2). The increase in stomatal density in the basal region was highly significant and changed the course of the longitudinal gradient on both leaf sides (Fig. 3).

Table 1. Leaf area and size of the second maize leaf.

Variant	Leaf area [mm^2]	Leaf length [mm]	Leaf width [mm]	
			base	middle
control	2190	189.0	9.0	12.8
hypoxical	1508	130.5	9.0	13.2

Whereas the leaf area was significantly diminished owing to hypoxia (Table 1), the total numbers of stomata per leaf were not changed significantly: 221 118 + 19 123 stomata in control plants, 198 935 + 16 994 in hypoxical plants.

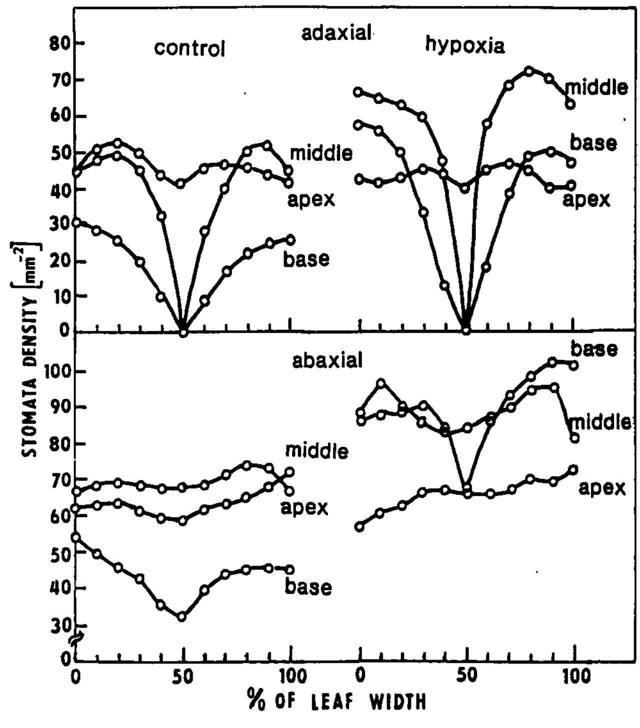

Fig. 2. Transverse gradients in stomatal density on adaxial side and on abaxial side. The leaf width taken as 100 %, $n = 5$.

The changes in stomatal density could be caused either by changes in the distance between rows of stomata or in the distance between stomata in rows. According to our results (Table 2) we can presume that both parameters were responsible for the changes in stomatal density.

The results showing changes in stomatal distribution were in accordance with the character of grass leaf growth and could be explained by growth changes caused by hypoxia. The growth of grass leaf ceased very early during the ontogeny of the leaf in its apical part. The second leaf which was studied originates still in the embryo.

Table 2. Distances between the stomata and stomata rows as affected by hypoxia

Leaf side	Leaf part	Distance between stomata [μm]		Distance between rows [μm]		Number of rows	
		control	hypoxical	control	hypoxical	control	hypoxical
adaxial	apex	130	140	169	175	-	-
	middle	140	123*	173	146	72	88
	base	194	123*	289	214*	35	46
abaxial	apex	123	132	114	118	-	-
	middle	108	114	125	104	-	127
	base	120	102	146	103*	61	90*

*significant against control at P=0.05

As at the beginning of flooding the coleoptiles were about 70 mm high, this leaf part was supposed to cease growing before the onset of hypoxia.

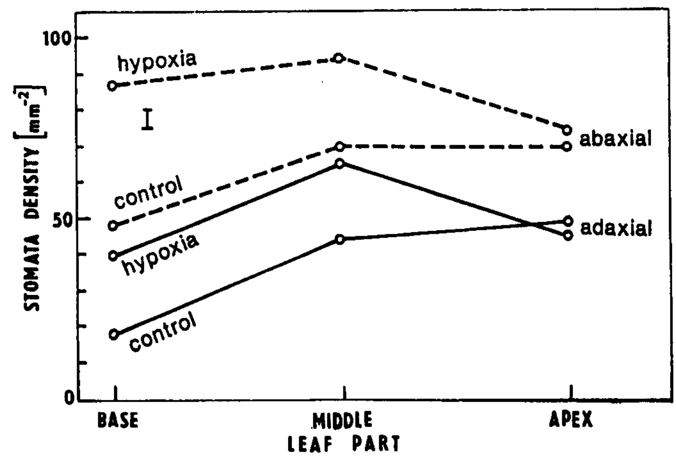

Fig. 3. Longitudinal gradients in stomatal density. The differences between control and hypoxical variant were significant at P = 0.05 the base on both leaf sides and at the middle on the abaxial side, n = 5.

That is why the character of stomatal arrangement in this area was not changed. On the contrary, the basal part continued growing for a long time and therefore the growth and its character was influenced by root hypoxia to a considerable degree. In accordance to this, the stomatal arrangement was altered. First of all, the distances between stomata in rows were smaller. Also the fact that distances between rows were shorter and their number across the leaf higher at the leaf base of hypoxically grown plants could be explained by retardation of growth in this area. The number of rows was not constant along the whole leaf. It was the lowest at the base and towards the middle their number increased. Between rows still existing new rows were inserted in some distance from the base. Owing to retarded elongation growth, this distance was much shorter in hypoxically grown plants and the number of rows in the basal part was larger.

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