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Communication

Growth Responses of *Plantago major* L. ssp. *pleiosperma* (Pilger) to Changes in Mineral Supply[1]

EVIDENCE FOR REGULATION BY CYTOKININS

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ABSTRACT

Plants of an inbred line of *Plantago major* ssp. *pleiosperma* were subjected to an alteration in mineral supply. Observed responses of growth rate and shoot to root ratio are thought to be induced by changes in endogenous cytokinin concentration and not by mineral concentration in plant tissue.

What is the earliest response of a plant to an altered mineral supply? Changes in kinetic parameters of uptake systems and enzymes, in mineral contents of plant tissue, in growth rate and in shoot to root ratio are mentioned. Clarkson (2, 3) concluded that modulation of the kinetics is an early response to nutrient stress and that it occurs before changes in growth rate can be measured. The kinetic adjustments are needed in order to maintain the internal ion concentrations in cytoplasm and in vacuoles, as close as possible, at preset values. Growth reduction would be initiated in case the internal concentrations decreased below the preset values (4). The present experiments indicate that such a concept of set point regulation does not apply for *Plantago*.

In order to discriminate between early events in the responses of plants to changes in mineral nutrition, genotypes of *Plantago major* were studied (7). For the present experiments, a genotype with fast growth responses to a reduction in mineral nutrition was selected. Plants of this genotype showed growth reduction within 4 d after transfer from a concentrated nutrient solution to a 50-times diluted one. Increased growth rates were observed within 10 d in plants transferred the other way around. Evidence for involvement of cytokinins in growth regulation of plants of this genotype was presented earlier: addition of BA could retard growth reduction when mineral nutrition was drastically lowered (8). This report deals with growth responses of individual plants measured in a nondestructive way. At the same time the contents of essential ions in shoot and roots were measured in order to establish whether the response in growth or in ions presented the earliest event. In addition, the effect of BA on both growth parameters was measured.

MATERIALS AND METHODS

Growth Conditions. A single inbred line of *Plantago major* was used in the present experiments: Z_2, belonging to subspecies *pleiosperma* (Pilger). Growth conditions were as described in a previous paper (8).

Growth. Plants were randomly chosen from each treatment. Plants were grown on a high nutrient solution (100%) or a diluted nutrient solution (2%). In addition, plants were transferred from one nutrient condition to the other, with 10^{-8} M m^{-3} BA in the nutrient solution (100→2%; 100→2% + BA; 2→100%; 2→100% + BA). The volume of the root systems and the total weight of the plants were measured 3 times a week. With the aid of previously determined linear correlations between root fresh weight and root volume, we were able to follow the shoot and root growth of individual plants (8). From these data, the shoot to root ratios and the RGR[3] values were calculated. All data were submitted to an analysis of variance for indices of significance.

Chemical Analysis. Nitrate was determined with a modification of the phenoldisulfonic acid method (6). Phosphate was determined colorimetrically (11) and potassium, magnesium, and calcium were determined by atomic absorption spectrophotometry.

RESULTS

The 100 → 2% Plants. Within 2 d after transfer from a concentrated to a diluted nutrient solution, plants had a significantly ($P \leq 0.05$) lower shoot RGR (Table I), a significantly lower shoot to root ratio (Fig. 1), and a significantly higher root RGR value (Table II). At the same time, no differences in the contents of five ions were observed between shoot and root tissue of 100% and 100 to 2% plants (Table III). Shoot RGR (Table I), root RGR (Table II), and shoot to root ratio (Fig. 1) were kept at the 100% level in the case where 10^{-8} M m^{-3} BA was added to the diluted nutrient solution.

The 2 → 100% Plants. Plants that were transferred from a diluted to a high nutrient solution tended to increase shoot growth (Table I), root growth (Table II), and shoot to root ratio (Fig. 1), but even after 7 d these increments were not significant ($P \leq 0.05$). The internal concentrations of $H_2PO_4^-$, NO_3^-, Mg^{2+}, Ca^{2+}, and K^+ of shoot and root tissue were raised to the level observed in 100% plants within 2 d (Table III). BA-treated 2→100% plants significantly increased ($P \leq 0.05$) their shoot growth (Table I) and shoot to root ratios (Fig. 1) within 2 d. Root RGR was hampered for 2 d, but it quickly increased during the following 4 d.

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[3] Abbreviation: RGR, relative growth rate.

Table I. *RGR Values (mg g^{-1} d^{-1}) of the Shoots as Affected by Changes in Mineral Nutrition*

Treatment	BA Addition	Days after Transfer 0	1	2	4	6
100%[a]	−	220 ± 14[b]	212 ± 17	208 ± 14	215 ± 18	213 ± 19
2%	−	50 ± 2	46 ± 5	49 ± 3	54 ± 3	51 ± 4
100 → 2%	−		120 ± 11	73 ± 4	48 ± 5	54 ± 5
100 → 2%	+		215 ± 16	220 ± 18	214 ± 14	187 ± 16
2 → 100%	−		51 ± 6	47 ± 3	53 ± 5	71 ± 8
2 → 100%	+		165 ± 12	210 ± 18	225 ± 18	210 ± 16

[a] 100% full nutrient strength; 2% 50 times diluted nutrient strength; 100 → 2% and 2 → 100% changes to low and high nutrient strength, respectively. [b] Mean of 10 determinations, ± SD.

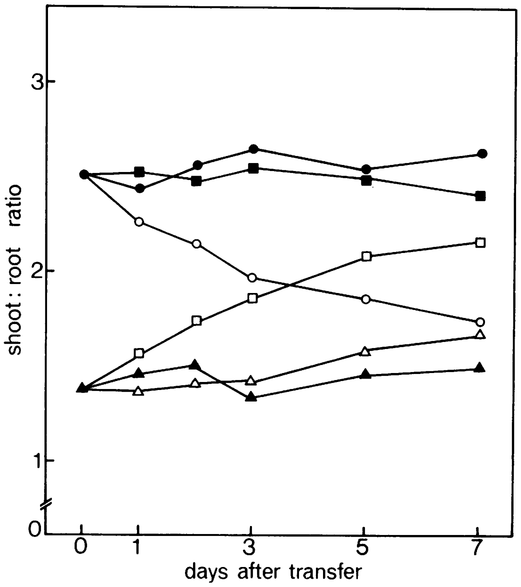

FIG. 1. Effects of changes in nutrient solution and BA addition on shoot to root ratios, measured during 7 d. (●), 100%; (▲), 2%; (○), 100 → 2%; (△), 2 → 100%; (■), 100 → 2% + 10^{-8} M m^{-3} BA; (□), 2 → 100% + 10^{-8} M m^{-3} BA, *n* = 10. SD ≤ 10%.

DISCUSSION

In agreement with previous reports (7, 8), a quick growth response was observed in plants of the genotype of *Plantago major* L. ssp. *pleiosperma* (Pilger). Upon a transfer from a concentrated to a diluted nutrient solution, shoot growth of 100→2% plants was almost immediately reduced and root growth was temporarily stimulated. This resulted in a rapidly decreasing shoot to root ratio. The fact that the contents of the studied cations, as well as NO_3^- and $H_2PO_4^-$, did not change the first days after transfer leads to the conclusion that ions were not primarily responsible for the quick growth response. The hypothesis that growth reduction would follow a decrease in mineral content did not apply to *Plantago*.

Plants transferred from a low to a high nutrient solution showed a very slow stimulation of growth, while the ion contents were elevated to the 100% level within 2 d after the transfer. Again, there was no direct relation between growth rate and ion contents of shoot and roots.

Ion contents may change locally in plant tissue and may induce some growth reactions in 100→2% plants. However, the use of juvenile plants and the observed root growth responses make this assumption doubtful.

The experiments with BA, added at the same time as the nutrient conditions were changed, indicated the importance of endogenous cytokinins in regulation of growth in *Plantago*. BA addition counteracted the transfer effects, delaying growth reduction in 100→2% plants and accelerating growth stimulation in 2→100% plants. BA addition showed that in 100→2% plants sufficient ions were available and utilized for growth. Logically, BA-induced growth (at low mineral nutrition) was of a temporary nature because of mineral limitation (8).

BA is readily taken up by roots (17) and metabolized into a large amount of cytokinin derivates (10). BA is known as a growth-promoting substance by overcoming growth limiting effects (1, 8). BA increases protein content (14) by stimulating protein synthesis and inhibiting protein degradation (9). From preliminary experiments, it was shown that in 100→2% plants BA was acting by causing enlarged endogenous cytokinin contents. BA addition to 2→100% plants accelerated growth without an increment in endogenous cytokinin levels (D Kuiper, unpublished data). Low salt plants have a limited number of active root tips, sites of cytokinin production. Thus, the BA addition formed a supplement to the natural cytokinins in 2→100% plants.

We suggest that cytokinin production in the root tips is sen-

Table II. *RGR Values (mg g^{-1} d^{-1}) of the Roots as Affected by Changes in Mineral Nutrition*

Treatment	BA Addition	Days after Transfer 0	1	2	4	6
100%[a]	−	160 ± 12	155 ± 16[b]	159 ± 13	165 ± 15	161 ± 12
2%	−	71 ± 5	74 ± 8	76 ± 9	69 ± 7	69 ± 6
100 → 2%	−		165 ± 12	183 ± 13	192 ± 12	180 ± 11
100 → 2%	+		159 ± 16	163 ± 14	165 ± 12	155 ± 13
2 → 100%	−		75 ± 6	83 ± 8	72 ± 7	91 ± 7
2 → 100%	+		60 ± 5	62 ± 8	83 ± 9	120 ± 13

[a] 100% full nutrient strength; 2% 50 times diluted nutrient strength; 100 → 2% and 2 → 100% changes to low and high nutrient strength, respectively. [b] Mean of 10 determinations, ± SD.

Table III. *Contents of Ions in Shoot and Root Tissue of P. major ssp. pleiosperma as Affected by Mineral Nutrition*

Treatment	t	Control[a] 100%	Transfer		Control 2%
			100 → 2%	2 → 100%	
		S/R	S/R	S/R	S/R
meq · (kg dry matter)$^{-1}$					
K^+	0	1230/1650			720/1130
	1	1350/1590	1280/1520	920/1390	780/1060
	2	1290/1510	1190/1530	1210/1450	740/1040
	4	1190/1500	1110/1510	1200/1560	640/920
	6	1210/1500	930/470	1300/1510	720/930
$H_2PO_4^-$	0	180/270			120/150
	1	190/290	190/290	160/230	110/140
	2	190/280	190/270	190/280	110/120
	4	200/270	180/270	190/270	100/90
	6	170/250	150/220	180/250	80/100
NO_3^+	0	1670/1180			1000/330
	1	1690/1025	1960/970	1500/830	710/430
	2	2020/1030	1840/1210	1900/1130	690/430
	4	1970/1230	1690/1230	2160/1400	600/370
	6	2190/1360	1270/740	1910/1160	660/490
Ca^{2+}	0	1100/210			810/200
	1	1140/190	1150/180	1000/200	830/200
	2	1190/190	1170/180	1130/190	830/210
	4	1210/170	1170/150	1190/190	820/180
	6	1190/190	980/180	1170/180	800/160
Mg^{2+}	0	280/870			270/870
	1	280/900	280/910	280/910	250/860
	2	285/890	270/905	290/915	265/850
	4	270/860	230/870	280/890	240/820
	6	280/820	240/850	260/840	250/830

[a] Control plants were for 14 d on the nutrient solution at $t = 0$. S, shoot; R, root; t, days after transfer. $n = 10$; SD $\leq 10\%$.

sitive to mineral strength and thus affects growth of shoot and roots and shoot to root ratio. Nitrogen may be the effector of the cytokinin production (5, 12, 13, 15) and thus regulate metabolic activity and RNA (16) and protein content of the plants (14), leading to adaptive responses of growth and shoot to root ratios to changes in the mineral nutrition of plants.

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