EFFECT OF NITRATE ON NODULATION IN A NITRATE REDUCTASE DEFICIENT PEA MUTANT

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Nitrate reductase (NaR) deficient mutants have recently been isolated in Pisum sativum (1,3). In NaR deficient mutant E_1 (1) nitrate reduction is impaired but uptake is intact. In wildtype peas nodule formation (5), nodule growth (5), and acetylene reduction (4) normally are inhibited after supplying the plant with nitrate. Therefore, NaR deficient mutants can be used to investigate whether the inhibition is caused by the presence of nitrate itself in the plant or is brought about by the reduction of this compound. In recent work (2) the effect of nitrate on acetylene reduction in the mutant was investigated. The results indicated that nitrate itself was not the inhibiting factor but that reduction is involved.

In the present study the effect of nitrate on nodulation and total nodule growth was examined. Preliminary results are shown in Tables 1 and 2. A concentration of 15 mM nitrate was chosen (5). Under these conditions, the old leaves in some mutant plants became necrotic before nodulation characteristics were examined as a result of nitrate accumulation. In the control, nitrogen-free mineral solution (SMS,1)+ 15mM KC1 was used. Nodulation of E_1 was 2 days later and nodule weight per plant was higher in cv. 'Rondo' (cv. Rondo 0.34g; E. 0.28g). In ether studies, with lower KC1 concentration in the control, nodulation claracteristics in cv. 'Rondo' and did not differ, indicating that t.e difference in nodulation with 15 mM presumably was due to the high concentration used.

Table 1. Nodulation of cv. 'Rondo' and mutant E. after supply of 15 mM KC1, 7.5 mM NH_4NO_3 or 15 mM KNO_3.

Line	Number and total weight of nodules (g) on main root					
	SMS+KCl		SMS+NH_4NO_3		SMS+KNO_3	
Rondo	34.4	(0.193)	11.7	(0.024)	11.1	(0.015)
	100	100	34	12	32	8 %
E_1	29.1	(0.119)	5.4	(0.003)	7.1	(0.004)
	100	100	19	3	24	3 %

Total 20 plants per class in two experiments.

In the studies on nodule formation, seeds and seedlings were treated with SMS+15 mM KC1, SMS+7-5 mM NH_4NO_3, or SMS+15 mM KNO_3 from the onset of the experiment and were analyzed^ 30 days after germination. Treatment with NH_4NO3 as well as with KNO_3 affected both the number of root nodules and the nodule weight (Table 1). Nodulation on lateral roots was almost completely absent (data not shown). In E_1 the inhibition of nodulation on the main root was more severe. It is known that nitrate is accumulated in E_1 (1). Therefore these results indicate that

for nodule formation, nitrate itself probably is the inhibiting factor and under such circumstances nodule development is affected too.

Nodulation starts on the main root. Therefore, probably the best impression about the effect of nitrate on nodule growth alone can be obtained by studying weight increase of young nodules on the main root. Application of nitrate to plants with young nodules simultaneously affects other factors, especially on the lateral roots. The effect on nodule growth has been investigated by treating nodulated plants 20 days after germination with 15 mM KNO_3. Table 2 shows that 8 days after treatment with nitrate nodule growth was impaired. There are indications that in E_1 growth inhibition was less in nodules on the main root.

Table 2. Nodule weight per plant 8 days after treatment of nodulated plants with 15 mM KCl or 15 mM KNO_3.

	Nodule weight (g)			
Line	On main root	On lateral root	Total	
Rondo	0.252	0.100	0.352	KCl
	0.109	0.048	0.157	KNO_3
	43	48	45	% of control
E_1	0.146	0.136	0.282	KCl
	0.105	0.054	0.159	KNO_3
	72	40	56	% of control

Total 20 plants per class in 2 experiments.

Further studies on nodule growth have been started in which the onset of nodulation has been synchronized by growing all plants on SMS without addition of KC1 until the time of nitrate application. We tried to avoid the development of necrotic symptoms completely by using lower nitrate concentrations.

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