FURTHER CHARACTERIZATION OF MUTANT nod-3, HIGHLY NODULATING IN THE PRESENCE OF NITRATE

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In pea, nodulation is strongly inhibited when plants are grown and inoculated on a nitrogen-containing medium (1). A mutant (nod-3) was found which is highly nodulating both on nitrate-free and on nitrate-containing media, whereas acetylene reduction is much higher than that of parent variety 'Rondo' (2).

The effect of ammonium and nitrate on nodulation and on taproot length of both genotypes is shown in Table 1. In these experiments, nodulation of cv Rondo on nitrogen-free medium was relatively high and the negative effect of nitrate on nodule number and nodule mass was stronger than that of ammonium. In mutant nod-3, nodulation expressed in nodule number and nodule mass is also inhibited by nitrate and ammonium, but to a much lower extent. In contrast with Rondo, nodulation of nod-3 seems to be affected more by ammonium than by nitrate. It is clear that mutant nod-3 is highly nodulating on nitrogen-free medium and in the presence of both nitrate and ammonium.

The taproot length of plants of Rondo is essentially the same in all experiments (Table 1). Mutant nod-3 had a much shorter taproot compared with Rondo, and showed differences in length when grown on ammonium (43.3) than on KC1 or KNO_3 (29.8 cm and 32.2 cm respectively). Root branching of mutant nod-3 and Rondo was compared after growth of seedlings without *Rhizobium* on nitrate-free and on nitrate containing medium. The number of secondary lateral roots appeared to be much higher in mutant nod-3 (data not shown). All of these observations could be an indication that the phytohormone balance in the two genotypes is different.

Table 1. Nodulation of cv Rondo and mutant nod-3 inoculated with R_. *leguminosarum* strain PF„ and cultured on standard mineral solution (SMS) plus 15 mM KC1, 15 mM KNO_3 or 7.5 mM $(NH_4)_2S0_4$-(n=5).

	Total nodule		Taproot	
Genotype	Number	Mass(g)	length	Medium
nod-3	>400	.743 ± .137	29.8 ± 5.0	15 mM KC1
Rondo	>140	.341 ± .035	50.4 ± 6.2	15 mM KC1
nod-3	>300	.564 ± .137	32.2 ± 2.4	15 mM KNO_3
Rondo	17	.021 ± .012	51.4 ± 7.3	15 mM KNO_3
nod-3	>250	.391 ± .048	43.3 ± 1.3	7.5 mM $(NH_4)_2SO_4$
Rondo	44	.068 ± .028	49.0 ± 3.8	7.5 mM $(NH_4)_2SO_4$

Mutant nod-3 was originally selected on nitrate containing medium ai i er inoculation with R. leguminosarum strain PF2 (2). An important question is whether the mutant properties of nod-3 are restricted to strain PF2. Therefore, the mutant and Rondo were inoculated with strain PRE (W. Europe), TOM (Turkey) and P8 (kindly supplied by Dr. Lie, Wageningen, the Netherlands). Rondo was nodulated by strain P8, but the nodules were ineffective (3). Table 2 shows that Rondo was nodulated by all strains used and that nodulation was always strongly affected by nitrate. Mutant nod-3 was highly nodulating with all strains on nitrogen-free medium, whereas the effect of nitrate on nodulation was comparable with the relatively weak effect found for strain PF2. Acetylene reduction was measured for Rondo and mutant nod-3 after nodulation with strain P8. In both genotypes, the nodules appeared to be ineffective, indicating that strain P8 is acting in the same way in both genotypes.

Table 2. Nodulation of cv Rondo and mutant nod-3 cultured on nitrogen-free and on 15 mM KNO_3 containing standard mineral solution (SMS), inoculated with different strains of Rhizobium.

Genotype	Total nodule number		Total nodule mass(g)		Bacterial strain
	KCl	KNO_3	KCl	KNO_3	
nod-3	>300	188	.546	.304	PF_2
Rondo	75	11	.231	.025	PF_2
nod-3	>300	>300	.750	.513	PRE
Rondo	98.7	28	.199	.029	PRE
nod-3	95.3	137.7	.342	.274	TOM
Rondo	51	8	.269	.010	TOM
nod-3	>400	93.8	.580	.200	P8
Rondo	122.7	3.8	.140	.004	P8

The observations (1) that mutant nod-3 is highly nodulating in the presence of both nitrate and ammonium, (2) that the mutant characters of nod-3 are strain independent, and (3) that the taproot length of nod-3 is reduced, are of importance when considering the use of nod-3 in agriculture and breeding.

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:. Jacobsen, E. and H. Nijdaai. 1983. PNL 15:31-32.
3. Lie, T. A., D. Hille, R. Lambers, and A. Houwers. 1975. In: Nutman, P. S. (ed.). Symbiotic Nitrogen Fixation in Plants. JBP 7, Cambridge University Press, London, p. 319-333.