A MUTANT SHOWING EFFICIENT NODULATION IN THE PRESENCE OF NITRATE

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In legumes, symbiotic N_2-fixation is an important source of nitrogen for the plant. However, nitrate, administered at the moment of sowing, inhibits nodulation (8), and when added after nodulation inhibits N -fixation (5). Using a nitrate reductase (NaR) mutant which also is nitrate accumulating, we ascertained that nitrate itself, when present in the plant, inhibits nodule initiation (6), and that the decrease in N -fixation is brought about by the reduction of nitrate (3). In the literature ineffective nodulation (7), resistance to nodulation (7), and high nodulation (4) are described as genetically determined variation in nodulation behavior of pea but, to our knowledge, no data are available regarding variation in the reaction to nitrate. We therefore searched for EMS-induced mutants that showed nodulation in the presence of nitrate. M2, seedlings were screened for nodulation on aerated liquid standard mineral solution (SMS, 2) supplemented with 15 mM KNO_3 and Rhizobium leguminosarum strain PF . Under these conditions nodulation of cv 'Rondo' is strongly inhibited. The seeds were from the same M2families as used for the selection of an NaR-deficient mutant (2). Among 222 M2families, one distinct nodulating mutant was found.

In the M3 M4 and M5progenies of that mutant only mutant phenotypes appeared; after crossing with cv Rondo as male parent, nodulation of the F1progeny on SMS + 15 mM $KNO_3$was inhibited as in the wild type; and in the F2the mutant appeared to be monogenic and recessive. According to the Rules of Genetic Symbols (1) its designation will be nod-3.

Preliminary nodulation data of nod-3 is given in Table 1. Nodulation of nod-3 on nitrate-containing medium is striking and even better than nodulation of cv Rondo on SMS. Also, on SMS nodulation of nod-3 is much better than that of cv Rondo. In nod-3 the appearance of nodulation is accelerated, number of nodules is much higher, and total nodule weight and acetylene reduction per plant (Table 2) are increased, whereas acetylene reduction of nodules per g fresh weight is lower.

Further investigations on nod-3 will include continued genetic analysis, its behavior with other bacterial strains, the morphological and/or physiological basis for its aberrant nodulation, and the effect of nitrate on the level of acetylene reduction. The double mutant with E1[NaR-deficient (2)] will be constructed, in order to study the effect of increased nitrate levels in the plant. Mutant nod-3 is interesting for studying the effect of efficient nodulation on yield, as Gelin and Blixt (1) did with their high nodulating genotypes, and additionally for studying the effect on yield of nitrate fertilization which does not inhibit nodulation in this mutant.

Table 1. Nodulation of cv Rondo and mutant nod-3 after culturing on medium containing strain PF_2 bacteria with or without 15 mM KNO_3.

	Number of nodules	Total nodule fresh weight (mg)	Culture conditions
cv Rondo	59.3	125	Germination in water;
nod-3	>300	589	Culture on SMS*
cv Rondo	16.6	26	Germination in water;
nod-3	>250	682	Culture on SMS + 15 mM KNO_3

*SMS = standard mineral solution (1).

Table 2. Acetylene reduction in cv Rondo and mutant nod-3, nodulated on medium without nitrate.

	Acetylene reduction	
	Per plant	Per g fresh weight
cv Rondo	2.0*	16.1*
nod-3	6.8	11.6

*u moles of C_2H_4 produced per hr.

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Nil

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