

## A CHLORATE RESISTANT NITRATE REDUCTASE-DEFICIENT PEA MUTANT

Feenstra, W. J. and E. Jacobsen

University of Groningen, Haren, The Netherlands

Kleinhoffs et al. (3) isolated nitrate reductase (NaR)-deficient mutants of Pisum by screening M2 families for a lower in vivo NaR-activity. A less laborious way for the isolation of NaR-deficient mutants is selection for chlorate resistance. This method has been applied successfully to bacteria (2) and to the higher plants Arabidopsis thaliana (5) and Nicotiana tabacum (4). We have carried out this method on Pisum and now want to report the isolation of a NaR-deficient mutant.

Seeds of cv 'Rondo' were treated with EMS. Among 7630 seedlings belonging to 1090 M2 families one chlorate resistant mutant was found. Experimental details will be published elsewhere. The mutant proved to be monogenic and recessive. Biochemical characterization of leaf material from 16-day-old plants yielded the following data (the comparable data of the parent variety, Rondo, are given between brackets): in vitro NaR-activity 0.08 (2.2) mkmol  $\text{NO}_2^-/\text{g}$  fresh weight/h; in vivo NaR-activity 0.19 (0.86) mkmol  $\text{NO}_2^-/\text{g}$  fresh weight/h; protein content 2.9 (3.6) mkg/g fresh weight; nitrate content 75.2 (7.2) m aeq/g fresh weight. The original mutant plant and its selfed progeny showed poor growth in soil and necrosis of leaflets. The necrosis started with the oldest leaves when the plants were still very young. Consequently the plants flowered poorly and yielded only a few seeds.

Mutant plants cultured on a liquid nutrient medium with  $\text{NH}_4\text{NO}_3$  as a nitrogen source showed vigorous growth and no necrosis, and yielded about as many seeds as cv Rondo cultured in the same way.

Preliminary studies showed that necrosis does not appear in plants grown on a liquid culture medium without nitrogen, but it is readily evoked by growing the mutant on a nutrient solution containing  $\text{NO}_3^-$  as the sole nitrogen source. The results suggest a toxic effect of accumulated nitrate, whereas the protecting influence of  $\text{NH}_4^+$  may either be brought about via an improved protein synthesis or by an inhibition of nitrate uptake (1).

1. Doddema, H. and G. P. Telkamp. 1979. *Physiol. Plant.* 45:332-338.
2. Groot, G. N. de and A. H. Stouthamer. 1969. *Arch. Mikrobiol.* 66:220-233.
3. Kleinhoffs, A., R. L. Warner, F. J. Muehlbauer, and R. A. Nilan. 1978. *Mutation Res.* 51:29-35.
4. **Mueller**, A. J. and R. Grafe. 1978. *Molec. Gen. Genet.* 161:67-76.
5. Oostindier-Braaksma, F. J. and W. J. Feenstra. 1973. *Mutation Res.* 19:175-185.