NITRATE REDUCTASE-DEFICIENT MUTANTS IN PISUM SATIVUM

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Three nitrate reductase (NR)-deficient pea mutants were induced by sodium azide in the cultivar 'Juneau' and were selected for a segregating M2 population by a qualitative in vivo NR assay. The NR activities of mutants A300, A317, and A334 are approximately 20, 1, and 5 percent, respectively, of the wild-type. After several generations of seed increase in the greenhouse, crosses were made among the mutants and Juneau; and the mutants were characterized for NR-associated enzymatic activities.

NR analysis of the F1 and F2 seedlings indicated that the three mutants are representative of two loci. Mutants A317 and A334 are allelic and have provisionally been assigned the gene designation nar-1. Mutant A300 is representative of a second locus and is designated nar-2. Segregation in the F2 from crosses of A300/A317 and A300/A334 indicated that nar-1 and nar-2 are not closely linked. Both nar-1 and nar-2 are codominant as indicated by intermediate NR activity in the F1 hybrids of A300/Juneau, A317/Juneau, and A334/Juneau.

Research with NR from fungi and higher plants has demonstrated that NR-deficiency can be caused by mutations in the NR structural gene, mutations in the NR regulatory gene, mutations in the gene(s) controlling synthesis of molybdo-cofactor components of NR, and mutations in the gene(s) controlling nitrate uptake. Enzymatic characterization of the NR partial activities and of enzymes associated with NR can be used to predict the nature of nar-1 and nar-2 genes. Nitrate reductases from most organisms can use reduced FMN, FAD, and viologens in addition to NAD(P)H to reduce nitrate in vitro. Most NR-deficient mutants lack all these activities; however, some NR structural gene mutations permit the synthesis of an enzyme which lacks NAD(P)H NR activity but has $FMHN_2$ NR activity. However, the $FMNH_2$ NR activities of all three mutants were lower than the respective NADH NR activities. Consequently the $FMNH_2$ NR characteristic was not helpful in determining the nature of either nar-1 or nar-2.

NR also has a diaphorase activity and can reduce other substrates such as cytochrome c. Plants of course have cytochrome c reductases other than NR; however, NR is the only enzyme with cytochrome c reductase activity that is known to be induced by nitrate. All three mutants have at least some nitrate inducible cytochrome c reductase activity which suggests that the NR gene product is being synthesized in response to nitrate but lacks the ability to reduce nitrate. This further suggests that neither nar-1 or nar-2 are regulatory genes since NR regulatory gene mutations result in the loss of the NR gene product which also eliminates the nitrate induced cytochrome c reductase, component of the enzyme. Furthermore, all three mutants have very high nitrate reductase activities (190-230% of the wild-type). In fungi, NR and nitrite reductase are both controlled by the same regulatory gene and loss of NR also results in the loss of nitrite reductase. However NR regulatory gene mutants have not as yet been reported in higher plants and the mechanism of control in higher plants may differ from fungi.

Another enzyme that can provide insight into the nature of the NR mutants is xanthine dehydrogenase. Xanthine dehydrogenase is also a molybdo-enzyme and appears to have the same molybdo-cofactor component as NR. The mutant

A300 does not have xanthine dehydrogenase activity which strongly suggests that nar-2 is a gene involved with .the production of the molybdo-cofactor.

Although direct evidence is not yet available, nar-1 is most likely the NR structural gene. The leaf nitrate concentrations in the mutants are 8 to 10 times greater than the wild-type indicating that nitrate uptake is not a causal factor in the NR-deficiency. Since nar-1 does not have the characteristics usually associated with NR regulatory gene mutants and nar-2 is a molybdo-cofactor gene, the only other known gene in which mutations cause loss of NR activity is the NR structural gene.

Editor's Note: Happily, a number of workers have isolated mutants that control nitrate reductase activity. Apparently, however, there has not been any coordinated effort to name mutants or assign symbols according to an agreed upon scheme. I strongly urge workers in this area to communicate with each other and with Dr. Blixt who is overall coordinator of gene symbols in Pisum. We want to avoid the duplication of symbols or the use of different symbols for the same mutants. It is much more convenient to avoid the problem in the first place than to try to correct a situation that is entrenched in the literature. Perhaps someone will volunteer to write an article summarizing the reports on this subject to date?