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HOST PLANT CONTROL OF THE INHERITANCE OF DINITROGEN FIXATION IN THE *PISUM-RHIZOBIUM* SYMBIOSIS[1] [2]

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SUMMARY

The contribution of the host plant genome in symbiotic dinitrogen fixation has received little attention. In examining more than two thousand samples from the USDA Plant Introduction Collection, host-determined genetic variation in dinitrogen fixation has been found. In genetic analysis of a mutant line of *Pisum* resistant to nodulation, two genes designated Sym_2 and Sym_3 have been detected; Sym_2 affects nodulation while Sym_3 influences fixation. The two genes segregate independently as dominant Mendelian characters. Effective symbiosis requires the presence of at least one dominant gene at each locus.

INTRODUCTION

Symbiotic dinitrogen fixation requires the cooperative interaction of two different genomes, one plant and the other bacterial. Despite the biological and economic importance of this process we have only a meagre knowledge of its genetics. Moreover, most genetic investigations have been directed toward the bacterial partner, although it is probable that at least some factors controlling the initiation, amount and termination of fixation are located in the plant. Plant genes affecting fixation have been reported for clovers (*Trifolium* sp.), soybean (*Glycine max* (L.) MERR.) and peas (GELIN & BLIXT, 1964) (for review see HOLL & LARUE, 1975). Other instances of host effects have been observed or inferred, but genetic analysis is lacking (see NUTMAN, 1956).

Field peas exhibit variation among cultivars in the rate and amount of nitrogen accumulation via dinitrogen fixation. We are identifying the genetic factors in field peas which influence the symbiotic process as a necessary prelude to exploiting the available genetic potential for crop improvement. In this paper, the selection of genetic variants and the detection of two plant genes affecting dinitrogen fixation are described.

MATERIALS AND METHODS

Selection Experiments. More than two thousand lines of the USDA Plant Introduction Collection of *Pisum* (peas) obtained from Dr A. E. Slinkard, Crop Development

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[2] Preliminary report of some of this data has been made at the International Symposium on Nitrogen Fixation, Pullman, Washington, June 1974.

Centre, Saskatoon, Canada, were examined for resistance to nodulation, or inability to fix nitrogen as measured by the acetylene reduction technique. Initial greenhouse selections were retested under both greenhouse and field conditions and these final selections were increased for genetic analysis. Growth and assay of the plant material was carried out as described for the hybridisation experiments.

Hybridisation Experiments. The wild type parent (nodulating, dinitrogen fixing) used in this investigation was Trapper, a small-seed field pea variety grown in Western Canada. Afghanistan (PRL H722), a mutant line resistant to infection by *Rhizobium* and unable to form an effective symbiosis, was obtained from Dr T. A. Lie (Laboratory of Microbiology, Agricultural University, Wageningen, The Netherlands) (LIE, 1971).

In both field and greenhouse experiments, seeds were inoculated with a commercial *Rhizobium leguminosarum* inoculum (Hansen Inoculator Co., Milwaukee, Wisconsin). Over the duration of the experiments (1972–1974) several batches of inoculum were used; no effect of different batches was observed.

In the greenhouse, inoculated seeds were planted and grown in a mixture of equal parts by volume of fine silica sand and vermiculite. Plants were watered weekly with nitrogen-free medium (WILSON & REISENAUER, 1963) and when necessary in the intervening periods, with tap water.

Field grown plants were inoculated at seeding and grown under natural field conditions except when additional water was provided during extended dry periods.

After 3–5 weeks growth (6–12 node stage) intact plants were examined visually for nodules and assayed for nitrogen fixation using the acetylene reduction technique as described by LARUE & KURZ (1973). The assay is non-destructive and allows selected plants to be grown to maturity. The basis of this method is the ability of the enzyme nitrogenase, responsible for dinitrogen fixation, to reduce alternative substrates including acetylene. The reduction product, ethylene, can be separated and quantified by gas chromatography. Gas chromatography was initially performed on a Carle Model 9000 gas chromatograph (Carle Instruments Inc., Fullerton, California, USA). Subsequent analyses of approximately 200–300 samples were carried out on the more sensitive Varian Series 204C instrument (Varian Aerograph Ltd., Walnut Creek, California, USA). Some nodulated plants showed no acetylene reducing activity on the Carle instrument. Analysis on the more sensitive Varian Gas Chromatograph revealed that some of these non-fixing, nodulated plants were capable of a low level of acetylene reduction, 10–25% of the normal wild type plants. The consequences of this difference are considered in the interpretation of the results.

The cross Trapper × Afghanistan (PRL H722) and its reciprocal were made in the greenhouse using manual transfer of pollen to the emasculated female parent.

Seed from the crosses was grown in the field in 1972 and examined visually for nodulation.

F_2 progeny were grown under greenhouse conditions and assayed for nodulation and fixation. Chi-square analysis was applied to the F_2 data.

RESULTS AND DISCUSSION

After three generations of testing, eleven selected lines of *Pisum* sp. have been obtained from the USDA Plant Introduction Collection. These lines are either resistant to nodulation, or after infection, form an ineffective symbiosis. Analysis of the host genetic component of these mutants is in progress. We have estimated that mutation in at least ten genes could alter the symbiotic association (HOLL & LARUE, 1975). The variation we have observed in field peas suggests that much of this genetic variability may be available in natural populations.

F_1 progeny of the Trapper × Afghanistan (and reciprocal) cross were grown in the field in 1972. High soil nitrogen completely suppressed nodulation and prevented any classification of the F_1 phenotype.

The data and statistical analysis of the greenhouse-grown F_2 progeny are summarised in Table 1, 2 and 3. Nodulation and fixation are inherited as dominant characteristics. Nodulation segregates independently as a simple Mendelian trait (Table 1). The combined data for fixation (Table 2) show a good fit to the theoretical 9 :7 ratio of a two gene difference in which one dominant allele at each locus is required for phenotypic expression. We have designated these two genes Sym_2 (nodulation) and Sym_3 (fixation) (HOLL & LARUE, 1975).

During the latter portion of this investigation, gas chromatographic assays were carried out on the more sensitive Varian instrument and it was possible to discriminate quantitatively four phenotypic and their corresponding genotypic classes (Table 3). These latter data are consistent with the initial conclusion that two unlinked genes are responsible for the wild type phenotype.

Table 1. Chi-square analysis of F_2 progeny of the cross Trapper (nodulated) × Afghanistan (non-nodulated).

Phenotype	Phenotypic frequency		X_1^2 (3 :1)
	observed	expected	
Nodulation	417	430	0.393
Non-nodulation	156	143	1.182
	573	573	1.575
			$0.3 > P > 0.2$

Table 2. Chi-square analysis of F_2 progeny of the cross Trapper (fixing) × Afghanistan (non-fixing).

Phenotype	Phenotypic frequency		X_1^2 (9 :7)
	observed	expected	
Fixation	330	322	0.199
Non-fixation	243	251	0.255
	573	573	0.454
			$0.6 > P > 0.5$

Table 3. Chi-square analysis of F_2 progeny of the cross Trapper (nodulating, fixing) × Afghanistan (non-nodulating, non-fixing).

Phenotype	Genotype	Phenotypic frequency		X_3^2 (9:2:1:4)
		observed	expected	
Nodulating, fixing	Sym_2——Sym_3——	140	140	–
Nodulating, non-fixing	$Sym_2sym_2sym_3sym_3$	33	31	0.129
Nodulating, low fixing (10–25% of wild type)	$Sym_2Sym_2sym_3sym_3$	18	16	0.250
Non-nodulating, non-fixing	sym_2sym_2——	58	62	0.258
		249	249	0.637
				$0.9 > P > 0.8$

In nodulated non-fixing lines (Genotype Sym_2–sym_3sym_3) a functional nitrogenase can be detected in vitro (HOLL, 1973). The defect specified by sym_3 appears to be in the supply of photosynthate material to the nodule. The physiological significance of the relationship between photosynthesis and dinitrogen fixation has received considerable attention in recent years (STANIER, 1974). It is probable that the physiological interactions involved in symbiotic dinitrogen fixation reflect the complexity of the genetic interaction of two different genomes. Two plant genes affecting the symbiotic process in field peas are described in this paper; a further eleven selected genetic variants are undergoing analysis. These data emphasize the involvement of the plant host, and the potential for manipulation of the genetic complement of field peas for improvement of symbiotic dinitrogen fixation.

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