

PEAS (*PISUM SATIVUM* L.)
WITH STRAIN SPECIFICITY FOR
RHIZOBIUM LEGUMINOSARUM

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SUMMARY

Seven varieties of *Pisum sativum* from Afghanistan, Iran, Tibet and Turkey were tested for nodulation by 25 diverse strains of *Rhizobium leguminosarum*. The pea varieties were resistant to nodulation by 13 rhizobial strains from temperate regions, and formed few or no nodules. The varieties nodulated with 4 of 12 rhizobial strains from Middle Eastern soils. The plant-rhizobia specificity was identical to *Pisum sativum* var. "Afghanistan".

Crosses were made among five nodulation resistant varieties and "Afghanistan". Genetic analysis indicates that the strain specific nodulation resistance in each of the five pea varieties is allelic with the *sym-2* gene in "Afghanistan".

Surveys of worldwide pea collections have failed to discover varieties which are never nodulated by any rhizobial strain. There appears, however, to be a class of nodulation resistant peas from the Middle East which have the same restricted strain specificity.

1. INTRODUCTION

The wild pea *Pisum sativum* var. "Afghanistan" is resistant to nodulation by strains of *Rhizobium leguminosarum* from temperate soils. Generally no or only a few nodules are formed (Lie *et al.*, 1976). Lie reported that a single gene was involved, but genetic analysis was not published. Holl (1975) reported that F₂ plants from crosses of "Afghanistan" with nodulating cultivar "Trapper" segregated 3:1 nodulated to non-nodulated. A recessive allele (*sym-2*) was associated with non-nodulation.

Nodulation on "Afghanistan" is rhizobial strain specific. Lie (1978a) found a *R. leguminosarum* strain, TOM (from Tomek, Turkey), which formed effective nodules with "Afghanistan" under aseptic conditions. Lie isolated other infective strains from soil samples from Middle East locations. Lie *et al.*, (1978b) found that European strains of *R. leguminosarum* blocked the formation of nodules on "Afghanistan" by strain TOM, though the European strains themselves were not infective on "Afghanistan". The gene(s) responsible for the ability of strain TOM to nodulate "Afghanistan" peas are carried on a plasmid, pRL5JI, as shown by conjugation experiments (Brewin *et al.*, 1980).

Other nodulation resistant peas were discovered in a survey of 2000 *Pisum* cultivars from the USDA Plant Introduction collection (Holl, 1975; Holl and LaRue, 1976). They were obtained by screening with a commercial inoculant mixture, and not tested further for strain specificity. More recently, a smaller screen of 375 pea varieties was conducted at the John Innes Institute (Young *et al.*, 1982a, 1982b). Twenty-six lines, nearly all from Afghanistan, were resistant to a strain of *R. leguminosarum* from England,

but were nodulated by TOM. Further tests indicated those 26 varieties were nodulated by another strain containing the TOM plasmid, pRL5JI. The pea lines were phenotypically similar to each other, and to "Afghanistan", being characterised by small flowers and pods, brown marbled seeds, and resistance to powdery mildew. No crosses were made to determine if there was a common genetic basis for the strain specificity.

Six other putative non-nodulating pea varieties identified by Holl (1975) were tested for strain-specific nodulation using 25 diverse strains of *R. leguminosarum*. F₁ and F₂ progeny of crosses made among the nodulation resistant pea varieties were scored for nodulation using a single strain of *R. leguminosarum*. The pattern of strain specificity and the genetic analysis reported here indicate that nodulation in these peas is under control of the same gene (*sym-2*) as in "Afghanistan".

2. MATERIALS AND METHODS

(i) Pea varieties

Seven nodulation-resistant varieties of *Pisum sativum* from the Middle East, and two nodulating, North American cultivars (table 1) were tested for nodulation.

TABLE 1
Origin and characteristics of Pisum sativum lines

Variety	Characteristics	Source
"Sparkle"	garden pea dwarf, white flowered; green, wrinkled seeds	1
"Trapper"	field pea tall, white flowered; seeds smooth, yellow	1
"Afghanistan"	nodulation-resistant (JI 1357, SVP 57425)* tall, anthocyanin; smooth, yellow, mottled seeds	2
PI 173055	ecotype elatius from Dara, Turkey, "Hatun Bakleri" tall, anthocyanin, branching, serrated leaves, smooth, yellow, dark, gritty, hard coated seeds	3
PRL 71HS2	non-nod selection from PI 140165, from Afghanistan, "Moshung" (JI 95, WS 1418)*	4
PRL 71HS5	tall, anthocyanin; smooth, yellow, mottled, speckled seeds non-nod selection from PI 175228, from Takhlakot, Tibet "Shratma"	4
PRL 71HS7	tall, anthocyanin; smooth, yellow, mottled seeds non-nod selection from PI 198735, from Ghazni, south of Kabul (JI 97, WS 1420)*	4
PRL 72HS1	tall, anthocyanin; smooth, yellow, mottled, speckled seeds non-nod selection from PI 210631, "Early Wales"	4
PRL 72HS2	tall, anthocyanin; smooth, yellow, mottled seeds ecotype elatius, non-nod selection from PI 227258, from Esfahan, Iran tall, anthocyanin; smooth, yellow, mottled seeds	4

Sources: 1. Commercial cultivars 2. T. Lie, Wageningen, The Netherlands 3. USDA Plant Introduction Station, Geneva, New York 4. Prairie Regional Lab, NRC, Saskatoon, Canada.

* References to numbers used in *Pisum* collections: PRL = Prairie Regional Lab, NRC, Saskatoon, Canada PI = USDA Plant Introduction Service JI = John Innes Institute, England WS = Weibullsholm, Sweden SVP = Stichting voor Plantenveredeling, Wageningen, Netherlands

(ii) *Rhizobium leguminosarum* strains

The sources of the rhizobial strains and their countries of origin are shown in table 2. Commercial pea inoculant was from the Nitragin Co., Milwaukee, Wisc.

TABLE 2
Strains of Rhizobium leguminosarum tested for infectivity

Strain	Country of origin	Source*
ATCC 10004	U.S.A.	1
128C53-smr	U.S.A.	2
300	England	3
NA501-1, NA502-1, NA503, NA504-2, NA505, NA525, NA533, CC1019, SU391, TA101	Australia	
BB54b, L28a, PS3a	Syria	5
510P, 511P	Israel	6
TOM	Turkey	7
[Bez3, F72, F77, F81, FPec1, FPec4, FPec16]		8

* Source 1, American Type Culture Collection, Beltsville, MD, USA; 2, J. Burton, Nitragin Co., Milwaukee, WI, USA; 3, J. Beringer, John Innes Institute, Norwich, UK; 4, Prairie Regional Lab, NRC, Saskatoon, Canada; 5, R. Islam, ICARDA, Aleppo, Syria; 6, Volcani Institute, Israel; 7, T. Lie, Wageningen, The Netherlands; 8, N. Ulgen, Soil and Fertilizer Research Institute, Yenimahalle, Ankara, Turkey.

The *R. leguminosarum* strains were derived from single colonies on yeast-mannitol Congo red agar (YMA-CR) plates (Vincent, 1970). All, except CC1019, PS3a, Bez3 and F72 which form white nodules, form pink nodules on cultivars "Sparkle" (>50) and "Trapper" (>90). Cultures were maintained on yeast-mannitol agar slants, and inoculum for infectivity tests was grown at 30° for 2 days in yeast-mannitol broth (YMB).

(iii) *Infectivity tests*

Surface sterilised seeds were planted in autoclaved 180 ml Dispo bottles (American Scientific Products, Rochester, NY) containing vermiculite and 100 ml nitrogen-free nutrient solution (KH₂PO₄, 0.27 g; K₂SO₄, 0.35 g; MgSO₄.7H₂O, 0.25 g; CaSO₄.2H₂O, 0.37 g; FeC₆H₅O₇, 0.03 g; 1 ml of micronutrient stock (containing H₃BO₃, 4.0 g; MnCl₂.4H₂O, 1.0 g; ZnSO₄.7H₂O, 0.575 g; CuSO₄.5H₂O, 0.125 g; Na₂MoO₄.2H₂O, 0.103 g; 1 l H₂O); 1 l H₂O; pH 6.8). Seedlings were inoculated 4 days after planting with 4 ml of rhizobial culture (10⁸ cells/ml), the surface covered with parafinised sand (Vincent, 1970), and seedlings grown in temperature controlled lightrooms. A combination of metal halide and high pressure sodium lamps provided 555 m E/m² s. The light/dark regime was 16 h/8 h and 21 C/18 C. Nodules were counted 17 days after inoculation. Each treatment was replicated at least three times. Strains which nodulated any Middle Eastern pea line were retested on "Sparkle", "Afghanistan", 71HS2, 71HS5, 71HS7 and 72HS2.

(iv) *Genetic analysis*

To test for allelism, "Afghanistan", 71HS2, and PI 173055 were reciprocally crossed in a diallel mating design, and each crossed to "Sparkle". Lines 71HS5, 71HS7 and 72HS2 were crossed with "Afghanistan". Progenies were scored for seed traits, then planted in 7 cm pots of sand/vermiculite 1 : 2, and fertilised with nitrogen-free nutrient solution in the lightrooms. Seedlings were inoculated with *R. leguminosarum* 128C33-smr 4-7 days after planting, and scored for shoot characteristics and nodulation 17-23 days after inoculation. The F₁ seedlings were then transferred to complete substrate (Cornell mix) and grown to maturity in the light rooms. Their progenies were scored for seed traits, and the F₂ seedlings tested for nodulation.

3. RESULTS

(i) *Infectivity tests*

The nodulation resistant varieties formed none or very few nodules with the rhizobial strains from temperate regions (table 3). The occasional nodules yielded rhizobial isolates which nodulated "Sparkle", but did not again form nodules on the resistant varieties. In addition to TOM, three rhizobial strains from the Middle East formed pink nodules on all the nodulation-resistant varieties. Two of the infective strains were from Israel, and one from Syria. It is noteworthy that the seven pea lines showed nearly identical patterns of strain specificity (table 3) with the 25 rhizobial strains tested.

(ii) *Genetic analysis*

R. leguminosarum 128C53-smr usually formed at least 30 pink nodules on "Sparkle" controls, while few (<5), if any, nodules developed on "Afghanistan", 71HS2, 71HS5, 71HS7, and 72HS2. PI 173055 occasionally had as many as 25 nodules. All F₁ and F₂ progenies of crosses among nodulation-resistant lines fell within the "non-nod" parental class (table 4). The lack of nodulating recombinants in the progeny indicates the same gene controls nodulation resistance in the five lines crossed to "Afghanistan".

In comparison, crosses between nodulation-resistant lines and "Sparkle" produced nodulating F₁ progeny. F₂ segregation ratios for nodulation were not clear, since progeny included seedlings with intermediate numbers of nodules, as well as nodulation resistant and well nodulated parental types. Testing for nodulation with two other strains of *R. leguminosarum*, PRE and 300, has not resulted in clear segregation ratios in "Sparkle" × "Afghanistan" F₂ progeny.

4. DISCUSSION

The lack of nodulating recombinants in progeny of crosses among nodulation-resistant lines and the nearly identical rhizobial strain specificity shown by them is convincing evidence that the strain-specific nodulation resistance genes in these seven lines are allelic.

TABLE 3
Infection of Pisum lines by Rhizobium leguminosarum

<i>R. leguminosarum</i>	Pea varieties						
	Afghanistan	71HS2	PI173055	71HS5	71HS7	72HS1	72HS2
"Temperate" strains							
128C53-smr	-	-	-	-	-	-	-
ATCC 1004	-	-	-	-	-	-	-
300	-	-	-	-	-	ND	-
NA501-1	-	-	-	-	-	ND	-
NA502-1	-	-	-	-	-	ND	-
NA503	-	-	-	-	-	ND	-
NA504-4	-	-	-	-	-	ND	-
NA505	-	-	-	-	-	ND	-
NA525	-	-	-	-	-	ND	-
NA533	-	-	-	-	-	ND	-
CC1019	-	-	-	-	+	ND	+
SU391	-	-	-	-	-	ND	-
TA101	(+)	-	-	-	-	ND	-
"Nitragin"	-	-	-	-	-	ND	-
Middle Eastern Strains							
PS3a	-	-	ND	-	-	-	-
Bez3	-	-	-	-	-	ND	-
F72	-	-	-	-	-	ND	-
F81	-	-	-	-	-	ND	-
FPec 1	-	-	-	-	-	ND	-
FPec 4	-	-	-	-	-	ND	-
FPec 16	-	-	-	-	-	ND	-
L28a	-	(+)	-	-	-	ND	-
510P	+	+	+	(+)	+	+	+
511P	+	(+)	+	(+)	+	+	+
BB54b	+	+	+	+	+	+	+
TOM	+	+	+	+	+	+	+

- = nodulation resistant, <5 nodules.

(+) = occasional nodulation, >5 nodules on some plants.

+ = normal nodulation, >5 nodules on at least 5-6 replicates

number of nodules per plant = 5-20' on 71HS5, 173055; 5-25 on Afghanistan; 5-35 on 71HS2, 72HS1; 10-45 on 71HS7, 72HS2, >50 on "Sparkle"; >90 on "Trapper"

ND = not determined

Analysis of "Afghanistan" × "Trapper" F₂ progeny showed that nodulation-resistance was associated with a single recessive gene designated *sym-2* (Holl, 1975). Lie reported that segregation for nodulation of F₂ progeny from "Afghanistan" × "Rondo" crosses was strain dependent. When *R. leguminosarum* PRE was used as the test strain nodulation resistance was due to a single dominant gene (Lie *et al.*, 1976). We have not been able to confirm that resistance to nodulation is controlled by a single gene in "Afghanistan" × "Sparkle" progeny. Non-parental plant types with intermediate numbers of nodules are found in F₂ progeny scored with *R. leguminosarum* strains 128C53, 300 or PRE.

Nodule number may be modified by genetic background. Even with the rhizobial strain TOM, nodule number on "Afghanistan" is lower than on "Sparkle". We are, therefore, backcrossing "Sparkle" × "Afghanistan" F₂

TABLE 4
Segregations for nodulation in the F₁ and F₂ generations in crosses among nodulation resistant pea lines

Cross	F ₁		F ₂		
	No. of crosses	Ratio (nodulation resistant: normal)	No. of crosses	No. of F ₁ plants	Ratio (nodulation resistant: normal)
71HS2 × Afghanistan	6	19:0	2	2	95:0
Afghanistan × 71HS2	18	43:0	3	7	194:0
173055 × Afghanistan	4	13:0	2	2	36:0
Afghanistan × 173055	2	2:0	1	2	132:0
71HS2 × 173055	6	13:0	4	4	83:0
173055 × 71HS2	1	5:0	1	2	33:0
71HS5 × Afghanistan	1	3:0	1	1	63:0
71HS7 × Afghanistan	2	3:0	2	3	75:0
72HS2 × Afghanistan	1	4:0	1	4	84:0

non-nodulating selections to "Sparkle", in an attempt to separate the effects of the *sym-2* gene from other genetic modifiers of nodule number.

Not all rhizobial strains from Middle East soils formed nodules on the nodulation-resistant pea lines. The four infective strains are from Turkey, Syria and Israel, and they differ in some cultural characteristics (Kneen and LaRue, 1983). The infectivity of TOM is associated with an atypical plasmid (Brewin *et al.*, 1980). It would be interesting to learn if a similar plasmid is present in the three additional strains we found to infect "Afghanistan".

After examining the putative non-nodulating peas reported by Holl (1975), we find that all nodulate with appropriate strains of rhizobia. In agreement with Young *et al.*, (1982*b*), we conclude that there is a class of peas, exemplified by "Afghanistan", with a restricted strain specificity. Additional pea genotypes defective in the symbiosis are desirable in order to study the host plant contribution to nitrogen fixation. These may have to be obtained by artificial mutagenesis.

Little is known about the plant or rhizobial genes which control the specificity of nodule formation. The "Afghanistan"-TOM (*sym-2*, *sym-2*—pRL5J1) association is one in which something is known of the genetics of both symbionts.

Devine and Weber (1977) proposed for agriculture the development of legume cultivars which exclude indigenous rhizobial strains while selectively forming nodules with specific introduced strains. A model for this conditional specificity system may be a strain specific (*sym-2*, *sym-2*) pea which would exclude indigenous rhizobia in temperate soils, but admit introduced competitive strains bearing the pRL5J1 plasmid. Clearly, the "Afghanistan"-like peas deserve further study.

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