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Rhizobium — Host Specificities in *Phaseolus coccineus* L. and *Phaseolus vulgaris* L.[1]

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ABSTRACT

The introduction of desirable agronomic characteristics into *Phaseolus vulgaris* L. is being attempted by interspecific hybridization with *P. coccineus*, but the effects on root nodulation are unknown. Accordingly, cross-inoculation relationships between *P. vulgaris* 'Red Kidney' and *P. coccineus* 'Scarlet Runner' were tested in the greenhouse with a group of 16 Rhizobium strains effective on Red Kidney and a group of 12 strains effective on Scarlet Runner. Both groups had diverse origins, including commercial sources and isolations from bean fields and other sites in California.

Only six strains, all isolates from Scarlet Runner, were effective on both hosts. Fifty percent of Scarlet Runner isolates were as effective on Red Kidney as the more effective of Red Kidney's homologous strains. Thirty percent of the California isolates tested on Red Kidney were more effective than two commercial inocula.

Strain rejection in Red Kidney was expressed in 75% of all cases as failure to form nodules. In contrast, strain rejection by Scarlet Runner was expressed in 64 to 68% of all cases as ineffective nodulation (nodule malfunction). If Scarlet Runner is representative of *P. coccineus* in this respect, interspecific hybridization is likely to increase rather than reduce the tendency of *P. vulgaris* to nodulate with ineffective rhizobia.

Additional index words: Rhizobium phaseoli, Phaseolus vulgaris, Phaseolus coccineus, Cross-inoculation, Nodulation effectiveness, Isolation of soil rhizobia.

MODERN cultivars of *Phaseolus coccineus* L. and *P. vulgaris* have evolved from related wild forms of the genus *Phaseolus* (6, 7, 10, 11, 17, 18). Cultivars of *P. vulgaris* exhibit a greater diversity of habit and other characteristics than do those of *P. coccineus* (6, 18), but the latter are thought to show greater vegetative vigor, seed size, disease resistance, and adaptation to cooler climates. These have been desirable characteristics for breeding programs aimed at improving *P. vulgaris* cultivars (13, 16).

Graham and Halliday (8) have suggested interspecific hybridization as a means of increasing host nodulation specificity in *P. vulgaris* and improving symbiotic performance under field conditions. *Phaseolus vulgaris* has often been noted for its promiscuity (1, 8, 9, 20, 21), and for the detrimental effects of ineffective nodulation (1, 2, 12, 14, 15). *Phaseolus coccineus* may be resistant to nodulation by strains of *Rhizobium phaseoli* effective on *P. vulgaris*, but current information is limited (1, 8, 21).

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Table 1. California soils sampled for rhizobial isolations.

Soil #	Location and use	Strains isolated: Red Kidney	Strains isolated: Scarlet Runner
1	Davis, bean field	†	C1a,b
2	Davis, bean field	V2	C2a,b,c
3	Davis, university farm	V3a,b,c	C3a,b,c
4	Davis, university farm	V4a,b,c,d,e,f	†
5	Winters, bean field	V5a,b	C5a,b,c
6	Woodland, alfalfa field	†	C4a,b
7	Cache Creek river wash	†	C7a,b
8	Lodi, bean field	V8a,b	C8a,b,c
9	Stockton, bean field	no nodules	‡
10	Cal Poly campus	no nodules	C10
11	Shafter, cotton field	V11	C11a,b
12	Shafter, alfalfa field	‡	C12
13	Clovis, vineyard	no nodules	C13
14	Clovis, peach orchard	V14	no nodules
15	Chino, fallow field	‡	C15a,b
16	Gorman, high desert	V16a,b	‡
17	Davis, tomato field	no nodules	no nodules
18	Livingston, peach orchard	no nodules	no nodules

† Host variety not evaluated.
‡ No viable strains retained.

In view of these breeding interests and concern with improving N fixation in *P. vulgaris*, a study of nodulation characteristics in *P. coccineus* seems appropriate. This paper presents data on rhizobial nodulation specificities for one cultivar of each species: *P. vulgaris* 'Red Kidney' and *P. coccineus* 'Scarlet Runner'. Nodulation characteristics and overall effectiveness of each host were tested with commercial bean rhizobia and native strains from 16 California fields.

MATERIALS AND METHODS

Effectiveness of selected rhizobial strains was determined on *P. coccineus* 'Scarlet Runner' and *P. vulgaris* 'Red Kidney' under greenhouse conditions. Three trials were conducted in the spring and early summer of 1978 and 1979. Scarlet Runner seeds were obtained commercially from Northrup King Co. for the host isolation trial and screening Trial 1, and from Lagomarsino Seed Co. for screening Trial 2. Red Kidney seed was supplied by C. L. Tucker, Dep. of Agronomy and Range Science, Univ. of California, Davis.

Eighteen California soils were sampled (Table 1). Two pots were filled with 2 kg of each soil, air-dry and unamended. Pots were maintained at approximate field capacity by watering to weight with distilled water. Two surface-sterilized seeds of each cultivar were planted in a pot. After emergence, seedlings were thinned to one plant per pot. Plants were harvested 30 days after emergence, and crushed nodule isolates were obtained from the

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Table 2. Established *Rhizobium* strains used in cross-inoculation trials.

Strain #	Strain	Source
19	CIAT 73	*R. phaseoli* for *P. vulgaris* from
20	CIAT 135	Centro Internacional de Agricultura
21	CIAT 404	Tropical (CIAT) Cali, Colombia
22	CIAT 160	
23	CIAT 247	
24	NA 575	
25	127 K17	Commercial *P vulgaris* strains from
26	127 K44	NITRAGIN Co., Inc., Milwaukee, Wis.
27	127 K46	
28	127 K30	
29	TAL 98	Cowpea miscellany strains, from
30	TAL 209	University of Hawaii, NifTAL Project, Paia, Hawaii

largest three proximal nodules on each plant by the procedure of Vincent (19). Uniform, single-celled colonies were cultured on yeast mannitol agar. Cultures were discarded if they were not short, gram negative rods. Forty-four strains were retained. In addition, established strains for *P. vulgaris* and *Vigna* spp. were included (Table 2).

In all trials, seeds were surface sterilized by immersion in 5% H_2O_2 for 5 min, and planted at 5 cm depths at a rate of four per pot. Seedlings were thinned for uniformity to two per pot. Treatments were replicated by four pots in Trial 1 and by three pots in Trials 2 and 3. Pots were arranged in randomized complete blocks and watered to weight with distilled water throughout growth.

Plant color, budding, and flowering were noted relative to that of the uninoculated control treatments, with and without NH_4NO_3. After harvesting, plant tops were dried at 65 C and weighed. Nodule count, weight, color, and distribution were recorded. Acetylene reduction rate was measured during harvest between 1000 hours and 1400 hours. For this purpose, plant root systems were cut off below the cotyledon, placed in 970 ml canning jars fitted with serum stoppers, and incubated for 1 hour in a 10% carbide-generated acetylene atmosphere. Samples were then withdrawn for gas chromatography.

Trial 1. Test of effective common bean rhizobia on Scarlet Runner.

The growth medium was river outwash from Cache Creek, Calif., with sandy loam texture and pH 7.8. Pots with plastic liners were filled with 2 kg of dry, autoclaved soil. A basal fertilizer was mixed with the soil in each pot 1 day prior to planting, providing 3 mmole/pot of both KH_2PO_4 and K_2SO_4, 10 ppm Zn as $ZnSO_4$, and 0.1 ppm Mo as Na_2MoO_4.

Inoculum was prepared from fresh rhizobial suspensions estimated to provide 10^8 viable cells/ml, and 2 ml/seed were applied at planting. Nitrogen fertilized controls received 4 mmole NH_4NO_3 2 weeks after germination.

Soil temperature fluctuated between 20 and 28 C. A 15% moisture content was maintained throughout growth. Plants were harvested 47 days after emergence.

Trial 2. Test of Scarlet Runner isolates on Scarlet Runner.

Pots containing a mix of 2:1 horticultural perlite and fine quartz sand were watered with 400 ml of nutrient solution containing 3.75 m *M* $CaCl_2$, 1.25 m *M* $MgSO_4$, 1.25 m *M* K_2SO_4, 0.38 m *M* KH_2PO_4, 0.25 m *M* K_2HPO_4, 62.5 μ M Fe-EDTA, and micronutrients. The growth medium was autoclaved for 2 to 3 hours. Nutrient solution was again added 19 days after germination.

Inoculum was prepared from fresh rhizobial suspensions estimated to provide 10^9 viable cells/ml, and seedlings each received 1.5 ml 3 to 5 days after emergence and thinning. The N fertilized controls received 5 mmole of NH_4NO_3 both 15 and 29 days after germination.

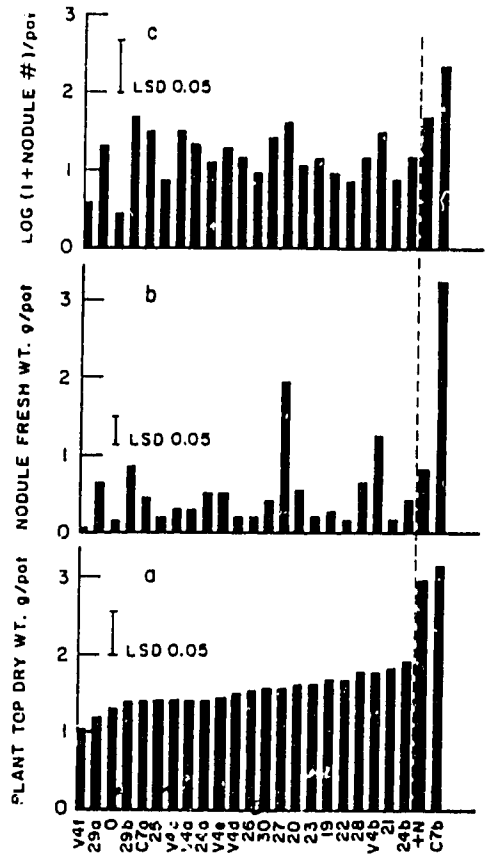

Fig. 1. Growth and nodulation of Scarlet Runner with rhizobia effective on *P. vulgaris*, *P. coccineus*, and *Vigna* spp. Strains identified by numbers according to Tables 1 and 2. Dotted line separates effective from ineffective symbioses, based on differences in plant top dry wt. (P + 0.05).

Soil temperatures were kept between 23 and 28 C by placing pots in constant temperature water baths. Forty percent moisture content was maintained in the pots. Plants were harvested from 34 to 36 days after emergence.

Trial 3. Test of Scarlet Runner and Red Kidney isolates on Red Kidney.

A combination of medium sand, 10% by volume spaghnum peat, and distilled water was autoclaved, placed in polyethylene lined pots and planted as in Trial 2. After emergence, the pot surfaces were covered with sterilized coarse perlite to reduce cross-contamination. Nutrient solution containing 1 m *M* $CaCl_2$, 1 m *M* $MgSO_4$, 1 m *M* KH_2PO_4, 10 μ *M* FE-EDTA, and micronutrients was added in increments; 0.25 strength the 1st week after germination, 0.50 strength during the 2nd week, and full strength during the remaining 3 weeks. The water soluble N content of the sand was below detectable levels (< 0.016 mmole/100 g soil) and that of the peat was 1.06 mmole N/pot. The N fertilized controls received an additional 5 mmole NH_4NO_3 13 and 28 days after emergence.

Soil temperatures fluctuated from 24 to 31 C during the day and from 17 to 21 C during the night. Pots were maintained at 20% moisture. Plants were harvested 34 days after emergence.

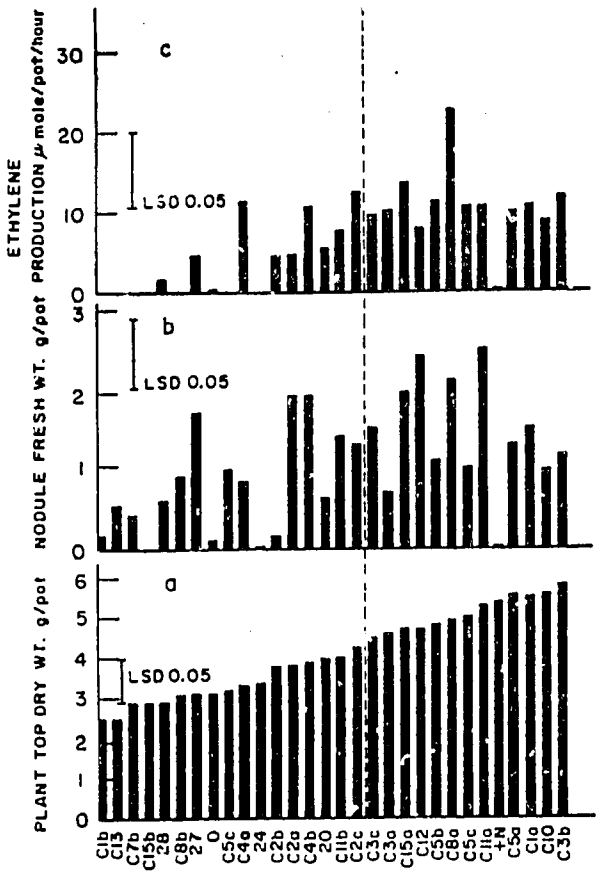

Fig. 2. Growth and nodulation of Scarlet Runner with soil isolates. Strains identified by number according to Tables 1 and 2. Dotted line separates effective from ineffective symbioses, based on differences in plant top dry weight (P + 0.05).

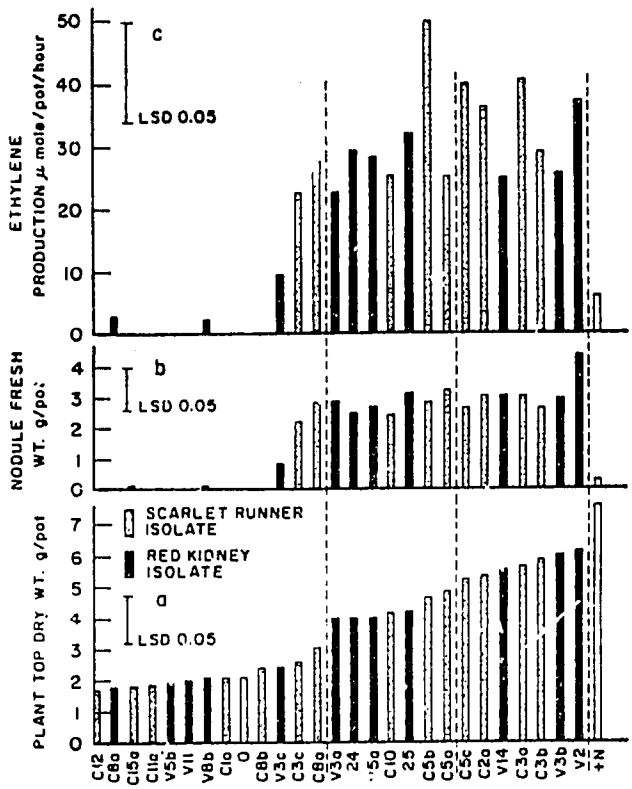

Fig. 3. Growth and nodulation of Red Kidney with soil isolates. Strains identified by number according to Tables 1 and 2. Dotted lines separate effectiveness classes based on differences in plant top dry weight (P + 0.05).

RESULTS

In all soils but no. 17 and 18, 10 to 80 nodules were produced by at least one of the host species. Nodule numbers of the two hosts correlated linearly ($r^2 = 0.64$). Red Kidney nodulated more heavily than Scarlet Runner on soils that were being cropped with beans at the time of sampling.

Trial 1. Test of effective common bean rhizobia on Scarlet Runner.

Strains that did not produce significantly better plant growth ($P < 0.05$) than the uninoculated control were classed ineffective; those not significantly different from the +N control were classed effective. Only one inoculum, C7b (a Scarlet Runner isolate) produced an effective symbiosis (Fig. 1a); but all produced nodules (Fig. 1b and c). Two kinds of nodules were apparent: small white nodules (diameter less than 1 mm) scattered throughout the lower root system; and larger tan, pink, and white nodules (diameter larger than 1 mm) scattered in the upper half of the root system. The uninoculated controls had mainly the former type, probably from aerial sources of contamination. Only the larger nodules were counted, weighed, and included in the nodulation data.

Strain C7b gave significantly higher nodule numbers than all other treatments (Fig. 1c). Fourteen other strains also formed significantly more nodules than the control at the 0.05 probability level. These strains included 10 known to be effective on *P. vulgaris* (including two cultures of NA575), three from the cowpea miscellany (including two cultures of TAL98), and one Scarlet Runner isolate. Nine of these strains also had significantly higher nodule weights than the controls (Fig. 1b).

Ineffective nodules were often red, indicating the presence of leghemoglobin. Several symbioses produced plants which appeared moderately green from the 23rd day of growth until flowering, which began on the 28th day. These strains included CIAT73, CIAT135, NA575, 127K30, TAL98, V4a, and V4b.

Trial 2. Test of Scarlet Runner isolates on Scarlet Runner.

The growth of Scarlet Runner with its homologous soil isolates showed great variability in effectiveness (Fig. 2). Fifty percent of the strains were not significantly better (P + 0.05) than the control based on plant top dry weight. They were classed ineffective. The remaining 50% were not significantly different from the +N control and were classed effective.

Only two or three ineffective Scarlet Runner isolates had negligible nodule weight and acetylene reduction. These were strains C1b, C15b, and perhaps C2b. The remaining ineffective symbioses had substantial nodule weights and acetylene reducing activities at harvest (Fig. 2b and 2c). Some showed sporadic periods of greening, others began turning green just before harvest. Ineffec-

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Table 3. Nodulation characteristics of Scarlet Runner and Red Kidney.

Host cultivar	Source of *Rhizobium*	Total # of strains tested	Insignificant or no nodulation	Ineffective nodulation	Effective symbioses
Scarlet Runner	*P. vulgaris*	16	7	9	0
	P. coccineus	24	2-3	9-10	12
Red Kidney	*P. vulgaris*	12	4	1	7
	P. coccineus	14	5	2	7

tiveness could then have been caused by delay in nodule formation or early nodule malfunction.

Four *R. phaseoli* strains repeated from trial 1 again produced variable levels of ineffective nodulation.

Trial 3. Test of Scarlet Runner and Red Kidney isolates on Red Kidney.

Nodulated plants fell into three groups, based on the L.S.D. for plant growth (P + 0.05). Symbioses in the group including the control were considered ineffective, while the other two groups were considered generally effective although variably so. Each effectiveness class contained nodule isolates from Scarlet Runner as well as those from Red Kidney (Fig. 3). Thirty-five percent of all isolates were more effective than the two commercial inocula.

Optimum growth in effective treatments was well correlated with earliness of fixation, as detected by change in plant color. All replicates of the top 10 treatments turned green 23 days after germination. Treatments V3a, NA575, and V5a were not green until Day 28, and produced less plant growth than others.

Of the 12 ineffective treatments, nine were nonnodulating. Strain V3c nodulated only slightly, and plants remained yellow throughout growth. Ineffective strains V3c and C8a produced significant nodule weights and acetylene reduction rates comparable to those of the effective strains, but greening was uneven and late (after 26 days).

DISCUSSION

Cross-inoculation relationships between Scarlet Runner and Red Kidney were determined by screening 16 rhizobial strains effective on Red Kidney with Scarlet Runner as host plant, and, conversely, screening 12 strains effective on Scarlet Runner with Red Kidney as host. Both groups of strains had random and diverse origins. Plant growth differences were used to determine differences in nodulation effectiveness.

Scarlet Runner formed effective symbioses only with its own nodule isolates, and with none of the strains isolated from *P. vulgaris*. Insofar as no such specificity has been described in the literature amongst cultivars of *P. vulgaris*, it appears that species differences are important.

Scarlet Runner combined selectivity in effectiveness with high levels of ineffective nodulation (Table 3). Of all the strains found ineffective on Scarlet Runner, 64 to 68% formed nodules resulting in variable nodule weight and activity, and variable and sporadic greening of shoots. Strain rejection by Scarlet Runner was expressed largely as nodule malfunction and not failure to form nodules.

Red Kidney showed a different pattern of specificity (Table 3). Fifty percent of the Scarlet Runner isolates were as effective on Red Kidney as Red Kidney's homologous strains. Six Scarlet Runner isolates were effective on both species. Strain rejection by Red Kidney was expressed in 75% of all cases as failure to form nodules.

Evaluating the relative performance of each host with native strains from various California soils was less conclusive. Symbiotic performance of Scarlet Runner was not optimal, nor were sufficient numbers of Red Kidney's homologous isolates tested.

The Red Kidney isolates tested on that host came primarily from four local bean fields. Together with the Scarlet Runner isolates from the same fields, 50% were ineffective. Burton et al. (4) found that only 16% of the *R. phaseoli* in Midwestern bean fields were ineffective, and isolates from all plant nodules were evaluated. This could imply high numbers of ineffective strains in California. However, 67% of the Scarlet Runner isolates from bean fields were effective on Red Kidney, with effectiveness levels equal to or significantly greater than the effectiveness of two commercial strains. These data could imply poor selective ability in Red Kidney, rather than the absence of effective strains.

Fifty percent of Scarlet Runner's isolates were ineffective on Scarlet Runner. Effective strains, however, were isolated from California soils under a wide variety of uses at the time of sampling. Scarlet Runner nodulated as effectively as Red Kidney in soils from local bean fields, where rhizobial effectiveness would most likely have been biased in favor of Red Kidney, a dominant local cultivar. Scarlet Runner combined widespread ability to nodulate effectively with high levels of ineffective nodulation.

Although *P. vulgaris* has often been described as highly promiscuous, both Burton (3, 5) and Vincent (20) have stressed its selective ability to nodulate effectively. The cross-inoculation relationships found here support such selectivity, relative to the performance of Scarlet Runner. Interspecific hybridization could increase the relative competitiveness of ineffective rhizobia for infection of *P. vulgaris* rather than reduce it as suggested (8). But nodulation characteristics within *P. coccineus* may be variable. Both this study and that of Beckham (1) are too limited to indicate to what extent the reported characteristics reflect rhizobial and/or cultivar differences. Evaluation of cultivar differences may provide more successful germplasm for interspecific crosses.

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REFERENCES

1. Beckham, L. S. 1970. Nodulation studies on *P. vulgaris* L. and *P. coccineus* L. M.S. Thesis, Cornell, Univ., Ithaca, N.Y.
2. Brakel, J. 1966. La fixation symbiotique de l'azote chez le haricot (*Phaseolus vulgaris* L.). Bull Rech. Agron. Gembloux 1(4):525-533.
3. Burton, J. C. 1975. Pragmatic aspects of the Rhizobium: leguminous plant association. p. 429-446. *In* W. E. Newton and C. J. Nyman (ed.) Proc. 1st Int. Symp. on Nitrogen Fixation, Vol. 2. Wash. State Univ. Press.
4. ----, O. N. Allen, and K. C. Berger. 1952. The prevalence of strains of *Rhizobium phaseoli* in some Midwestern soils. Soil Sci. Soc. Am. Proc. 16:167-170.
5. ----, ----, and ----. 1954. Response of beans (*Phaseolus vulgaris* L.) to inoculation with mixtures of effective and ineffective rhizobia. Soil Sci. Soc. Am. Proc. 18:156-159.
6. Evans, A. M. 1976. Beans, *Phaseolus* spp. (Leguminosae-Papilionatae) p. 168-172. *In* N. W. Simmonds (ed.) Evolution of crop plants. Longman, N.Y.
7. Gentry, M. S. 1969. Origin of the common bean, *Phaseolus vulgaris*. Econ. Bot. 23:55-69.
8. Graham, P., and J. Halliday. 1976. Inoculation and nitrogen fixation in the genus *Phaseolus*. p. 313-334. *In* J. M. Vincent, A. S. Whitney, J. Bose (ed.) Exploiting the *legume-Rhizobium* symbiosis in tropical agriculture. College of Tropical Agriculture mis. pub. 145, Univ. of Hawaii.
9. Graham, P. H., and C. A. Parker. 1964. Diagnostic features in the characterization of the root-nodule bacteria of legumes. Plant Soil 20:383-396.
10. Harlan, J. R. 1975. Crops and man. ASA, CSSA publication, Madison, Wis.
11. Kaplan, L. 1965. Archaeology and domestication in American *Phaseolus* (beans). Econ. Bot. 19:358-368.
12. Keya, S. O. 1977. Nodulation and nitrogen fixation in legumes in East Africa. p. 233-243. *In* A. Ayanaba and P. J. Dart (ed.) Biological nitrogen fixation in farming systems of the tropics. J. Wiley and Sons, Ltd.
13. Lapinskas, P., and A. M. Evans. 1976. The potential of *P. coccineus* as a grain legume in Great Britain. Annual Report of the Bean Improvement Coop.
14. McCoy, E. R. 1929. A cytological and histological study of the root nodules of the bean, *Phaseolus vulgaris* L. Centbl. Bakt. 79:394-412.
15. Pessanha, G. G., A. A. Franco, J. Dobereiner, A. Groszmann, and D. P. P. de Souza Britto. 1972. Correlacao negativa da nodulacao com a producao de feijao (*Phaseolus vulgaris*) em solos onde nitrogenio nao e fator limitante. Pesqui. Agropecu. Bras. 7:49-56.
16. Rutger, J. N., and L. S. Beckman. 1970. Natural hybridization of *Phaseolus vulgaris* × *Phaseolus coccineus* L. J. Am. Soc. Hortic. Sci. 95:659-661.
17. Smartt, J. 1976. Tropical pulses. Longman, London.
18. ----. 1978. The evolution of pulse crops. Econ. Bot. 32(2): 185-198.
19. Vincent, J. M. 1970. A manual for the practical study of root-nodule bacteria. IBP Handb. No. 15, Blackwell Scientific Publications, Oxford.
20. ----. 1974. Root nodule symbioses with *Rhizobium*. p. 265-241. *In* Quispel (ed.) The biology of nitrogen fixation. North-Holland Publishing Co., Amsterdam.
21. Wilson, J. K. 1944. Over five hundred reasons for abandoning the cross-inoculation groups of the legumes. Soil Sci. 58:61-69.

Variation and Correlations of Proline Accumulation in Spring Wheat Cultivars[1]

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ABSTRACT

Water-stressed plants accumulate considerable amounts of free proline in their leaf tissues. The physiological significance of this metabolic response to water deficit is, however, contentious.

Proline accumulation in *in vitro*-stressed seedling leaf segments and its associations with a number of physiological responses induced by water deficit was studied in 14 wheat (*Triticum aestivum* L.) cultivars with known drought resistance rankings. The cultivars differed (at $P < 0.05$) in their capacity to accumulate proline, which was also intercorrelated with increases in total catabolic amino acids and sugars during stress.

The study did not corroborate the purported association of high proline concentration in stressed leaf tissues with drought resistance, severity of leaf firing, or post-stress recovery. High relative growth rate of seedlings subjected to moderate water stress was found to be strongly correlated with drought susceptibility.

Additional index words: ***Triticum aestivum*** **L., Drought resistance, Chlorophyll, Leaf firing, Relative growth rate, Water stress.**

WITH a few exceptions, crop plants accumulate free proline in their leaves when exposed to moderate to severe water stress (16). The physiological significance of this metabolic response to water deficit is, however, contentious.

In *Hordeum vulgare* L., for instance, the amount of accumulated proline in water-stressed leaves was shown to be correlated with yield stability over a wide range of environments (18), and it was suggested that proline-accumulating potential could serve as an index of drought resistance. This proposition appears to be tentative in the light of more recent studies which suggested that the extent of proline accumulation reflected the degree of internal water deficit as evidenced by the decline in leaf water potential (9,10). According to the latter studies, drought resistant genotypes should accumulate *less* proline than susceptible ones under identical stress conditions. In other studies (4,20) the extent of accumulation was not related to the degree of reduction in leaf water potential, even though genotypic differences for proline accumulation were significant.

Hanson *et al.* (10) further considered the build-up of free proline in water-stressed leaf tissue to be a deleterious consequence of internal water deficit, as supported by an apparent association between proline concentration in the tissue and the severity of post-stress "leaf-firing." Their hypothesis contradicted the claim that high proline content in the leaf during stress confers a survival value on that leaf (20), or that a high accumulator recovers more rapidly upon relief of stress (4).

The present study attempted to clarify the relationship between *in vitro* proline-accumulating potential and drought resistance in *Triticum aestivum* L. The cultivars chosen had previously been ranked for field drought

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resistance in Mexico (7) and their ratings provided the *ad hoc* basis for exploring possible associations between these characters and other measurable physiological responses to water stress.

MATERIALS AND METHODS

In vitro Stress of Excised Seedling Leaves

Seedlings were raised in PVC tube trays (Growing Systems Inc., Milwaukee, WI), one seedling per cell, using vermiculite rooting medium. Daily irrigation was supplemented with complete nutrients (Aquasol, Horico, Melbourne, 0.04% w/v) every 4th day. When 8 to 9 days old the primary leaf-blades (L1) were excised, weighed, and cut approximately into 2 cm segments. The pieces were floated on their adaxial surfaces on aerated polyethylene glycol (PEG) 6000 (Ajax, Sydney, 29.6% w/w, or approx.-11 bars), 10 ml per leaf-blade, contained in 55-mm plastic petri dishes. In the control group, distilled water was used in place of osmoticum. Stress was maintained for 3 days in a controlled environment with a 12-hour photoperiod, an ambient temperature of 25 C, 60% R.H., and light intensity of approx. 140 $\mu E\ cm^{-2} sec^{-1}$ at the petri dish surface. The leaf segments were dabbed dry with tissue paper and killed in liquid N. The second leaf-blades (L2) of the same seedlings were harvested 7 to 8 days after the first and stressed in the same procedure described above.

The experiment was split into four sowings with all 14 cultivars represented in each sowing by a different plant progeny of 15 seedlings, 10 of which were subjected to the stress treatment with the remainder constituting the control group. The positions of the petri dishes were completely randomized.

Chlorophyll Stability

Changes in chlorophyll contents of detached L1 and L2 segments floated on osmoticum or distilled water were studied in a separate experiment with three replications. In each replicate three L1 blades of 8-day-old seedlings were divided systematically into three subsamples of approximately 1 cm segments. Their fresh weights were determined. One subsample (control) was immediately killed in liquid N, while the remaining two were floated on either distilled water (senescence) or -11 bar PEG solution (senescence plus stress) under the experimental conditions described above in the stress experiment. The procedure was repeated for L2, harvested 7 days after the first. The first subsample provided an estimate of the cultivar's chlorophyll content, and chlorophyll loss was estimated from the relative difference between the second and third subsamples. Stability was expressed as 100% − chlorophyll loss %.

Leaf-Firing

Leaf-firing reactions (10) induced in intact two-leaf seedlings were studied in a separate sowing. Sowing was completely randomized over the tube tray with five replications, each comprising four seedlings per cell. Growing conditions were similar to those described in the stress experiment. Stress was abruptly induced 10 days after sowing by flooding each cell with 10 ml of PEG solution (40% w/w, or approximately -18 bars); a daily dose of 10 ml of the osmoticum was applied for the next 2 consecutive days. Stress was relieved on the 4th day by flushing each cell with excess water. Normal daily irrigation was observed thereafter until leaf reaction was scored 5 days after relief of stress. Prior to stress relief one seedling (L1 + L2) per replicate was harvested for proline assay. Proline was expressed on per g "wilted fresh weight" basis with the accompanying assumption that relative water content in the semi-wilted state was similar for all cultivars.

Growth Rates

Relative growth rates (RGR) exhibited by stressed and unstressed seedlings were compared in an experiment with five replications of three seedlings each. A replicate was represented by a tube-tray ("block") divided column-wise into four "plots" each of 14 cells ("subplots") sown to as many cultivars. Sowing was completely randomized within the plot, as were the plots within the block.

At the start of the experiment, one plot per block was harvested 10 days after sowing (Day 0) to determine the initial dry weight (DW) of shoots plus roots. Three treatments were imposed thereafter: 1) daily irrigation (unstressed control); 2) irrigation only every 3rd day (moderate stress); and 3) irrigation only every 6th day (severe stress). The second and final harvest was made of all plots 12 days after Day 0. Natural log transformation was applied on the DW data.

The ratio(s) of RGR exhibited in the stress treatment(s) to that of control was assumed to be a measure of the cultivar's relative capacity to recover from stress.

Chemical Analyses

Free amino acids and sugars were extracted by grinding the leaf tissue in a mortar and pestle with 3% 5'sulfosalicyclic acid in an approx. ratio of 1 ml extractant : 25 mg fresh weight of tissue (minimum : 2 ml). The homogenate was filtered through Whatman #2 filter paper.

A suitable duplicated aliquot of the filtrate was assayed for proline according to the modified acid ninhydrin method of Bates *et al.* (3). It was not essential to extract the chromophore in toluene, and absorbance was read at 515 nm in an automated-sampling spectrophotometer (Hitachi Model 101). Total amino acids were assayed by the ninhydrin method of Yemm and Cocking (23) with glycine as standard; the absorbance (at 570 nm) for proline was only about 25% those of equimolar solutions of other common amino acid (see Yemm and Cocking, 23). Total sugar was spectrophotometrically determined at 480 nm after reaction with indole reagent (2) with sucrose as standard.

In chlorophyll assay the tissue was finely cut into 1 to 2 mm segments and leached in 80% aqueous acetone at 4 C in the dark for 3 days. Absorbance was read at 652 nm (1) from which total chlorophyll extracted, expressed as mg per g fr. wt. tissue, was calculated according to the formula:

Statistical Analysis

Angular transformation (Arcsine $\sqrt{\ }$) was made when the original data were expressed as percentages.

Simple correlation coefficients were computed for proline, total amino acids, and total sugars within plants over families, and those among physiological characters were computed from the means of the 14 cultivars.

RESULTS AND DISCUSSION

In Vitro Water Stress

Proline accumulated in the leaf tissue after about 24 hours, and accumulation continued for up to 6 days by which time the tissue was generally chlorotic. With a few exceptions, the second leaf (L2) consistently accumulated more proline than the primary leaf (L1) (Table 1), and this consistency was also indicated in the nonsignificance of

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DIS file ARDA

Date: 12 Mar 84

FROM: S&T/AGR/RNR, Lloyd R. Frederick

SUBJECT: Reprints of Articles with Partial Support of AID Contracts/Grants.

TO: S&T/DIU, ~~Natalie Thompson~~ Lida Allen

Attached are four (4) copies of the following article(s) with the supporting grant/contract number(s)

S&T/A (1) Hohenberg, J.S., D.N. Munns and C.L. Tucker. 1982 Rhizobium - Host Specificities in *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. Crop Science 22: 455-459

Grant/Contract Number AID/ta-C-1207 (NifTAL - U. Hawaii)

Attachments: a/s

S&T/A 180 (2) Uratsu, S.L., H.H. Keyser, D.F. Weber and S.T. Lim. 1982 Hydrogen Uptake (HUP) Activity of *Rhizobium japonicum* from Major U.S. Soybean Production Areas. Crop Science 22: 600-602. (USDA RSSA 4-76) (Beltsville World Rhizobium)

S&T/A (3) Graham, Richard A. and Alfred L. Donawa. 1982. Greenhouse and Field Evaluation of *Rhizobium* strains nodulating groundnut (*Arachis hypogaea* L.). Tropical Agriculture 59: 254-256. (USDA PASA AG/TAB 610-9-76 subgrant 59-2361-012-1-5-012-0 (Cornell Univ.) (Limiting Factors)

cc: T. Gill
A. Bertrand

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