Genetic Linkage Between Isozyme, Morphological, and DNA Markers in Tepary Bean

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A genetic map of tepary bean (*Phaseolus acutifolius* A. Gray) may be useful to plant breeders attempting to transfer desirable genes from this species to other *Phaseolus* species. In order to expand the genetic information available for tepary bean, the inheritance of and linkage relationships among 23 morphological, isozyme, and RFLP markers were determined. All but one of the characters segregated in a monogenic fashion, and low levels of segregation distortion were observed. New two-locus linkages identified included *Aat-2*/*Gpi-c2*, *Aco-2*/*Dia-3*, and *Dia-3*/*Idh-x*. Nine of the 23 loci exhibited linkage to other loci analyzed and could be assigned to one of three distinct linkage groups. Two tepary bean linkage groups (*Adh-1*/*Aat-2*, *Aco-2*/*Dia-3*) appear to be conserved in common bean, although the linkage estimates for *Adh-1*/*Aat-2* are dramatically different in these species. The *Adh-1*/*Aat-2* linkage also appears to be conserved in lentil and pea. Additionally, the *Gpi-c1*/*Pgd-3* linkage has a possible counterpart in soybean, and the *Fdh-1*/*Gpi-c1* linkage in tepary bean is maintained in chickpea.

Plant breeders have long recognized that exotic germplasm may serve as a source of valuable genes with which to enhance crop performance. Common bean (*Phaseolus vulgaris* L.) breeders have identified the tepary bean, *Phaseolus acutifolius* A. Gray, as a source of genes which might improve the drought and heat tolerance of common bean (Thomas et al. 1983). The tepary bean consists of two distinct varieties (var. *acutifolius* and *tenuifolius*) distinguished by differences in leaflet morphology. Domesticated landraces of tepary bean also exist. This species is extraordinarily well adapted for growth in hot, dry climates, reportedly tolerating ground temperatures as high as 53°C (Nabhan et al. 1981). In addition to drought and heat tolerance, the tepary bean also has attracted the attention of plant breeders because of its resistance to bacterial diseases and other pests (e.g., Coyne and Schuster 1973; Omwega et al. 1989; Shade et al. 1987). Several attempts to transfer genes from tepary to common bean have been made, with limited success (Waines et al. 1988).

Genetic maps are currently available for a number of legumes, including common bean (Nodari et al. 1993; Vallejos et al. 1992), soybean (*Glycine* spp.) (Keim et al. 1990), cowpea (*Vigna unguiculata*), and mung bean (*Vigna radiata*) (Fatokun et al. 1992). Although most of these maps have been generated only recently, they already have proven useful for identifying molecular markers for commercially important monogenic traits (Diers et al. 1992) and for analyzing the genetic basis of more complex characters (Fatokun et al. 1992; Keim et al. 1990).

A tepary bean genetic linkage map may be useful in an interspecific bean breeding program involving this species, both for selection of desired genes and for elimination of deleterious genetic material (Tanksley and Rick 1980). However, to date there is a paucity of genetic information available for this potentially useful crop. To begin to fill this void and build a framework for future gene mapping in tepary bean, we report here the inheritance and linkage relationships of 23 characters in tepary bean, including 14 new isozyme, morphological, and DNA marker loci.

Materials and Methods

Plant Material

Seed samples were obtained from Dr. J. G. Waines (University of California, Riverside). Accessions were originally from the University of California, Riverside legume collection, the Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia), or the United States Department of Agriculture (USDA, Pullman, Washing-

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Table 1. Tepary bean parents used to generate segregating progenies

Accession	Origin	Classification
L177	Sonora, Mexico	wild *acutifolius*
L320	Arizona, U.S.A.	wild *tenuifolius*
L474	Arizona, U.S.A.	wild *tenuifolius*
L556	Jalisco, Mexico	wild *tenuifolius*
L581	Sonora, Mexico	*acutifolius*[a]
G40100	Durango, Mexico	wild *tenuifolius*
G40115	U.S.A.	wild *tenuifolius*
PI201268	Chiapas, Mexico	*acutifolius*[a]
PI319443	Sonora, Mexico	*acutifolius*[a]

[a] Domesticated landraces.

ton). Designations for each are reflected by the prefix L, G, or PI, respectively, in the name of the accession. Plants from selected accessions (Table 1) were crossed to generate F_1 hybrids. F_1 hybrids were subsequently grown to maturity and allowed to self-pollinate to generate F_2 populations. Some F_2 populations were grown to maturity to obtain F_3 seed. Between 40 and 58 individuals from six different populations were used in our analysis.

Data Collection

Three morphological characters were scored for segregation in F_2 families. The first, stem anthocyanin (SAN), was scored on F_2 plants with fully expanded primary leaves. Individuals were scored for the presence or absence of red coloration on the stem. The second morphological character scored, leaf anthocyanin (LAN), was scored on F_2 plants at the same stage as for stem anthocyanin. The positive LAN phenotype is characterized by the presence of a distinct red hue on the abaxial surface of the primary leaves, while the alternate phenotype is the absence of such coloration. The third morphological character scored was pod dehiscence (DPO). This trait was scored for segregation using pods from individual F_2 plants. Scoring was based upon the extent to which pod valves curled along their axis when dehisced, with positive dehiscence characterized by at least 360° of curling in the valves after pods were split open.

Isozyme polymorphisms were scored for segregation primarily in F_2 populations. However, the F_3 generation was used to infer F_2 genotypes in a few cases. Imbibed seeds and leaf tissue were prepared for starch gel analysis by grinding in either a 0.1 M potassium phosphate buffer, pH 7.0, containing 10% PVP, 10% glycerol, and 0.1% mercaptoethanol, or a 0.05 M Tris maleate buffer, pH 8.0, containing 0.1% mercaptoethanol and 0.2% Triton X-100. Paper wicks were dipped in the sample slurry and applied to a vertical slice in the gel. Two starch gel systems were used. The first was a histidine citrate gel, pH 6.5 (Stuber et al. 1977). The second was a discontinuous Tris citrate/lithium borate system, pH 8.1 (Selander et al. 1971). The histidine citrate gel was run for 4–5 h at 275 volts, whereas the Tris citrate/lithium borate system was run for 5–6 h at 35 milliamps (275 volts maximum). Gel electrophoresis was conducted at 4°C.

After electrophoresis, gels were sliced horizontally, and slices were stained for enzyme activity at 37°C. The enzyme systems analyzed included aspartate aminotransferase (AAT), aconitase (ACO), alcohol dehydrogenase (ADH), diaphorase (DIA), alpha esterase (EST), formate dehydrogenase (FDH), glucosephosphate isomerase (GPI), isocitrate dehydrogenase (IDH), *N*-acetyl glucosaminidase (NAG), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SKD), and triosephosphate isomerase (TPI). Systems analyzed on the histidine citrate system included ACO, ADH, DIA, FDH, IDH, and PGD. The enzymes scored on the Tris citrate/lithium borate system included AAT, EST, GPI, NAG, SKD, and TPI. PGM was resolvable in both systems. Imbibed seed extracts were used to analyze ACO, ADH, DIA, EST, FDH, GPI, IDH, PGD, PGM, and SKD. Leaf tissue was used to score AAT, NAG, and TPI. In a few instances, enzymes normally scored in seed tissue were resolved in leaf tissue (DIA, GPI, PGD, SKD).

Recipes used for ACO, ADH, FDH, IDH, PGD, and SKD were essentially those reported by Garvin et al. (1989). NAG was stained with the assay of Weeden (1986). AAT, EST, and TPI were stained according to Wendel and Weeden (1989), with minor differences in component concentrations and pH of buffers. GPI and PGM were stained using modifications of recipes presented by Ignart and Weeden (1984). DIA was stained according to Wendel and Weeden (1989), or with an assay containing 0.1 M potassium phosphate, pH 7.0, 0.2 mg/ml MTT, 0.6 mg/ml menadione, and 0.2 mg/ml NADH.

The subcellular location of AAT, PGD, PGM, and TPI isozymes was examined as follows: 3-week-old plants of the tepary bean accessions L320 and PI319443 were placed in the dark for 3 days. Leaves from each accession were then harvested and ground with ice cold extraction buffer (0.01 M Tricine, pH 7.9, 0.3 M sorbitol, 1% Ficoll, 1 mM isoascorbic acid) in a blender, using five 1-s bursts. The slurry was filtered through several layers of cheesecloth, and centrifuged for 30 s at 500 *g* to remove nuclei and cellular debris. The supernatant was sequentially centrifuged for 1, 3, and 10 min at 1,000, 3,000, and 10,000 *g*, respectively. Pellets were lysed with 0.05 M Tris-HCl, pH 7.5, with 0.01% mercaptoethanol, and analyzed by starch gel electrophoresis along with crude leaf extracts. The marker enzymes for the subcellular compartments were the plastid and cytosolic GPI isozymes (Weeden et al. 1989).

The inheritance of the 45S ribosomal repeat was examined by RFLP analysis. DNA was extracted from F_2 plants and parents, using the procedure of Doyle and Doyle (1987). Approximately 5 μg of DNA from each parent and F_2 individual was digested with the restriction enzyme *Xbal*, separated by agarose gel electrophoresis, and transferred to nylon membrane (Genescreen Plus, Du Pont). Cloned ribosomal DNA from soybean (provided by Dr. J. J. Doyle, Cornell University), was ^{32}P-labeled using the method of Feinberg and Vogelstein (1984). Hybridization between membranes and probe DNA was conducted overnight at 65°C in essentially the same buffer described elsewhere (Bernatsky and Tanksley 1986). Filters were washed at 65°C with 2 × SSC/0.1% SDS before autoradiography.

Inheritance and Linkage Analysis

Segregation data obtained for all the characters scored were tested for goodness-of-fit to a monogenic segregation model using the χ^2 statistic ($\alpha = 0.05$). Morphological characters and two isozymes (DIA-1, DIA-2) were tested against a 3:1 expected segregation ratio. The remaining isozymes and the ribosomal RFLP data were tested against an expected segregation ratio of 1:2:1.

Contingency χ^2 analysis was used to reveal possible linkage between loci segregating in a monogenic fashion. Maximum likelihood linkage estimates and their associated standard errors were generated for pairs of loci that exhibited significant contingency χ^2 values ($P < .05$). Linkage was rejected if a significant χ^2 was not detected for a given locus pair in all populations testing the linkage, or if the sum of the linkage estimate and two standard errors approximated or exceeded 50% recombination.

All inheritance and linkage analyses were conducted with the Macintosh format LINKAGE-1 program (Suiter et al. 1983).

Results

Characterization of Enzyme Systems

Subcellular localization. The relative intensities of the GPI isozyme bands in the 1,000 *g* and 3,000 *g* pellets did not differ markedly from those observed in the raw leaf extract. These pellets should have contained the majority of intact chloroplasts in the preparation and therefore should have shown enrichment for the plastid isozyme relative to the cytosolic GPI isozymes. As the "chloroplast" pellet did not exhibit enrichment for the plastid marker enzyme, we were not able to confidently identify the isozyme compartmentalization for AAT, PGM, PGD, or TPI.

Inheritance of new isozymes. Aspartate aminotransferase: Parents examined for AAT activity consistently exhibited three zones of activity, AAT-1, AAT-2, and AAT-3. Segregation for both AAT-1 and AAT-2 was consistent with monogenic control (Table 2). The genes encoding these two isozymes are designated *Aat-1* and *Aat-2*. Further, both isozymes appear to be dimeric, since we observed heterodimers in heterozygous individuals.

Diaphorase: Gels stained for DIA revealed a complex banding pattern. Variable numbers of bands were resolved on the anodal slice, and approximately half of the tepary accessions also possessed a strongly-staining cathodal band. Segregation analysis revealed that this cathodal zone was allelic to the least mobile anodal zone of activity found in accessions lacking the cathodal zone (Table 2). These two zones were designated allozymes of the locus *Dia-3*. Two zones in the anodal slice, DIA-1 and DIA-2, also appeared to segregate. These polymorphisms were scored as dominant traits because heterozygotes were sometimes difficult to distinguish from homozygotes. Genetic analysis of DIA-2 segregation ratios revealed simple monogenic control of this isozyme. We designated this gene *Dia-2* (Table 2). In the progeny L474 × L581, segregation was observed for DIA-1, a zone of activity that migrated more rapidly than other DIA zones. The χ^2 analysis of segregation data obtained for DIA-1 rejected a monogenic hypothesis (Table 2).

Esterase: A rapidly developing zone of EST activity that migrated to the cathodal region of gels was detected in tepary bean. Segregation for this zone, EST-C, was analyzed in three F_2 families. In all three, segregation appeared to be monogenic and codominant (Table 2). The gene encoding EST-C was designated *Est-c* (cathodal).

Table 2. Single locus goodness of fit tests for characters segregating in tepary bean populations

F_2 family	Character	Number with observed phenotype: Presence or fast	Heterozygous	Absence or slow	χ^2
L177 × L556	AAT-2	9	25	5	3.92
	DIA-3	10	16	13	1.72
	EST-C	10	20	9	0.08
	FDH-1	10	20	10	0.00
	GPI-c1	9	18	12	0.69
	GPI-c2	4	29	7	8.55*
	PGD-1	9	18	13	1.20
	PGD-3	9	23	7	1.46
	rDNA	5	21	13	3.51
	SKD	8	18	13	1.51
PI319443 × G40115	AAT-1	9	20	15	2.00
	ACO-2	13	17	15	2.87
	ADH-1	6	23	15	3.77
	SAN	33	—	11	0.00
	DIA-3	11	25	8	1.23
	EST-C	10	21	7	0.89
	GPI-c1	6	23	9	2.16
	GPI-c2	15	23	6	3.77
	NAG	4	28	11	6.21*
	PGM-1	10	24	10	0.36
PI201268 × L556	AAT-1	8	19	13	1.35
	AAT-2	11	19	10	0.15
	ACO-2	12	22	12	0.09
	DIA-3	10	25	11	0.39
	FDH-1	15	26	5	5.13
	GPI-c2	16	24	6	4.43
PI201268 × L320	AAT-1	15	33	10	1.96
	ACO-2	13	32	13	0.62
	ADH-1	12	28	18	1.31
	DIA-2	44	—	11	0.73
	DIA-3	11	31	16	1.14
	DPO	33	—	19	3.69
	EST-C	20	27	11	3.07
	GPI-c1	13	27	16	0.39
	GPI-c2	18	28	12	1.31
	IDH-X	10	34	12	2.71
	NAG	13	28	17	0.62
	PGD-1	9	27	19	3.65
	PGD-3	12	30	14	0.43
	rDNA	5	30	16	6.33*
	SKD	14	32	12	0.76
L474 × L581	AAT-1	12	17	11	0.95
	ACO-2	14	21	5	4.15
	ADH-1	9	21	10	0.15
	DIA-1	16	—	24	26.1***
	FDH-1	9	7	24	28.1***
	GPI-c1	19	11	10	12.1***
	GPI-c2	10	21	9	0.15
	SKD	13	22	5	3.60
	TPI-1	8	19	13	1.35
G40100 × G40115	AAT-1	8	21	11	0.55
	ADH-1	8	27	5	5.35
	DIA-3	10	20	10	0.00
	GPI-c1	7	27	6	4.95
	GPI-c2	5	27	8	5.35
	NAG	9	19	12	0.55
	PGM-1	8	21	11	0.55
	PGM-2	10	21	9	0.15
	LAN	31	—	9	0.13

* $P < .05$.
*** $P < .001$.

N-acetyl glucosaminidase: The fluorescent assay used to detect NAG resolved one anodal zone of activity in parental tissues which segregated as a codominant trait in two of the three of the populations tested (Table 2). In the other population, the segregation ratio deviated significantly from the expected ratio. The locus encoding NAG activity was designated *Nag*.

6-Phosphogluconate dehydrogenase: All tepary beans exhibited at least two zones of PGD activity. We were able to resolve three zones of activity in certain parents used to generate segregating populations. The additional zone resolved in these parents was more anodal to the other zones, and was denoted PGD-1. The zone of intermediate mobility, formerly known as 6PGD-1 (Garvin et al. 1989), was designated PGD-2, and the least mobile zone of

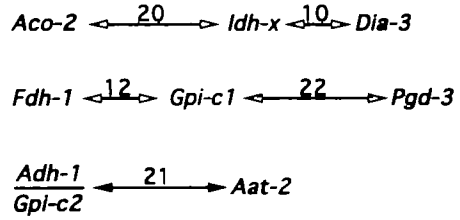

Figure 1. Defined linkage groups in tepary bean

PGD activity, formerly referred to as 6PGD-2, was designated PGD-3 (Garvin et al. 1989). Segregation for PGD-1 was detected in two families, and was not significantly different from that expected for a codominant trait controlled by a single gene (Table 2). Apparently, the alternative allele of *Pgd-1* encodes an allozyme which comigrates with PGD-2. We inferred this from segregation patterns for PGD-1, which revealed that heterozygotes possessed a heterodimer of intermediate mobility between PGD-1 and PGD-2. This heterodimer is absent from individuals homozygous for the allele encoding the fast PGD-1 allozyme.

Phosphoglucomutase: Two of our crosses involved a parent possessing a rare slow mobility variant of PGM-1, the more mobile of the two PGM isozymes. In both progenies segregating for PGM-1, a 1:2:1 ratio was observed for parental and hybrid phenotypes (Table 2). PGM-1 is encoded by a locus designated *Pgm-1*.

Triosephosphate isomerase: All tepary parents analyzed possessed two distinct zones of TPI activity. The two zones were labeled TPI-1 and TPI-2 to reflect relative mobility during electrophoresis, with TPI-1 migrating more rapidly than TPI-2. A rare alternate form of TPI-1 that migrated more slowly than TPI-2 was also identified in a few accessions, including L474. In L474 × L581, the lone progeny segregating for TPI-1, the pattern of segregation indicated that TPI-1 is encoded by a single nuclear gene designated *Tpi-1* (Table 2).

Inheritance of Previously Described Isozymes

Our analysis of nine previously characterized isozymes (Garvin et al. 1989), including ACO-2, ADH-1, FDH-1, GPI-c1, GPI-c2, IDH-X, PGD-3, PGM-2, and SKD, confirmed that all are encoded by single discreet genes (Table 2). Gene designations used for the genes encoding these isozymes are consistent with previous research (Garvin et al. 1989), with the exception of genes encoding PGD isozymes, as discussed earlier.

Inheritance of Morphological Characters

Morphological characters included in this study were scored in separate F_2 populations. The segregation of SAN and LAN, the two anthocyanin characters, each fit ratios expected from single dominant genetic characters (Table 2). In both cases, the presence of anthocyanin was dominant to the absence of the pigment. Pod dehiscence (DPO) also exhibited a segregation pattern consistent with a monogenic model, with positive dehiscence dominant to the lack of dehiscence (Table 2). Loci encoding these morphological characters were designated *San, Lan,* and *Dpo,* respectively.

Inheritance of the 45S Ribosomal Repeat

The inheritance of the 45S ribosomal repeat (rDNA) was examined in two progenies. The soybean rDNA probe cross-hybridized with fragments of ~9.0 and 2.0 kb in *Xba*I-digested DNA from each parent, with distinct size polymorphism detected for each fragment among the parents. In segregating populations, the two fragments cosegregated completely with each other, indicating that they are located at the same locus. Codominant monogenic inheritance of the ribosomal repeat, *rDNA,* was suggested by segregation ratios, though segregation distortion for rDNA was observed in one of the two families analyzed (Table 2).

Linkage

Pairwise linkage tests between 229 two-locus pairs were conducted, and several linkages were detected (Table 3). Linkages involving new loci included *Aat-2/Gpic-2, Aco-2/Dia-3,* and *Dia-3/Idh-x.* We also detected the weak potential linkages *Aat-1/Aat-2, Idh-x/Nag,* and *Nag/Lan.* In addition, several linkages which had previously been reported or suspected (Garvin et al. 1989) were confirmed, including *Aco-2/Idh-x, Adh-1/Gpi-c2, Fdh-1/Gpi-c1, Fdh-1/Pgd-3,* and *Gpi-c1/Pgd-3* (Table 3). Ultimately, we were able to organize all linked loci into three distinct linkage groups, and were also able to determine gene orders for these linkage groups. The first linkage group consisted of *Aco-2/Idh-x/Dia-3,* the second included the loci *Adh-1/Gpi-c2/Aat-2,* and the third consisted of the loci *Fdh-1/Gpi-c1/Pgd-3* (Figure 1).

The linkage detected between the loci *Idh-x* and *Nag* was ultimately discounted since *Nag* was not linked to either *Aco-2* or *Dia-3,* loci which flank *Idh-x.*

Discussion

Segregation Analysis

Genetic analysis of six tepary bean F_2 families identified 13 new polymorphic loci, including three controlling morphological characters, nine isozyme loci, and the 45S ribosomal array. A 10th polymorphic isozyme was identified in the analysis, but this isozyme did not display the expected monogenic ratio and requires further study before its genetic basis is determined. In addition, the monogenic inheritance of nine previously described isozymes (Garvin et al. 1989) was confirmed.

A relatively low level of segregation distortion was observed in our study; only ~10% of the loci exhibited significant skewing as revealed by the χ^2 tests for monogenic segregation. This estimate is similar to results commonly observed in intragenic analyses (Zamir and Tadmor 1986). Sampling error rather than a biological cause was the apparent basis of the observed segregation distortion, because in no case was segregation distortion for a particular locus observed in more than one segregating population. In all crosses examined, the hybrid and subsequent generations appeared to be as vigorous as either parent and were fully fertile. Thus, intraspecific incompatibilities do not appear to be as important a problem in tepary bean as in crosses among common bean varieties, particularly when common bean parents are from different geographic origins (Gepts and Bliss 1985).

Linkage

Theoretically, linkage analysis of 195 two-locus pairs could be conducted among the 13 new loci described here, and between these loci and the nine previously described isozyme loci included in our analysis. Because of the lack of appropriate crosses to test linkage between rare alleles and the exclusion of some pairwise comparisons due to segregation distortion, linkage analysis was completed for only two-thirds of the possible combinations, though in some cases we were able to infer linkage relationships because of tests with flanking loci. Even so, of the 18 isozyme loci that were used in the linkage analysis, nine could be assembled into three separate linkage groups (Figure 1). This clustering of 50% of the isozyme loci is somewhat surprising considering that tepary bean has 11 chromosome pairs.

Several studies have examined synteny in related species with molecular markers (e.g., Bonierbale et al. 1988; Hulbert et al.

Table 3. Linkage estimates for locus pairs deviating from independent segregation

Locus pair	F_2 family	χ^2 [b]	Progeny scores[a]				Recombination fraction ± SE
			NR	SR	DR	DH	
Aat-1/Aat-2[c]	PI201268 × L556	12.43*	10	14	4	12	0.34 (0.07)
Aat-2/Gpi-c2	PI201268 × L556	24.68***	13	11	2	14	0.21 (0.05)
Aco-2/Dia-3	PI319443 × G40115	16.84**	13	16	2	13	0.26 (0.06)
	PI201268 × L556	33.71***	16	11	1	18	0.15 (0.04)
	PI201268 × L320	19 55**	14	19	3	22	0.26 (0.05)
Aco-2/Idh-x	PI201268 × L320	24.00***	14	18	1	23	0.20 (0.04)
Adh-1/Gpi-c2	L474 × L581	80.00***	19	0	0	21	0.037[d]
	G40100 × G40115	80.00***	13	0	0	27	0.037[d]
	PI319443 × G40115	88.00***	21	0	0	23	0.033[d]
	PI210128 × L320	116.00***	30	0	0	28	0.025[d]
Dia-3/Idh-x	PI201268 × L320	55.52***	18	11	0	27	0.10 (0.03)
Fdh-1/Gpi-c1	L177 × L556	35.53***	16	9	0	14	0.12 (0.04)
Fdh-1/Pgd-3	L177 × L556	11.38*	8	18	1	12	0.31 (0.06)
Gpi-c1/Pgd-3	L177 × L556	19.84**	11	15	0	13	0.21 (0.05)
	PI201268 × L320	21.20***	16	19	2	19	0.23 (0.05)
Nag/Lan[c]	G40100 × G40115	9.15*	5	4	0	(31)[e]	0.22 (0.07)

[a] Four classes of genotypes used to generate maximum likelihood estimates of linkage. NR, SR, DR, and DH = the nonrecombinant parental genotype, single recombinant, double recombinant, and double heterozygote classes of F_2 individuals, respectively.

[b] Contingency χ^2 values * $P < .05$; ** $P < .01$; *** $P < .001$.

[c] To be considered tentative linkages until further evidence is available.

[d] Theoretical maximum recombination frequency, estimated with the formula of Hanson (1959).

[e] Individuals for which genotypes could not be determined, potentially including NR, SR, DR, and DH progeny genotypes.

1990). Isozyme information has been similarly employed (Kazan et al. 1993; Weeden et al. 1992). Though there is limited isozyme linkage information available for common bean, comparisons of linkage involving orthologous isozyme loci in tepary and common bean did reveal two cases of synteny. Two isozyme linkage groups were reported by Vallejos and Chase (1991), *Aco-2/Dia-1* and *Adh-1/Got-2* (*Aat-2*), and are likely orthologous to the *Aco-2/Dia-3* and *Adh-1/Aat-2* linkages detected in tepary bean. Linkage estimates for the *Aco-Dia* linkage, ~20 cM, were similar in both common and tepary bean. In contrast, the 2 cM linkage estimate between *Adh-1* and *Got-2* (*Aat-2*) in common bean (Vallejos and Chase 1991) is an order of magnitude smaller than the *Adh-1/Aat-2* linkage in tepary bean. A genome-wide picture of the extent of synteny between these species should be revealed by inclusion of molecular markers from the common bean molecular maps (Nodari et al. 1993; Vallejos et al. 1992) in further tepary bean gene mapping studies. Such information should be useful for assessing the feasibility of transferring tepary bean genes to common bean by interspecific hybridization.

Several of the isozyme linkages in tepary bean appear to be conserved in other legume genera as well. For instance, linkage between *Got-3* and *Adh* has been detected in lentil (Zamir and Ladizinsky 1984), and in pea *Aat-c* and *Adh-1* are known to be on the same linkage group (Weeden et al. 1993). These linkages are likely orthologous to the tepary bean linkage between *Gpi-c2* (and by extension *Adh-1*) and *Aat-2*. In lentil, these loci were found to be separated by 21 cM (Zamir and Ladizinsky 1984), as was the case in tepary bean. In chickpea, the loci *Fdh* and *Gpi-c* are separated by 7 cM (Gaur and Slinkard 1990). Our study also detected linkage between *Fdh-1* and *Gpi-c1* in tepary bean, and found that these loci are separated by 12 cM. Lastly, the 22-cM *Gpi-c1/Pgd-3* linkage of tepary bean may be orthologous to the *Gpi-1/Pgd-1* linkage (16 cM) reported in soybean (Palmer and Hedges 1993).

The conserved linkages we identified between *Phaseolus* and other legume genera are interesting from an evolutionary standpoint, for all involve a gene encoding cytosolic GPI, which has been shown to have been duplicated in the legume subfamily Papilionoideae (Weeden et al. 1989). Subsequent silencing of one of the duplicate loci has occurred in pea, lentil, chickpea, and a variety of other papilionoid legumes. Our result of linkage between *Fdh-1* and *Gpi-c1* in tepary bean suggests that the silenced *Gpi-c* in chickpea is orthologous to the tepary bean *Gpi-c2* gene and that this *Gpi-c* pseudogene may be located near *Adh-1*.

To date, few of the isozymes or other markers analyzed here have been incorporated into either of the molecular common bean linkage maps (Nodari et al. 1993; Vallejos et al. 1992). If the level of isozyme polymorphism we observed in tepary bean correlates with the level of RFLP polymorphism, it should be feasible to incorporate the available common bean molecular markers into a tepary bean map in order to create a composite *Phaseolus* genetic map that includes both molecular markers from common bean and many isozyme marker loci from tepary bean. Such a map should be useful for interspecific bean breeding programs involving the tepary bean.

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