Theor Appl Genet (1987) 73:410–418

The inheritance of a urease-null trait in soybeans*

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Received August 1, 1986; Accepted August 20, 1986
Communicated by A. L. Kahler

Summary. Four soybean seed urease nulls (lacking both the activity and antigen of the embryo-specific urease) were intermated and the F_1 and F_2 seed examined for urease activity. Both generations were without urease activity, and the nulls were therefore considered non-complementing. In crosses of each null line to cultivars homozygous for the allelic, codominantly inherited urease slow or fast isozyme, the F_1 seed expressed the embryo-specific urease isozyme of the urease-expressing parent. A 3 : 1 segregation for presence and absence of urease was observed in progeny from F_1 and heterozygous F_2 plants. The F_2 and F_3 from fast × null combinations revealed that urease-positive seed were all phenotypically urease fast, while the same seed from slow × null combinations showed a segregation of one seed containing a fast urease, either exclusively or in a heterozygous state with the slow isozyme, for every 69 phenotypic slows. Data pooled from F_2 plants which segregate for both the presence (*Sun*) and absence (*sun*) of urease and for the fast (*Eu*1-b) or slow (*Eu*1-a) urease allele indicate that the null lesion (*sun*) is linked to *Eu*1 by approximately one map unit. The evidence is consistent with two models: (1) *sun* is an allele at the embryo-specific urease isozyme locus (*Eu*1) and that a high degree of exchange (and/or conversion) within the locus results in a 1% recombination frequency between the null trait and urease allozyme; (2) *sun* is at a distinct locus which is separated by one map unit from the embryo-specific urease isozyme locus (*Eu*1) upon which it acts in the cis position. Polyadenylated embryo RNA from one of the null lines, PI 229324, exhibited no urease template activity in vitro. Thus, the lack of urease antigen is due to lack of accumulation of translatable urease mRNA. The availability of soybeans lacking seed urease should be extremely useful to breeders as a trait for linkage studies and to geneticists as a transformation marker.

Key words: *Glycine max* (L.) Merr. – Linkage – Recombination – Urease activity – Urease antigen – Urease mRNA – *Sun* – *Eu*1

Introduction

Many members of the Leguminosae have an abundant seed urease (EC 3.5.1.5). In the soybean [*Glycine max* (L.) Merr.] the enzyme represents 0.2% to 0.4% of the extractable seed protein (Polacco and Havir 1979; Winkler et al. 1983) and is synthesized during the same period that the bulk of the seed protein is deposited (Polacco and Sparks 1982). Urease antigen is detectable 11 days after pollination, reaches a peak 20 days after pollination, and declines over the next 30 days to a level in mature seeds which is 50% of the peak value (as percent total protein) (Polacco and Sparks 1982). Urease activity increases continually from the eleventh day after pollination, but lags behind the antigen level until the 20th day (Polacco and Sparks 1982).

Polacco et al. (1982) identified a line, PI 229324 (Itachi), which lacked seed urease activity normally detectable by colorimetric assay. Further characterization of this null by

* Portions of this work were funded by the Illinois and Missouri Agricultural Experiment Stations, the SOHIO-University of Illinois Center of Excellence in Crop Molecular Genetics and Genetic Engineering and by grants PCM-8219652 from the National Science Foundation and USDA/SEA-CRCR-1-1374 from the USDA Competitive Grants Office

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rocket immunoelectrophoresis revealed the lack of seed urease antigen (Polacco et al. 1982). Three additional null lines have been identified and shown to be phenotypically similar to PI 229324 (Kloth et al. 1984).

A physiological study of PI 229324 has shown that callus derived from cotyledon, shoot tip, or root tip has a ureolytic activity level comparable to callus derived from lines which express a typical level of seed urease (Polacco et al. 1982). The thermal stability, immunological properties and hydroxyurea sensitivity of urease from callus-derived suspension culture were similar in the seed urease expressing and seed urease-null lines (Polacco et al. 1982; Polacco and Winkler 1984).

Extracts of urease-null seeds (PI 229324) exhibit a small amount of urease activity when assayed with radiolabelled urea (Polacco et al. 1982). This residual urease activity in the seed of the null and the activity in suspension cultures of both null and standard varieties represent a second urease that resembles the leaf urease more than the abundant seed urease (Kerr et al. 1983; Polacco and Winkler 1984). The abundant seed urease was termed embryo-specific by Polacco et al. (1985), while the seemingly identical ureases of leaf, seed coat, suspension culture and of urease-null embryos are collectively termed the ubiquitous urease.

Polacco and Havir (1979) found that the embryo-specific urease can exist in at least two states of aggregation. They concluded that the electrophoretically slow form of the embryo-specific urease is a hexameric aggregate of 93,500 dalton subunits and that active fast, trimeric, aggregate can be derived in vitro from the hexamer.

The degree of aggregation is also genetically controlled. Buttery and Buzzell (1971) first described two heritable seed (embryo-specific) urease isozyme differentiated by their relative mobility in polyacrylamide gels, the "fast" isozyme being half the size of the "slow" isozyme. Kloth and Hymowitz (1985) demonstrated that the fast and slow embryo-specific ureases are inherited as codominant alleles and assigned the term *Eu*1-a to the electrophoretically slow allele and *Eu*1-b to the electrophoretically fast allele.

The objectives of this study were to understand the inheritance of the embryo-specific urease null and its relationship to the alleles of the *Eu*1 locus.

Materials and methods

Biological material

Adapted cultivars and introduced soybean lines used in this study were obtained from the United States Department of Agriculture Northern (Urbana, IL) and Southern (Stoneville, MS) Soybean Germplasm Collections and are described in Table 1.

The identification of the line PI 229324 (Itachi) as a seed urease-null has been described by Polacco et al. (1982). Three additional urease-null lines have been isolated by Polacco and coworkers (Table 1). All four seed urease nulls originate from Japan. To determine their allelism, the four null lines were intermated.

Unless stated otherwise, F_1 plants were pot-grown in a greenhouse or, during the summer, in a sheltered location outdoors. The F_2 populations from the crosses of the maturity group III cultivars were field-grown at Urbana, Illinois. The crosses of the maturity group V cultivars and lines were pot-grown.

Urease assays and electrophoretic analysis

F_2 seeds were analyzed for embryo-specific urease variants by using a small chip from each seed. As described below, the presence of urease was determined directly on the chip and the electrophoretic form was determined from chip extracts. Such non-destructive sampling made it possible to plant the F_2 seeds in field plots according to cross and presumed urease genotype after gel electrophoresis.

To detect embryo-specific urease isozymes, whole seed or seed chips (removed from opposite the hilum with a razor blade) were crushed with a pliers and homogenized 5 s with a Polytron (Brinkman) at room temperature in 60 mM Tris-HCl, pH 8.2 with 15 mM $CaCl_2$, 390 mM sucrose and 10 mM dithiothreitol (DTT). Three milliliters of grinding buffer were used for whole seed and 1 ml for seed chips. The extracts were centrifuged (Beckman TJ-6) for 15 min at 5,000×g at 4 °C. The supernatant was saved and, if not used immediately, was stored overnight at −20 °C. To be able to distinguish the isozyme phenotypes, it was important to add DTT to the extraction buffer immediately before use.

Table 1. Characteristics of soybean cultivars and introductions used in this study

	MG[a]	Fl. color[b]	Eu[c]	Seed urease[d]
Cultivar				
'Columbia'	III	W_1	*Eu*1-a	*Sun*
'Prize'	III	W_1	*Eu*1-a	*Sun*
'Essex'	V	W_1	*Eu*1-a	*Sun*
'Forest'	V	w_1	*Eu*1-b	*Sun*
'Williams'	III	w_1	*Eu*1-b	*Sun*
Introduction (Name)				
PI 229324 (Itachi)	III	W_1	*Eu*1-b	*sun*
PI 416975 (Kairyou Kakushin)	V	w_1	*Eu*1-b	*sun*
PI 417073 (Kounou-1)	V	w_1	*Eu*1-b	*sun*
PI 417074 (Kounou-2)	V	W_1	*Eu*1-b	*sun*

[a] MG = maturity group
[b] Fl. color = flower color; W_1 = purple; w_1 = white
[c] *Eu*1-a = electrophoretically slow urease allele; *Eu*1-b = electrophoretically fast urease allele
[d] *Sun* = presence of urease in the seed; *sun* = absence of urease in the seed

The urease isozymes were separated by polyacrylamide gel electrophoresis with a pH 8.3 system using a 7% (w/v) total acrylamide, 4% bisacrylamide, 1.5 mm × 140 mm × 140 mm slab. The buffer and gel solutions were those of Davis (1964) as modified by Gabriel (1971). Twenty-five microliters of extract (66 to 150 µg protein) from whole seed and 40 µl of extract (66 to 150 µg protein) from seed chips were used in each sample well. Gels were run at room temperature without cooling at 30 mA per slab until the tracking dye had moved at least 4 cm.

Urease was located in gel slabs by the cresol red procedure of Buttery and Buzzell (1971) modified as follows: The buffer was 84 mM acetate, pH 5.0; the concentration of urea was increased to 333 mM; and the cresol red concentration was doubled to 0.1% (w/v).

The cresol red assay was extended to screen seeds for the presence or absence of urease. Seed chips were incubated in 100 µl of urease-staining buffer at room temperature and the change in color from orange to red-purple within 30 min indicated a urease-positive seed chip. Urease-catalyzed hydrolysis of urea raises the pH of the solution resulting in the cresol red color change.

RNA isolation and translation

Total RNA was isolated from embryos in mid-development (20–35 days after pollination) of field-grown PI 229324 (*sun, Eu*1-b) and 'Prize' (*Sun, Eu*1-a). The procedure of Polacco and Sparks (1982) was employed with the addition of the following treatments to remove polysaccharide: RNA was suspended in 1 mM Tris-HCl, pH 7.5 with 50 mM KCl and 1.5 mM $MgCl_2$ and precipitated at 4 °C overnight upon the addition of an equal volume of 4 M $LiCl_2$ (Barlow et al. 1963). Precipitated RNA was washed twice with 2 M $LiCl_2$, once with 3 M ammonium acetate, pH 5.5, and once with 95% ethanol. RNA was dried, resuspended in water to 10 A_{260} units/ml and subjected to oligo (dT)-cellulose chromatography (Aviv and Leder 1972) to isolate polyadenylated RNA. Polyadenylated RNA was size fractionated as described by Polacco and Sparks (1982).

In vitro translations were performed in the rabbit reticulocyte lysate system according to the manufacturer's instructions (Promega Biotec, Madison, WI). [^{35}S] methionine (100 Ci/mmole) was from New England Nuclear (Boston, MA). Urease translation product was immuneprecipitated, resolved, and detected on SDS-PAGE as described previously (Polacco and Sparks 1982).

Recombination likelihood for linkage analysis

Recombination between the *Eu* and the *sun* (null) element was estimated by the maximum likelihood method from a sample of 550 seeds. The expected and observed F_2 phenotypic frequencies are shown in Table 2. The maximum likelihood expression is (Allard 1956):

$$L(p) = \frac{(550!)}{(2!\,4!\,411!\,133!)} P_1^2 P_2^4 P_3^{411} (0.25)^{133}.$$

Taking logarithms, differentiating and setting the equation equal to zero,

$$0 = \frac{d \ln(L(p))}{dp} = \frac{4p+4}{p^2+2p} + \frac{4-8p}{p-p^2} + \frac{822p-1644}{3-4p+p^2}.$$

This equation was solved by successive approximation. The standard error was estimated by

$$Sp = (1/n I_p)^{0.5}, \quad \text{where } n = 550$$

and

$$I_p = \sum_{i=1}^{3} (1/P_i)(dP_i/dp).$$

Miscellaneous procedures

Protein concentration was determined by the Folin-Lowry method (Lowry et al. 1951) using lysozyme as a standard. Since the samples contained interfering DTT, protein was precipitated in an equal volume of cold 10% (w/v) TCA. Samples were refrigerated for 30 min and the protein pelleted by centrifugation as described above. Pellets were washed twice with 80% (v/v) acetone, air dried overnight and redissolved in 0.1 N NaOH. The presence or absence of seed lectin was determined by the Ouchterlony method (1948) as conducted by Stahlhut et al. (1981).

Table 2. Genotypes and phenotypic frequencies of seed from selfed plants heterozygous for the linked loci determining the urease isozyme form and the presence and absence of urease. F_1: *Sun Eu*1- a × *sunEu*1-b

Phenotype	Genotypes[a]	Phenotypic frequency	
		Expected[b]	Observed
Fast urease	*Sun Eu*1-b/*Sun Eu*1-b, *Sun Eu*1-b/*sun Eu*1-b, *Sun Eu*1-b, *sun Eu*1-a	$P_1 = 1/4\,(p^2+2p)$	2
Fast and slow urease	*Sun Eu*1-a/*Sun Eu*1-b	$P_2 = 1/2\,(p-p^2)$	4
Slow urease	*Sun Eu*1-a/*Sun Eu*1-a, *Sun Eu*1-a/*sun Eu*1-b, *Sun Eu*1-a/*sun Eu*1-a	$P_3 = 1/4\,(3-4p+p^2)$	411
Null	*sun Eu*1-a/*sun Eu*1-a, *sun Eu*1-a/*sun Eu*1-b, *sun Eu*1-b/*sun Eu*1-b	1/4	113
	Total	1	550

[a] *Sun* = presence of seed urease; *sun* = absence of seed urease; *Eu*1-a = slow urease isozyme; *Eu*1-b = fast urease isozyme

[b] p = cross-over frequency

Results

Non-complementing recessive mutations block embryo-specific urease expression

All F_1 seed from crosses of each of the four nulls to urease-expressing lines contained the active embryo-specific urease isozyme (*Eu*1-a or *Eu*1-b) of the urease-expressing parent. The absence of urease was therefore concluded to be recessive to the presence of the enzyme.

The recessive nature of the nulls allowed the determination of their allelism by examining the F_1 progeny of intermated null lines. The F_1 seeds were uniformly urease-negative and no F_2 seed from selfed F_1 plants contained the abundant (embryo-specific) urease. The four non-complementing urease null lines are therefore concluded to be allelic for alterations which block the expression of the embryo-specific urease. In all cases, the absence of urease had not maternal influences.

There is a null allele at a single locus

To test the hypothesis that alteration at a single locus is responsible for the null trait, we analyzed F_2 seed from selfed F_1 plants of null by urease-positive parents (Table 3). No deviation was found from a 3 : 1 (positive : null) hypothesis expected for assortment at a single locus. F_2 seed without enzyme activity were planted, allowed to self-pollinate and the resultant F_3 seed all lacked detectable embryo-specific urease activity. Heterozygous F_2 plants segregated progeny in a 3 : 1 ratio for the presence and absence of embryo urease (Table 4). Similar results were obtained when PI 416975, PI 417073, and PI 417074 were crossed to 'Essex' (*Eu*1-a) and 'Forrest' (*Eu*1-b) (data not shown).

Segregation of urease isozyme (Eu1-a, Eu1-b) from the null trait

The F_2 urease-positive embryos from the crosses of nulls with 'Williams' were examined for the electro-

Table 3. Observed and expected segregation of F_2 seed from selfed F_1 plants from the crosses 'Columbia' (*Eu*1-a) × PI 229324 (urease null), 'Williams' (*Eu*1-b) × PI 229324 and reciprocals

Cross	No. of F_2 seed		Urease activity		Chi-square	
			Present	Absent	3:1 ratio	
					Value	*P*
'Columbia' × PI 229324	73	obs.	59.00	14.00	1.18	0.30–0.20
		exp.	55.75	18.25		
PI 229324 × 'Columbia'	74	obs.	51.00	23.00	1.46	0.30–0.20
		exp.	55.50	18.50		
'Williams' × PI 229324	112	obs.	81.00	31.00	0.43	0.70–0.50
		exp.	84.00	28.00		
PI 229324 × 'Williams'	447	obs.	334.00	113.00	0.02	0.90–0.80
		exp.	335.25	111.75		

Table 4. Observed and expected segregation of F_3 from selfed F_2 plants heterozygous for the presence and absence of urease activity from the crosses 'Columbia' (*Eu*1-a) × PI 229324 (urease null), 'Williams' (*Eu*1-b) × PI 229324 and reciprocals

Cross	No. of F_2 seed		Urease activity		Chi-square	
			Present	Absent	3:1 ratio	
					Value	*P*
'Columbia' × PI 229324	204	obs.	145.00	59.00	1.67	0.2–0.01
		exp.	153.00	51.00		
PI 229324 × 'Columbia'	301	obs.	227.00	74.00	0.03	0.9–0.8
		exp.	225.75	75.25		
'Williams' × PI 229324	462	obs.	351.00	111.00	0.21	0.7–0.5
		exp.	346.00	115.00		
PI 229324 × 'Williams'	391	obs.	302.00	89.00	1.04	0.5–0.3
		exp.	293.25	97.75		

Table 5. Observed and expected segregation of F_3 seed from selfed F_2 plants of the cross 'Columbia' × PI 229324 presumed to be heterozygous for the urease isozymes

Plant	No. of seed		Urease genotype[a]			Chi-square	
			F	F/S	S	1:2:1 ratio	
						Value	*P*
822b-1-6	100	obs.	26	45	29	1.18	0.7–0.5
		exp.	25	50	25		
822B-1-44	100	obs.	33	43	24	3.58	0.2–0.1
		exp.	25	50	25		

[a] F = fast genotype; S = slow genotype; F/S = heterozygote

Table 6. Expected and observed frequencies of seed from selfed plants of the cross PI 229324 by 'Columbia' which are heterozygous for the loci determining the urease form and the presence of absence of urease[a]

Phenotype	Frequency	
	Expected[b]	Observed
Fast urease	171.88	2
Fast and slow urease	68.75	4
Slow urease	171.88	411
Null	137.50	133
Total	550.00	550

[a] See Table 2 for genotypes

[b] Expected frequency based on two, independently assorting loci

phoretic form of their abundant urease. Williams is homozygous for the fast urease (*Eu*1-b). Among 250 urease-positive F_2 seeds from crosses of 'Williams' with PI 229324 only the *Eu*1-b form was found. Similarly, only the *Eu*1-b form was observed among F_2 urease-positive seed from the crosses of PI 416975, PI 417073, and PI 417074 to Forrest (data not shown).

In contrast, from the cross 'Columbia' (*Eu*1-a/*Eu*1-a) × PI 229324, 3 *Eu*1-a/*Eu*1-b presumptive heterozygotes were identified among 56 urease-positive F_2 seeds. Two of these (822B-1-6 and 822B-1-44) were selfed and their seed showed no significant deviation from a 1 : 2 : 1 ratio (fast : fast/slow : slow) (Table 5) indicating that they were heterozygous for the codominant urease isozymes. *Eu*1-b progeny also were observed among the urease-positive F_2 from the crosses of the nulls PI 416975, PI 417073 and PI 417074 to 'Essex' (*Eu*1-a/*Eu*1-a) (data not shown).

The recovery of novel fast (*Eu*1-b) segregants from crosses between null phenotypes and *Eu*1-a homozygotes, while finding only nulls and slow (*Eu*1-a) phenotypes from crosses of nulls with *Eu*1-b homozygotes, indicates that the null lines carry an electrophoretic fast form (*Eu*1-b) of urease which is unexpressed or inactive. The low frequency of *Eu*1-b progeny from null × *Eu*1-a crosses indicates the existence of allelism or close linkage between the null lesion and the *Eu* locus.

Data were pooled from F_3 seed of isolated F_2 field-grown plants which segregate both for the presence and absence of embryo-specific urease and for *Eu*1 allele (Table 6). Linkage was estimated by maximum likelihood analysis (Table 2; "Materials and methods") which indicated that the null lesion is linked to *Eu*1 by 1 ± 0.44 map units.

Construction of a null, Eu1-a genotype

A two-step strategy was developed using F_2 plants from the PI 229324 × 'Columbia' (*Eu*1-a/*Eu*1-a) population to locate F_3 recombinant nulls bearing an unexpressed *Eu*1-a (slow) isozyme. Progeny testing identified F_2 plants segregating for the presence and absence of urease (genotypes I, III, VII, IX; Fig. 1). Those segregating predominantly slow urease (*Eu*1-a), were examined further (genotypes I and III). If, after exhaustive analysis, all urease-positive seed had a slow urease phenotype, then the parental F_2 was presumed to have the genotype [null(*Eu*1-a)/ + *Eu*1-a] (genotype III of Fig. 1). Obviously, many progeny must be tested to distinguish between genotype I [null(*Eu*1-b)/ + *Eu*1-a] and III [null(*Eu*1-a)/ + *Eu*1-a] (Fig. 1). A plant meeting the selection criteria [null-segregating, lack of fast (*Eu*1-b) urease-positive segregants] was located in the PI 229324 × 'Columbia' F_2 population.

To confirm the existence of the null(*Eu*1-a) genotype, plants were raised from the null progeny of the plant selected by the criteria described above, and were crossed to urease-positive *Eu*1-b homozygotes. Their hybrids were allowed to self-pollinate in isolation, and the F_2 progeny were screened for the predicted *Eu*1-a-expressing phenotypes. Three hundred and four F_2 seed from one test cross were analyzed and 2 *Eu*1-a recombinants were found.

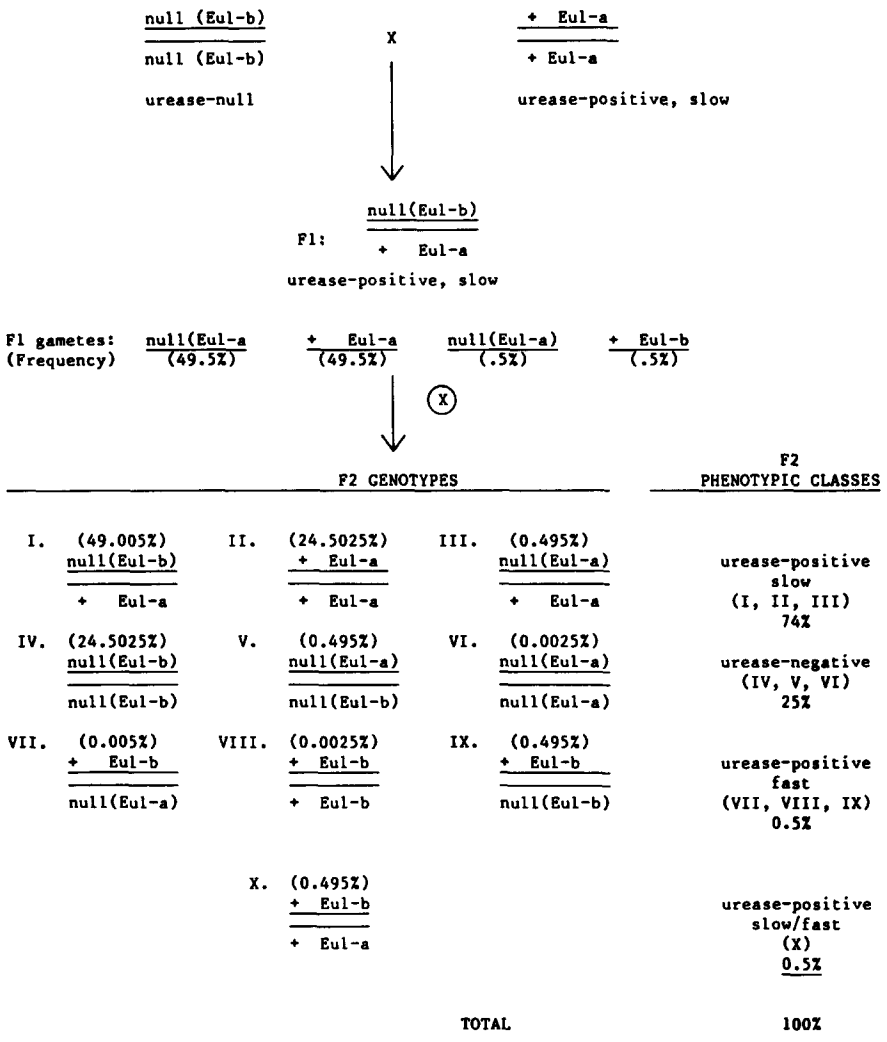

Fig. 1. Expected F_2 genotypes and phenotypes in the F_1 and F_2 of the cross 'Columbia' (urease positive, slow) ×PI 2293241 (urease-null). The urease null lesion is taken to be one map unit from the *Eu*1 (isozyme) locus and to block expression of the fast allele (*Eu*1-b) in *cis*. The phenotypic frequencies are predicted by the expression in Table 2 where p (the exchange rate) = 0.01

Lack of urease template activity in vitro in null embryo mRNA

PI 229324 lacks embryo-specific urease protein as well as activity (Polacco et al. 1982; Winkler et al. 1983). To determine whether this phenotype is due to a lack of urease translation polyadenylated mRNA from developing embryos of 'Prize' and PI 229324 was translated in vitro. Both RNA preparations were size-selected to enrich for urease template activity in the in vitro rabbit reticulocyte lysate system (Polacco and Sparks 1982). Figure 2 shows the similarity in the pattern of translation of both RNA preparations. Of total translation product (lanes TP, Fig. 2) resolved by SDS PAGE, the PI 229324 lane contained 44,500 DPM of incorporated [^{35}S] methionine while the 'Prize' lane contained 67,000 DPM. Equal amounts of radioactive protein (1.2×10^6 DPM) were subjected to immuneprecipitation by antiserum raised against purified embryo-specific urease (Polacco and Havir 1979). Only Prize translation product contained an immunoreactive species (lanes IP, Fig. 2). It was found to migrate closely with the in vivo-synthesized urease subunit (approximately 95 kilodaltons) and to be competed effectively from antibody binding by in vivo urease (lane IP/Ur, Fig. 2). Thus, the lack of urease accumulation in embryos of PI 229324 is correlated with a lack of accumulation of translatable urease mRNA. This result is consistent with our recent results which indicate that Itachi embryos lack transcripts homologous to a urease genomic clone (Krueger et al. 1985).

Discussion

The data indicate that the urease-null phenotype is controlled by an element approximately one map unit from the urease isozyme locus, *Eu*1. It is proposed that the term *sun* (seed urease null) be used for the recessive

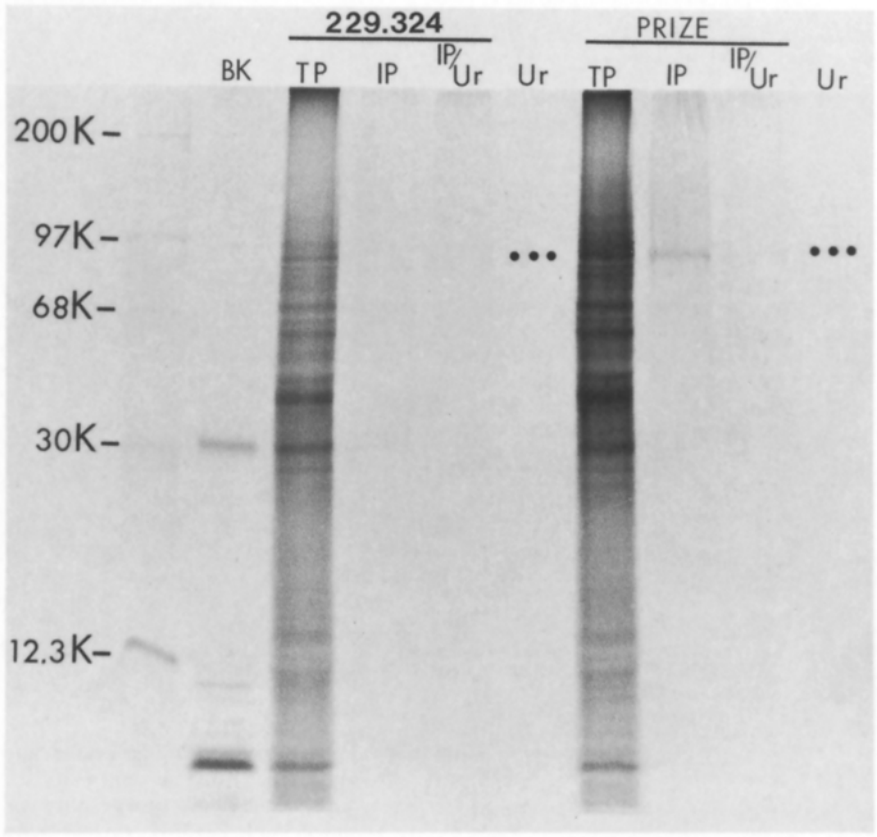

Fig. 2. Immunoprecipitation of urease translation product synthesized in vitro. Translation products (lanes TP) from a heavy size class polyadenylated RNA from embryos of PI 229324 and 'Prize' were immunoprecipitated with antiserum to seed urease and resolved on a 10% polyacrylamide SDS gel (lanes IP). Only 'Prize' translation product yielded a detectable urease-antigenic species. It closely migrated with the in vitro urease subunit (indicated by the three dots in lanes Ur). Lanes IP/Ur represent immunoprecipitations with 10 μg competing in vitro urease. Equal amounts of in vitro-synthesized protein (i.e. 1.2×10^6 DPM of incorporated [^{35}S] methionine, 1,000 Ci/mmole) were employed in each of the four immunoprecipitations. 'Prize' and PI 229324 translation product (lanes TP) contained 67,000 and 44,500 DPM, respectively, of TCA precipitable radioactivity

element that is phenotypically characterized by the absence of embryo-specific urease activity, antigen and translatable mRNA in soybean seeds. Our data do not distinguish between *sun* being an allele at the *Eu*1 locus or a lesion in a distinct, linked locus.

By definition a cross-over frequency of 1 equals 50 map units. Thus, the number of base pairs in the map unit separating *Eu*1 and *sun* can be calculated by dividing the quotient of the total genome size (in base pairs) and the total number of genetic exchanges per meiosis by 50 map units. Such values (Lewin 1980) range from 5×10^5 base pairs/map unit in *Drosophila* to 4×10^6 base pairs/map unit in maize (*Zea mays*). In *Drosophila,* the map unit size predicted from total cross-over figures (5×10^5 base pairs) has been confirmed by comparing the genetic (Chovnick et al. 1976) and physical (McCarron et al. 1979) map of the rosy (*ry*) locus by which one map unit is calculated to be 8×10^5 base pairs.

By extrapolating to soybean, *sun* would lie 1,000 kilobases from *Eu*1. However, in soybean, unlike *Drosophila,* there is no precedent for correlation between a physical distance and fine structure exchange frequencies. In maize, recombination frequencies within the alcohol dehydrogenase-1 (*Adh*1) and waxy (*Wx*) loci can approach 0.1 (Freeling 1978) and 0.3% (Nelson 1968), respectively. (Here we have doubled the frequency of "wild type" pollen grains produced by heteroallelic plants to include the undetected reciprocal double mutants.) The *Adh*1 transcriptional unit is approximately 3.4 kb (Dennis et al. 1985), while *Wx* is approximately 5 kb (Wessler and Varagona 1985). Freeling and Bennet (1985) estimated one map unit within *Adh*1 to be 21,700 bp for an effectively paired region. (Their estimate was actually twice as large because they chose to eliminate conversion events which they estimate to be responsible for half of their recombinants; but in any case this value is significantly less than 4×10^6 bp per map unit.) A similar size for a map unit within *Wx* is readily derived from the physical map (Wessler and Varagona 1985) of the genetic markers analyzed by Nelson (1968). Thus, our data are also consistent with *sun* and *Eu*1 being allelic. A high degree of homologous pairing within the *Eu*1 locus could result in a seemingly anomalously high exchange rate of about 1%.

Of course, the apparent recombination between *sun* and *Eu*1 could be an erroneous conclusion from pollen contamination or from an unstable *sun* allele. However, the consistent recovery of *Eu*1-b segregants from several crosses between homozygous nulls and *Eu*1-a plants was in contrast to the lack of *Eu*1-a segregants in several crosses of null$\times$*Eu*1-b plants and militates against pollen contamination. All F_1 plants were grown in the greenhouse where natural outcrossing has never been reported or in a sheltered and isolated location outside the greenhouse during the summer months.

Further evidence for the lack of pollen contamination as well as for a stable *sun* allele is provided by our never having observed urease-positive progeny of either type (*Eu*1-a or *Eu*1-b) from several hundred tested seed of selfed null plants. In addition, the null RNA employed in the in vitro translation of Fig. 2 was extracted from approximately 250 developing seeds from null plants. This seed extract was divided in two parts, one for RNA preparation and the other for urease purification (Polacco et al. 1985). Embryo-specific urease would have represented 75% of total residual (ubiquitous) urease activity if only one seed were urease-positive; however, only the ubiquitous isozyme (*Eu*2), and not the embryo-specific urease, was recovered (Polacco et al. 1985).

It has not yet been definitively demonstrated that *Eu*1 is the urease structural gene. To test whether *Eu*1 contains the urease coding region we are (1) comparing the segregation of urease restriction fragment length polymorphisms with that of the urease isozyme (*Eu*1-a versus *Eu*1-b), and (2) examining allelism between *Eu*1-a and a temperature-sensitive fast urease (*Eu*1-b) we have recently recovered from mutagen-treated seed (Meyer-Bothling and Polacco 1986).

In the absence of knowledge of the *Eu*1 function there are two disparate models for the *sun* phenotype: (1) *sun* and *Eu*1 are alleles at the structural gene locus. *sun* is either not transcribed or encodes an altered transcript which is rapidly degraded; (2) *sun* is at a distinct cis-acting locus which blocks the expression of the allele at the closely linked *Eu*1 locus (which could encode urease or a urease processing function).

A third model, that *sun* is the urease structural gene while *Eu*1 is a processing locus, predicts that the *Eu*1 gene product can operate on the *Sun* product in trans. If this were the case, the F_1 progeny of *sun Eu*1-b × *Sun Eu*1-a (e.g., PI 229324 × 'Columbia') should each contain both urease forms. In fact, they express only the slow (*Eu*1-a) form.

If *sun* is indeed a cis-acting element at a distinct locus 1,000 kilobases from *Eu*1, this distance is likely too large for *sun* to be an alteration in an enhancer element. A cis-acting element effective at long distances qualifies as a position effect. A possible explanation of the *sun* effect is that it represents a transposition, possibly of heterochromatin to within one map unit of the *Eu*1 locus. Obviously, further genetic and molecular analyses of the urease structural gene in null lines are necessary to elucidate the basis of the urease-null trait. These studies have several implications for plant breeders and geneticists. Among them is that the elucidation of the urease null trait will lead to the understanding of gene expression in a crop plant and secondly, the null urease trait will be useful for linkage studies.

Acknowledgements. The authors would like to thank Dr. Edgar Hartwig for his generosity in providing F_1 seed of crosses between maturity group V cultivars and introductions. Andy Thomas is thanked for performing the screens of the germplasm collections. Drs. A. Hepburn and Mark Holland are thanked for their helpful discussions.

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