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Mutational analysis of the embryo-specific urease locus of soybean

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Summary. By a non-destructive urease screen of M_2 soybean (*Glycine max* [L.] Merr. cv. Williams) seeds, four true-breeding mutants (n4, n6, n7 and n8) were recovered which lack most (n6, n8) or all (n4, n7) embryo-specific urease activity. This trait was due to a single, recessive lesion at the *Sun* (seed urease-null) locus identified earlier in an exotic germplasm (PI 229324, Itachi). All *sun* mutants produced normal ubiquitous urease, the low abundance isozyme found in all soybean tissues examined. Tight mutants n4 and n7 accumulated no detectable embryo-specific urease protein or mRNA; n6 and n8 accumulated normal or near normal levels of urease mRNA but had seed urease protein levels approximately 5% and 0.5%, respectively, of the progenitor. Mutant n8 appeared to produce a low level of fully active urease (approximately 0.7% activity level, approximately 0.5% protein level) while n6 produced a higher level of an altered, nearly inactive urease (0.09% activity level, approximately 5% protein level). Urease alterations in n6 were manifested by its increased temperature sensitivity and variation in aggregation state and pH preference. Thus, mutations in the *Sun* locus affected both the level and the nature of the embryo-specific urease gene products indicating that *Sun* encodes the embryo-specific urease. We reported earlier that the *Eul* locus, which controls the aggregation state of the embryo-specific urease, is one map unit from *Sun* and that the *Eul* allele *cis* to *sun* is not expressed (Kloth et al. 1987). That the level of urease gene product, its aggregation state and other enzyme properties can be affected by induced *sun* mutations, suggests that the *Eul* and *sun* alleles are at the same locus.

Key words: Urease – Null – Clone – Soybean – Isozymes

Introduction

The embryo-specific urease isozyme of soybean is synthesized exclusively in the developing embryo (Polacco and Winkler 1984; Polacco et al. 1985). A mutant (Polacco et al. 1982; Kloth et al. 1987) lacking the embryo-specific urease accumulates normal levels of the second urease isozyme, the ubiquitous urease, so named because it is found in leaves, cultured cells, seed coats and embryos (Polacco and Winkler 1984; Polacco et al. 1985). The presence or absence of embryo-specific urease (and its mRNA; Krueger et al. 1987) is under control of the locus designated *Sun* (seed urease-null) (Kloth et al. 1987). Cultivars homozygous for the recessive *sun* allele lack embryo-specific urease.

A second locus, *Eu1*, controls embryo-specific urease expression (Buttery and Buzzell 1971; Kloth and Hymowitz 1985). The *Eul* locus contains codominant alleles for the electrophoretic fast (*Eul-b*) and slow (*Eul-a*) forms of the embryo-specific urease. By native polyacrylamide gel electrophoretic (PAGE) mobility and gel elution profiles, it was concluded that the fast/slow forms represent trimeric/hexameric aggregates (Buttery and Buzzell 1971; Polacco and Havir 1979; Polacco et al. 1985).

The nature and relationship of the *Sun* and *Eul* loci are not well understood. In crosses of *sun/sun* plants with slow urease (*Eul-a/Eul-a*, *Sun/Sun*) plants, a low frequency of F_2 progeny contained fast- and slow-running urease or exclusively the fast urease. These F_2 recombinants were observed only when the null was crossed with *Sun/Sun, Eul-a/Eul-a* and not with *Sun/Sun, Eul-b/Eul-b*. The conclusion was that *sun/sun* plants were homozygous for a silent *Eul-b* allele which was revealed by exchange (1%) between *sun* and *Eul-a* (Kloth et al. 1987).

The single map unit between the *Eul* and *Sun* loci has been interpreted in terms of two alternative models (Kloth et al. 1987). In the first, *Eul* and *sun* are alleles at a single locus, within which there is a high (1%) frequency of genetic exchange. In the second, *Sun* and *Eul* are distinct loci and *sun* blocks the expression of the silent *Eul* allele in *cis*.

The first model predicts that some leaky *sun* mutants encode a structurally altered urease manifested by a change in electrophoretic migration (aggregation state) and other properties. The second model predicts that mutation at *Sun* will affect only the level and not the structure of the embryo-specific urease. We examined two leaky *sun* mutants to determine if they produced an embryo-specific urease altered in structure.

Materials and methods

Plant material. Soybean (*Glycine max* [L.] Merr.) cv. Williams 82 and Prize were obtained from commercial sources. Itachi (PI 229324) was recovered in a screen of the USDA Germplasm Collection (Polacco et al. 1982). Approximately

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Abbreviations: βME, β-mercaptoethanol; NMU, N-nitroso-N-methyl urea; TM, Tris-maleate

Table 1. Urease phenotype of seeds determined by the seed chip assay

Line	Genotype	Seed chip phenotype	(%)[a]
Williams 82	*Sun/Sun*	Purple in 10–30 min	(100)
n8	*n8/n8*	Pale pink in 4–6 h	(0.7)
n6	*n6/n6*	Pale pink in 8–15 h	(0.09)
n4	*n4/n4*	Pale pink in 3–5 days	(0.008)
n7	*n7/n7*	Pale pink in 3–5 days	(0.008)
Itachi[b]	*sun/sun*	Pale pink in 3–5 days	(0.013)
Williams[c]	*sun/sun*	Pale pink in 3–5 days	(0.012)

[a] Number in parentheses is the percentage of normal seed urease activity determined quantitatively for progeny of each M_2 seed (Table 4)

[b] Itachi, PI 229324

[c] The seed urease-null trait was introgressed into Williams by five backcrosses (a gift of T. Hymowitz and R.L. Bernard)

1000 bulked M_2 progeny of N-nitroso-N-methyl urea (NMU)-treated Williams seed (Ryan and Harper 1983) were generously provided by James Harper of the University of Illinois. The *sun* (Williams) line, i.e. Williams, into which the urease-negative trait of Itachi (*sun*) was backcrossed, was a gift of R.L. Bernard and T. Hymowitz (Illinois). Seeds were germinated in paper towels before transfer of seedlings to potting soil (inoculated with *Bradyrhizobium japonicum* USDA strain 143). All crosses were performed in a greenhouse under natural light.

Urease-deficient seed screen. A 5 to 20 mg chip cut from the side opposite the hilum of individual M_2 seeds was incubated in 1 ml 0.1 M urea, 5 mg/l cresol red, 10 mM potassium phosphate, pH 7.0 at room temperature. Standard urease-positive seed chips turned the assay solution from yellow to pale pink to purple within 30 min by alkalinization due to ammonia evolution. The allelic *sun* mutants required 4 h to 5 days to induce a color change (Table 1). The required time for color change was used as a phenotypic character among cross progeny.

Urease assays. Urease was measured in leaves (expanding trifoliates of vegetative plants) and mature seeds. Extracts were prepared as in Polacco and Winkler (1984) except that 10 vol. of buffer were added to seeds, 2 vol. to unwashed leaves, and all procedures were performed at 4° C. Urease specific activity was expressed as urea hydrolyzed per minute per milligram extract protein at 30° C. Activity was determined from CO_2 or NH_3 released from urea. Assay reactions in TM buffer [0.1 M Tris-maleate, 1 mM EDTA, 1–10 mM β-mercaptoethanol (βME), pH 7.0] were initiated by urea added to 9 mM.

To measure $^{14}CO_2$, 100 µl aliquots of crude extract (1–2 mg bean protein or 0.3–0.8 mg leaf protein) were placed into plastic scintillation minivials fitted with a 4 cm long "sleeve" of 13 × 3.2 mm amber latex tubing (Scimatco, Fisher Scientific). After addition of 0.9 ml substrate (10 mM [^{14}C]urea, 100 cpm/nmol in TM) the reaction was "capped" with a second minivial whose opening was tightly fitted into the rubber tubing. The upper vial contained a 1 × 1 cm glass fiber filter (GFA, Whatman) freshly wetted with 40 µl of 50% saturated NaOH (prepared each day from saturated NaOH). Triplicate reactions were terminated at times ranging to 6 h by injection of 0.5 ml 1 M H_2SO_4 through the rubber tubing with a tuberculin (1 ml) syringe. Zero time controls either were initiated and immediately terminated, or were produced by reversing the order of H_2SO_4 and urea additions. $^{14}CO_2$ trapped in the filter was determined by scintillation counting as described previously (Polacco and Winkler 1984). Urea hydrolysis was linear up to 6 h.

In determining ammonia by the Berthelot reaction Tris-maleate was replaced by 0.1 M potassium phosphate in the extraction and substrate buffers. Duplicate reactions were initiated by addition of 50 µl 27 mM urea in 0.1 M potassium phosphate, 1.0 mM EDTA, 10 mM βME, pH 7.0, to 100 µl extract and were terminated by the addition of 0.1 ml 0.1 M H_2SO_4. Phenol-nitroprusside reagent 1.25 ml (Sigma), diluted 1:5 in H_2O, was added and, following vortexing, 1.25 ml of a 1:5 dilution of alkaline hypochlorite reagent (Sigma) was added. Reaction tubes were vortexed, heated in a 65° C water bath for 5 min, cooled to room temperature, centrifuged for 2 min and the A_{625} of the solutions was compared with duplicate standard $(NH_4)_2SO_4$ samples containing 12.5 to 100 nmol NH_4^+. Time plots of NH_4^+ release had better correlation coefficients when ammonia was first volatilized by microdiffusion and trapped before determination: Urea (10 mM) in 180 µl TM was added to 20 µl crude extract in 0.5 ml capless aliquot tubes which were standing in 250 µl 0.1 M H_2SO_4 in 13 × 100 mm glass tubes. After incubation at 30° C for 0 to 75 min, 200 µl saturated sodium borate (pH 11) was added to each reaction mixture, and glass tubes were capped tightly with rubber stoppers. After diffusion of NH_3 overnight, the aliquot tubes were removed and NH_4^+ in the H_2SO_4 solution was measured by the Berthelot assay described above.

Protein electrophoresis and Western analysis. Crude seed extracts (8 mg/ml protein) were denatured by mixing with an equal volume of 0.1 M TM, 1 mM EDTA, pH 7.0, 1% (v/v) βME, 2% (w/v) SDS and heating at 65° C for 7 min. Denatured protein (40 mg) was resolved on a 10% acrylamide gel (Laemmli 1970) for 3 h at room temperature and 8 W constant power. SDS was excluded from the stacking gel while the resolving gel and upper running buffer contained 0.1% (w/v) SDS (Laemmli 1970). Native proteins (100–200 µg) were separated in the same gel system lacking SDS; the resolving gel was 7.5% acrylamide, and electrophoresis was for 3 h at 30 mA constant current at 4° C.

Native and denaturing gels were electroblotted (Towbin et al. 1979) to nitrocellulose which was reacted with a 1:400 dilution of rabbit antiserum raised against embryo-specific urease (Polacco and Havir 1979). [^{125}I]protein A (7.8 µCi/µg, New England Nuclear, 5×10^6 cpm/filter) was employed in place of second antibody. Filters were air dried and packaged with pre-flashed X ray film for autoradiography at −70° C with intensifying screens.

RNA isolation. RNA was isolated from frozen developing seeds using a scaled down procedure from Polacco and Sparks (1982). Tissue (7–9 g) was ground with 20 ml extraction buffer (60° C) in a small blender (350 ml) for 3–5 s. Agitation continued for an additional 3–5 s as 20 ml chloroform:phenol (1:1) at 60° C was added. Subsequent steps were as reported and poly(A)$^+$ RNA was recovered by the method of Aviv and Leder (1972).

Protein measurement. Protein was measured either by the Bradford method (according to instructions provided by

Table 2. Seed urease-deficient lines breed true

Line	M_3	M_4		M_5	
	No. seeds	No. parent plants	No. seeds	No. parent plants	No. seeds
n4	46	11	32	3	45
n6	46	14	123	8	123
n7	46	12	121	3	48
n8	46	11	75	2	32

A single M_2 seed gave rise to the M_3 progeny. All were tested by the seed chip assay (Table 1 and Materials and methods) and all had the urease-negative phenotype of the original M_2 seed

Table 3. F_2 transmission of seed urease-negative traits

Cross	Classes[a]		n	χ^2	P[b]
n4 × Williams 82 (*Sun/Sun*)	Standard 94	n4 34	128	0.167	>0.5
n8 × PI 229324 (*sun/sun*)	n8 99	Itachi 29	128	0.375	>0.5
n7 × n8	n8 208	n7 61	269	0.715	>0.3

[a] The classes are based on the seed chip phenotypes given in Table 1

[b] Probabilities for random deviation from a 3:1 ratio (high urease: low urease)

the reagent manufacturer, BioRad) or by the microbiuret method (Mokrasch and McGilvery 1956) which was modified for leaf protein: Two milliliters cold 10% (w/v) TCA was added to triplicate 100 µl aliquots of leaf extract. After standing on ice for 10 min followed by desktop centrifugation for 10 min, the supernatants were decanted and the pellets were washed with a mixture of 1 ml 10% TCA plus 4 ml cold acetone. After draining, the pellets were dissolved in 200 µl 10% (w/v) NaOH, heated for 3 min in a 100° C bath and cooled to room temperature. In duplicate tubes, a 50 µl aliquot of base-solubilized protein was added to 150 µl 10% NaOH. One tube received 0.8 ml water and the other, 0.8 ml microbiuret reagent. The A_{300} of these samples was measured versus appropriate blanks and absorbance due to protein was calculated from the difference of the two absorbance measurements.

Results

Recovery of true-breeding, seed urease-negative induced mutants

Eight seeds lacking most or all urease activity (Materials and methods) were recovered from a screen of approximately 1000 bulked M_2 progeny of NMU-treated Williams seed. Four (n4, n6, n7, n8) survived to maturity and their M_3, M_4 and M_5 progeny were all urease-negative (Table 2).

The mutants are allelic to sun

All four mutants when crossed to Williams 82 yielded urease-positive F_1 progeny. Selfed F_1 plants yielded F_2 populations containing approximately three urease-positive individuals for each urease-negative. (The data for mutant n4 are shown in Table 3.) Thus, recessive, single gene lesions are responsible for the low seed urease in the n4, n6, n7 and n8 mutants. All four mutants were crossed pairwise to each other and to PI 229324 (Itachi), which is homozygous for the recessive *sun* lesion (Kloth et al. 1987). None of the F_1 contained more urease than the more active parent, indicating allelism between *sun* and each induced mutant. None of the F_2 yielded standard (high) urease recombinants. Data for two F_2 populations are shown in Table 3. In the cross of PI 229324 × n8, the n8 and PI 229324 (*sun*) phenotypes segregated in a 3:1 F_2 ratio confirming the allelism of *sun* and n8. F_2 segregation patterns of the n7 × n8 cross (Table 3) followed a seed chip urease phenotypic ratio of 3:1 n8 to n7 levels, confirming the allelism of n7 and n8.

Table 4. Leaf and seed urease levels

Genotype	Seed		Leaf	
	n	Activity (µmol urea per min per mg protein)	n	Activity (nmol urea per min per mg protein)
Sun/Sun[a]	8	0.9 ± 0.4	4	1.2 ± 0.7
n4/n4	4	$7.8 \pm 3.2 \times 10^{-5}$	7	2.3 ± 1.2
n6/n6	4	$8.6 \pm 1.9 \times 10^{-4}$	3	2.7 ± 2.4
n7/n7	3	$7.4 + 2.3 \times 10^{-5}$	5	1.5 ± 0.9
n8/n8	3	$6.7 \pm 4.8 \times 10^{-3}$	1	0.3
sun/sun[b]	3	$1.2 \pm 0.5 \times 10^{-4}$	4	1.1 ± 0.96
sun/sun[c]	2	$1.1 \pm 0.3 \times 10^{-4}$	0	

Standard deviation is calculated using n-1 where n = number of determinations. Percentages of standard (*Sun/Sun*) seed activity are given in Table 1

[a] Williams 82

[b] PI 229324 (Itachi)

[c] Williams into which the Itachi urease-null trait was introduced

To summarize, we conclude that induced mutations in n4, n6, n7 and n8 are *sun* alleles because all are recessive, single gene lesions, because they do not complement *sun* or each other in the F_1, and because the F_2 of these crosses yield no urease-positive individuals.

We propose that the induced *sun* mutations be designated as *sun-n4*, *sun-n6*, *sun-n7* and *sun-n8*. However, for simplicity the working designation here will be *n4*, *n6*, etc. True-breeding mutants expressing the seed (embryo-specific) urease-null trait will be designated genotypically as *n4/n4*, *n6/n6*, etc., or phenotypically as n4, n6, etc.

Urease isozyme levels

Seed urease levels quantified for each mutant line (Table 4) ranged from 0.008% to 0.7% that of *Sun/Sun* (Williams 82) and thus corroborated the relative urease levels determined by the seed chip assay (Table 1). In contrast to their low seed activities the *sun* mutants have normal or near-normal leaf (ubiquitous) urease levels (Table 4). The greater variation observed in specific activity of leaf versus seed urease for each genotype is due, at least in part, to the observed dependence of leaf urease activity on leaf developmental and physiological state (our unpublished observations).

Mutants n6 and n8 had approximately 6 and 60 times

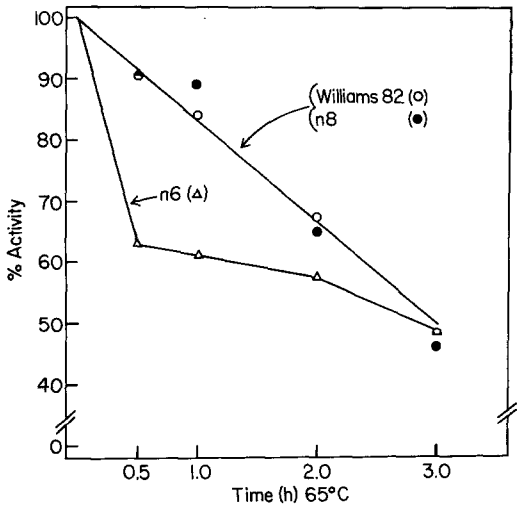

Fig. 1. Heat inactivation of seed urease. Residual urease activity in crude extracts of Williams 82 (o) and leaky mutants n6 (Δ) and n8 (•) was measured after heating at 65° C. Aliquots (0.3 ml) of crude seed extracts (approximately 4.5 mg protein in phosphate buffer) were heated at 65° C for 0.5 to 3 h, cooled on ice, and urease activity was assayed immediately after spinning out insolubles (Williams 82 supernatants were first diluted 1:200). One hundred microliters of 13.5 mM urea in phosphate buffer was added to triplicate 50 µl aliquots of extract and 15 min reactions (30 min for n6) (37° C) were terminated with 100 µl 0.1 M H_2SO_4 before the Berthelot NH_4^+ determination. Non-heated extracts were assayed in quadruplicate

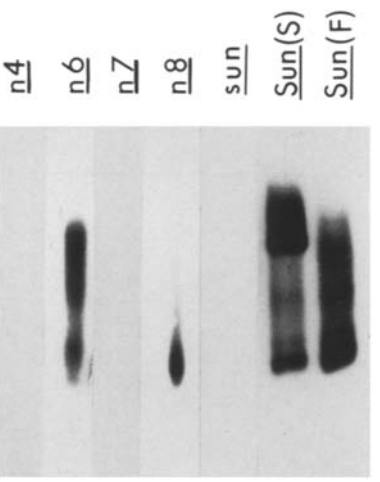

Fig. 2. Immunoblot of seed proteins separated by native polyacrylamide gel electrophoresis. Lane headings identify samples by homozygous *Sun* allele. All mutants (*n4, n6, n7, n8, sun*) are in the Williams background. *Sun* (F) is Williams 82 which makes predominantly fast (trimeric) embryo-specific urease (*Eul-b*) and *Sun* (S) is Prize which makes the slow (hexameric) urease (*Eul-a*). All samples contain 40 µg seed protein except *n6* (200 µg) and *n8* (160 µg). Proteins were blotted to nitrocellulose and reacted with a 1:400 dilution of embryo-specific urease antiserum (Polacco and Havir 1979; Materials and methods). Bound antibody was detected with [^{125}I]-protein A. Autoradiographs were exposed for 24 h except those for *Sun* (Fast) and *Sun* (Slow) which were exposed for 4 h. The smear of bands in lanes *Sun* (Fast) and *Sun* (Slow) is characteristic of immunoblots but is absent in activity stained gels (see, for example, Polacco and Havir 1979). The most intense bands obtained by both visualization methods seem identical

the seed urease activity, respectively, of the prototype *sun* mutant, Itachi (Tables 1 and 4), which contains the ubiquitous urease as its exclusive residual seed activity (Polacco and Winkler 1984, Polacco et al. 1985). We tested several criteria to determine whether n6 and n8 made an altered embryo-specific urease which was only partially active or, alternatively, produced low levels of normal embryo-specific urease.

Tests for structural alteration in embryo-specific urease from n6/n6 and n8/n8.

Temperature sensitivity. The *n8/n8* genotype produced a seed urease activity which was indistinguishable in heat lability from that produced by Williams 82 (Fig. 1). It appeared that a component of the activity in *n6/n6* extracts (ca. 40%) was rapidly inactivated while the residual activity was more heat resistant than progenitor urease. The complex inactivation profile of *n6/n6* seed urease is consistent with its complex aggregation state discussed below.

Holoenzyme aggregation state. The Williams progenitor produces the electrophoretically fast form of embryo-specific urease (Fig. 2, Polacco et al. 1985). Western blots of native polyacrylamide gels revealed that the residual embryo-specific urease in *n8/n8* was fast while *n6/n6* produced predominantly the slow urease characteristic of the cultivar Prize [*Sun* (S); Fig. 2]. The smear of bands in lanes *Sun* (Fast) and *Sun* (Slow) is characteristic of native immunoblots and unlike the sharper bands seen in activity stained gels (see, for example, Polacco and Havir 1979). The most intense bands obtained by both visualization methods seem identical and represent predominantly trimeric (Fast) and hexameric (Slow) holoenzyme states, respectively (Polacco et al. 1985). Thus, the urease subunits of *n6/n6* tend to form higher polymerization states than the predominantly trimeric embryo-specific urease of the progenitor. The propensity to form trimeric and hexameric species is normally specified by the codominant *Eul-b* and *Eul-a* alleles, respectively (Kloth and Hymowitz 1985).

Table 5. Ratio of urease activity at pH 7.0 and pH 8.8 in mature seed and leaves

Genotype	Seed		Leaf	
	n	S.A. pH 7.0/ S.A. pH 8.8	n	S.A. pH 7.0/ S.A. pH 8.8
Sun/Sun[a]	8	1.7±0.4	2	0.6±0.3
n4/n4	3	1.1±0.2	2	0.45±0.04
n6/n6	3	2.9±0.5	2	0.7±0.3
n7/n7	2	1.2±0.1	2	0.6±0.2
n8/n8	2	1.5±0.6	3	0.8±0.2
sun/sun[b]	2	0.9±0.3	1	0.7
sun/sun[c]	2	0.9±0.1	0	

S.A. pH 7.0/S.A. pH 8.8 = specific activity at pH 7.0 divided by specific activity at pH 8.8. Reported value is the average of ratios determined. Standard deviation is calculated using (n-1) where n = number of determinations

[a] Williams 82
[b] Itachi, PI 229324
[c] Williams into which the *sun* allele was backcrossed

pH dependence. The embryo-specific urease has a broad pH optimum near 7 while the ubiquitous urease exhibits

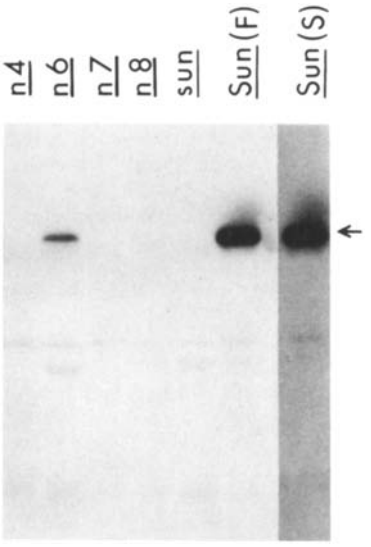

Fig. 3. Immunodetection of urease among seed proteins resolved by SDS-polyacrylamide gel electrophoresis. Crude protein (40 μg) from mature seeds of the "progenitor" *Sun* (Williams 82), of induced mutants homozygous for *n4, n6, n7* and *n8*, and of *sun* converged into the Williams background was resolved on a 10% acrylamide gel. Although this autoradiograph shows urease subunit only in mutant *n6/n6*, prolonged exposure revealed a low level of normally migrating subunit in *n8/n8* seeds (see Fig. 4). (F) refers to the fast-running urease (trimer) in Williams 82. (S) refers to the slow-running urease (hexamer) in Prize

double optima at pHs 5.5 and 8.75 (Kerr et al. 1983). The activity ratio at pH 8.8 versus 7.4 has been used as a criterion for identifying the urease isozyme in seeds, leaves, cell culture (Polacco and Winkler 1984) and in seed coats (Polacco et al. 1985). The *sun* allele, either in Itachi or in the Williams background, resulted in a seed urease activity ratio (pH 8.8 vs. pH 7.0) of 0.9 (Table 5). This is in general agreement with the ratios of 0.5 to 1.0 observed for leaves of all genotypes and suggests that the *sun/sun* seeds contain only the ubiquitous urease since the embryo-specific urease has an activity ratio of 1.7 (Table 5). The seed activity ratios of *n4/n4* and *n7/n7* were within the range of *sun/sun* suggesting that their seeds contained exclusively the ubiquitous urease which was in agreement with the inability to detect embryo-specific urease on their Western blots (Figs. 2, 3), even after overexposure. While there is too much standard error in the seed activity ratio determinations of *n8/n8* to draw a conclusion on the nature of its urease, the ratio of 2.9 for *n6/n6* embryos is significantly higher than 1.7 (Table 5) and suggests that *n6* encodes a qualitatively altered embryo-specific urease (which is six-sevenths of total seed activity; Table 4).

Embryo-specific urease protein and mRNA levels in sun *mutants*

By Western (Fig. 3) and Northern blot (Fig. 4) analysis we conclude that *n4/n4, n7/n7* and *sun/sun* lack both embryo-specific urease protein and mRNA. Mutants *n6/n6* and *n8/n8* had virtually normal levels of urease mRNA (relative to those of lipoxygenase mRNA; Fig. 4B) but accumulated very little urease protein.

We estimate from the band intensities of the gel of Fig. 3 and of other protein gels that *n6/n6* produces approximately

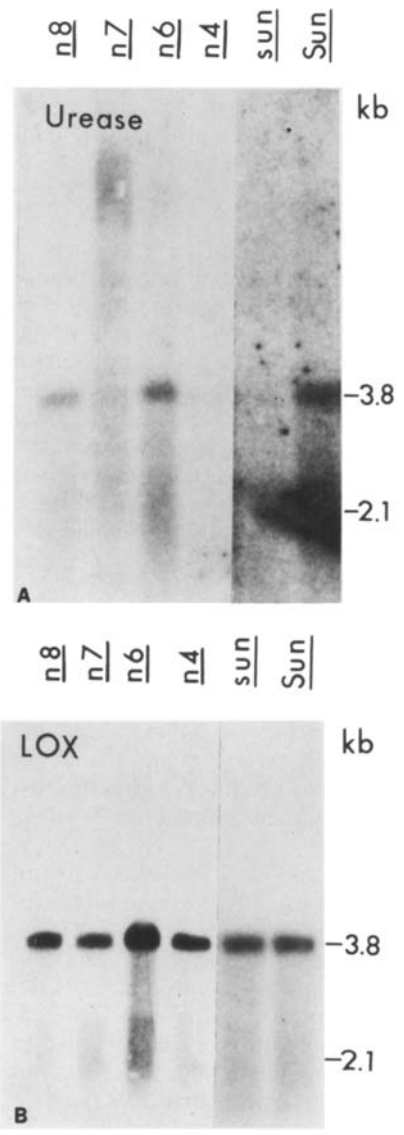

Fig. 4A, B. Northern blot analysis of mRNA from developing embryos (25 to 30 days after pollination). **A** Blots were hybridized with an 860 bp *Hin*dIII fragment of a soybean urease genomic clone (Krueger et al. 1987). The probe contains 222 bp of urease open reading frame equivalent to amino acids 699 to 772 of the 840 of Jack bean seed urease (Mamiya et al. 1985) to which it shows 83% deduced amino acid identity. **B** Duplicate blots were hybridized with a 479 bp cDNA (pAL-132) for soybean seed lipoxygenase-1 (Start et al. 1986). Lanes from mutants homozygous for *n4, n6, n7* and *n8* contained 2 μg mRNA while *sun* (Williams) and *Sun* (Williams 82) lanes contained 1 μg. Glyoxal RNA electrophoresis and Northern blotting were as described in Maniatis et al. (1982). Transfer of RNA to cationized nylon membrane (Zeta-Probe, BioRad) was in 20 × SSC at 4° C for 36 h. The post transfer (1 h in 2 × SSC, 1% w/v glycine) and prehybridization (4 h in 3 × SSC, 0.1% SDS, 5 × Denhardt's, 50 μg/ml denatured salmon sperm DNA) washes were at 65° C. [α^{32}P]dCTP (3,000 Ci/mmol) was incorporated overnight into 100 ng of purified urease genomic DNA and lipoxygenase cDNA by random oligonucleotide priming and extension using the oligolabeling kit reaction components and protocol provided by the manufacturer (Pharmacia). Radiolabeled probes were separated from unincorporated nucleotides by passage over a 60 × 5 mm column of BioGel P60 in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) buffer. After hybridization for 36 h at 65° C with boiled probe (7×10^8 cpm/μg), filters were washed at 65° C for 10 min (5 times) in 2 × SSC, 0.1% SDS, and packaged at −70° C with pre-flashed X ray film for autoradiography

5% the standard level of embryo-specific urease while *n8/n8* produces about one-tenth the level of *n6/n6* (i.e., about 0.5% standard). Only in *n8/n8* is there a parallel reduction in seed urease protein and activity: *n6/n6*, 5% urease protein, 0.09% urease activity; *n8/n8*, 0.5% protein, 0.7% activity (Table 1 and Fig. 3).

Discussion

We sought leaky *sun* alleles to test two alternative hypotheses proposed by Kloth et al. (1987) for the *Sun* locus. The first stated that *sun* is a lesion *within* the *Eul* locus which is most easily explained as encoding the primary sequence of the embryo-specific urease. In the second model *Sun* is a distinct control locus exerting *cis* effects on the tightly linked *Eul* locus (which specifies enzyme aggregation state). By the second model all leaky *sun* mutants should produce lowered levels of normal urease while by the first some leaky *sun* mutants should produce partially inactive urease. The altered embryo-specific urease characteristics of *n6/n6* tend to favor the first model.

The urease in *n6/n6* embryos has altered temperature stability (Fig. 1), aggregation state (Fig. 2) and pH dependence (Table 5) while the embryo urease of *n8/n8* is normal by the same criteria. In addition the *n8/n8* urease appears to have a higher turnover number (k_{cat}) than the *n6/n6* urease: crude extracts of *n8/n8* have seven times the urease specific activity of *n6/n6* embryos (Tables 1, 4) but at most only one-tenth to one-fifth the urease protein (see Western blot of Fig. 4).

We thus propose that the *sun* and *Eul* alleles are in the same locus in which lesions can affect urease transcript level (transcription, transcript stability or processing: *sun, n4, n7*), enzyme aggregation state (*Eul-a, Eul-b, n6*), and catalytic properties such as temperature sensitivity, pH dependence and k_{cat} (*n6*). To confirm that the "*Sun-Eul*" locus encodes urease, we are mapping this locus relative to RFLP (restriction fragment length polymorphic) loci revealed by cloned urease probes (Krueger et al. 1987).

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