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Characterization of the Maize *Globulin-2* Gene and Analysis of Two Null Alleles

Alan L. Kriz[1] and Nancy H. Wallace[1,2]

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*The most abundant proteins present in maize (*Zea mays* L.) embryos are saline-soluble globulins. A* M_r *45,000 globulin component, designated GLB2, is encoded by the* Glb2 *gene. A cDNA clone corresponding to* Glb2 *was used as radiolabeled probe to examine the expression of* Glb2 *in developing embryos and other maize tissues.* Glb2 *transcripts accumulate during embryo development and are not detectable in germinating kernels.* Glb2 *transcripts are found only in the developing embryo, and not in endosperm, seedling, or unfertilized ears. Analysis of globulin profiles in embryos homozygous for either a previously described null allele,* Glb2-0, *or a novel null allele,* Glb2-N1, *revealed that these embryos lack not only the GLB2 protein but also globulins of lower molecular mass which may represent processed forms of GLB2. Southern blot analysis of DNA from* Glb2-0/0 *and* Glb2-N1/N1 *plants in which a* Glb2*-specific clone is used as probe indicates that the two null alleles are genetically distinct.*

KEY WORDS: globulin; null allele; seed storage protein; *Zea mays* L.

INTRODUCTION

Maize globulins represent a group of saline-soluble proteins which accumulate primarily in the developing embryo. The most abundant globulin is GLB1, a M_r 63,000 protein encoded by the *Glb1* gene (Kriz, 1989) which was originally described by Schwartz (1979) as *Pro.* Another relatively abundant

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[1] Department of Agronomy, University of Illinois, Urbana, Illinois 61801.
[2] Present address: DEKALB Plant Genetics, Eastern Point Rd., Groton, Connecticut 06340.

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globulin found in the maize embryo is GLB2, a M_r 45,000 protein encoded by the *Glb2* gene. As described below, both GLB1 and GLB2 possess characteristics of seed storage proteins, which function to provide a source of nitrogen and carbon to the seedling during the early stages of seed germination.

A common characteristic of seed storage proteins described to date is that they are usually encoded by multigene families (Shotwell and Larkins, 1989). In contrast, the *Glb1* gene exists as a single copy in the haploid genome as determined by genetic analyses of several electrophoretic variants (Schwartz, 1979). This was recently confirmed at the molecular level by gene copy-number reconstruction analysis (Belanger and Kriz, 1989). Although we have not observed electrophoretic variants for GLB2, a null allele for *Glb2* has been identified (Kriz, 1989). GLB2 also appears to be encoded by a single gene as determined by genetic analyses in which the *Glb2-0* null allele segregates as a Mendelian recessive trait (Kriz, 1989).

Amino acid analysis of GLB1 and GLB2 reveals that both of these globulins contain high amounts of arginine, which can serve as a potential nitrogen source upon germination, and low amounts of cysteine and methionine (Kriz, 1989). Thus, the amino acid composition of GLB1 and GLB2 is similar to that of globulin seed storage proteins present in other plants. The developmental accumulation pattern of GLB1 and GLB2 was determined by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of total protein extracts from maize embryos at different stages of embryogenesis (Kriz, 1989). The proteins are detectable by 24 DAP and increase in amount throughout development. Neither GLB1 nor GLB2 is detectable in germinating maize kernels (Kriz, 1989; Kriz *et al.*, 1990). Such a regulated developmental expression of the maize globulin genes is similar to that observed for other seed storage protein genes. Maize embryo globulins appear to be unique among seed storage globulins in that each of the major components is encoded by only a single gene (Kriz, 1989). This is in contrast to the situation observed for 7 S storage globulins in dicots, which are encoded by small multigene families, as well as the maize endosperm alpha-zeins, the primary storage proteins of the maize kernel, which are encoded by a large multigene family (reviewed by Shotwell and Larkins, 1989).

The maize *Glb1* gene has been extensively characterized at the biochemical and molecular levels (Schwartz, 1979; Kriz and Schwartz, 1986; Belanger and Kriz, 1989). This gene is highly polymorphic, and several different *Glb1* alleles have been identified (Schwartz, 1979; Osterman, 1988). The three most commonly occurring alleles have the designations *Glb1-L, -I,* and *-S,* for *Large, Intermediate,* and *Small* proteins, respectively (Schwartz, 1979). The *Glb1* polypeptide gene product of each allele is processed by a combination of co- and posttranslational events to form the mature GLB1

protein (Kriz and Schwartz, 1986; Belanger and Kriz, 1989). The two major processing intermediates, designated proGLB1′ and GLB1′, often persist in mature embryos.

In contrast to the situation observed for *Glb1,* the only genetic variants identified for *Glb2* are the null alleles *Glb2-0* (Kriz, 1989) and *Glb2-N1* (this report). To extend our knowledge of maize *Glb* gene structure and expression, we initiated studies of the *Glb2* gene at the molecular level. Recently we reported the isolation and characterization of a cDNA clone corresponding to *Glb2* (Wallace and Kriz, 1991). We present here analysis of expression of the *Glb2* gene in the developing maize embryo and in other plant tissues. We also present comparisons of the two *Glb2* null alleles with each other, as well as with the normal *Glb2-+* allele, by Southern blot analysis, which indicates that the molecular defect in *Glb2-0* is distinct from that in *Glb2-N1.*

MATERIALS AND METHODS

Developmentally staged tissues were obtained from field-grown plants of appropriate genotypes as previously described (Belanger and Kriz, 1989). The maize inbred lines Va26 (*Glb1-S/S*) and W64A (*Glb1-L/L*) are both homozygous for a functional *Glb2* wild-type allele, designated *Glb2-+*. Maize lines homozygous for null alleles of either *Glb1* or *Glb2* have been described by Schwartz (1979) and Kriz (1989), respectively. A double null line of the constitution *Glb1-0/0 Glb2-0/0* was selected by screening kernels from selfed F_2 plants from an initial cross of a *Glb1-0/0 Glb2-+/+* plant by a *Glb1-L/L Glb2-0/0* plant.

Molecular weight standards of protein and RNA were obtained from Bethesda Research Laboratories (BRL; Gaithersburg, Maryland). Nitrocellulose and Magnagraph nylon membranes were from Micron Separations, Inc. (Westboro, Massachusetts). A Stratalinker 1800 apparatus (Stratagene, La Jolla, California) was used for the UV linkage of RNA and DNA to nylon membranes. DNA fragments from the cDNA clones pcGlb1S (Belanger and Kriz, 1989) and pcGlb2 (Wallace and Kriz, 1991), corresponding to the *Glb1* and *Glb2* genes, respectively, were isolated from agarose gels by using the Gel/X Extractor (Genex Corporation, Gaithersburg, Maryland) and radiolabeled with [α-^{32}P]dATP (3000 Ci/mmol; New England Nuclear, Boston) by using a commercial random priming kit (BRL).

Protein Extraction, SDS-PAGE, and Immunoblot Analysis

Proteins were extracted from individual mature embryos by using an SDS sample buffer at a ratio of 50 mg/ml as previously described (Belanger and

Kriz, 1989). Five microliters of each sample was loaded on a 12% polyacrylamide gel and subjected to SDS-PAGE (Laemmli, 1970). Gels were electroblotted onto nitrocellulose by using a semidry blotting apparatus (Polyblot; American Bionetics, Hayward, California). Whole globulin antiserum at a 1:1000 dilution was used for immunodetection of globulins as previously described (Puckett and Kriz, 1991). This antiserum reacts with GLB1, as well as the protein processing intermediates proGLB1′ and GLB1′, GLB2, and globulins of M_r 27,000 and M_r 26,000.

Isolation and Analysis of Maize DNA and RNA

Total RNA was extracted from frozen maize tissues by the method of Cox (1968). RNA was also extracted from dry mature kernels, mature kernels imbibed overnight in water, and kernels which were imbibed overnight and allowed to germinate for 3 days as previously described (Kriz *et al.,* 1990). For northern blot analysis, 10 μg of each RNA sample was subjected to electrophoresis on formaldehyde agarose gels. Hybridization with radiolabeled probes was as previously described (Belanger and Kriz, 1989).

Total maize DNA was extracted from frozen unfertilized ears or from leaf tissue of 14-day-old seedlings by the method of Dellaporta *et al.* (1983). Five micrograms of DNA was loaded on a 0.8% agarose gel and subjected to electrophoresis and Southern blot analysis as previously described (Belanger and Kriz, 1989). Both Southern and northern hybridization analyses were performed at $T_M - 25°C$.

RESULTS

SDS-PAGE and Immunoblot Analysis of *Glb* Variants

Variation in proteins encoded by the *Glb* genes is readily apparent from SDS-PAGE (Fig. 1a) and immunoblot analysis (Fig. 1b) of total embryo protein extracts. The GLB1 and GLB2 proteins are normally present at high levels in mature embryos possessing functional *Glb1* and *Glb2* alleles. Allelic variation is exhibited as either (1) size polymorphism, as in the case of *Glb1-S* (*Small* protein) and *Glb1-L* (*Large* protein) alleles, or (2) complete absence of protein product, as in embryos homozygous for either the *Glb1-0* or the *Glb2-0* null alleles. This variation in these polypeptides represents the only observable phenotype for plants of different *Glb* genotypes. No obvious differences in kernel or embryo size, development, or germination are detectable among the various genotypes, even in *Glb1-0/0 Glb2-0/0* plants which are completely lacking in embryo globulins (Fig. 1b). The immunoreactive polypeptides slightly larger than GLB2 which are present in the

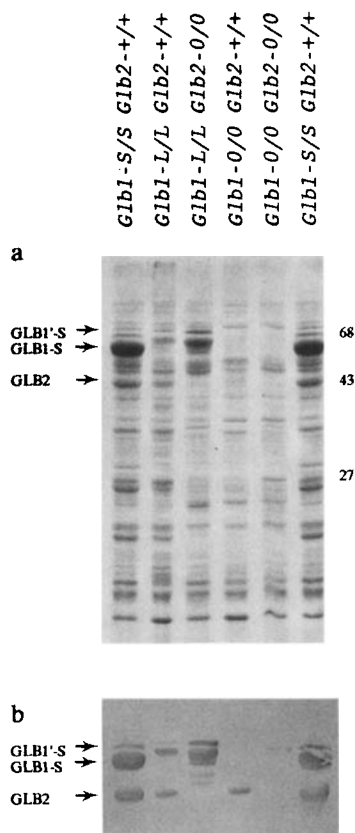

Fig. 1. SDS gel and immunoblot analysis of protein extracts from mature maize embryos of different *Glb* genotypes. (a) Total protein extracts prepared from individual embryos as described under Materials and Methods were subjected to electrophoresis in a 12% polyacrylamide gel which was subsequently stained with Coomassie blue. Positions of *Glb1*- and *Glb2*-encoded proteins are as indicated, and the positions of molecular weight markers in kilodaltons (kD) are provided on the right side of the gel. (b) Duplicate samples from the same gel were subjected to immunoblot analysis in which antiserum to whole globulin was used as probe. GLB1′-S is a protein processing intermediate of GLB1-S (Kriz and Schwartz, 1986).

Glb2-0/0 embryo extract (Fig. 1b) are most likely derived from *Glb1*-encoded polypeptides (Belanger and Kriz, 1989) and are apparently not related to the *Glb2* gene product. This is evident from analysis of extracts from embryos homozygous for both *Glb1-0* and *Glb2-0,* in which no immunoreactive polypeptides are present. A possible explanation for the presence of these polypeptides in the *Glb2-0/0* samples is presented in the Discussion. This analysis also demonstrates that the *Glb2* null phenotype is independent of the *Glb1* null phenotype. Embryos homozygous for *Glb2-0* contain *Glb1*-encoded polypeptides, and embryos homozygous for *Glb1-0* contain GLB2.

In addition to recognizing GLB1 and GLB2, antiserum to whole embryo globulin also reacts with two polypeptides of M_r 27,000 and M_r 26,000 (Puckett and Kriz, 1991) which are present in the whole globulin fraction of mature embryos (Kriz, 1989). These polypeptides, collectively referred to as M_r 27,000 globulins below, are absent in *Glb2-0/0* embryos (Fig. 2). The

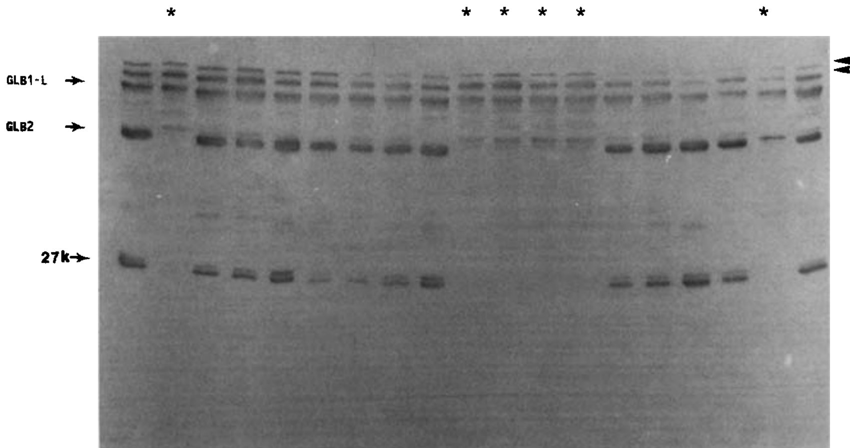

Fig. 2. Immunoblot analysis of protein extracts from embryos of an F_2 ear segregating for the *Glb2-+* and *Glb2-0* alleles. Extracts from embryos homozygous for *Glb2-0* are indicated by asterisks. Positions of GLB1-L, GLB2, and the M_r 27,000 globulins (27K) are as indicated. Positions of the larger M_r protein processing intermediates proGLB1′-L and GLB1′-L are marked by arrowheads on the right. Faint immunoreactive bands apparent in *Glb2-+* samples at positions intermediate to GLB2 and the 27K polypeptides are presumably GLB2 degradation products.

nature of these polypeptides has not been determined, but the presence or absence of these polypeptides is always correlated with the *Glb2* phenotype. In a screen of 259 F_2 progeny embryos obtained by initially crossing *Glb2-+/+* plants with *Glb2-0/0* plants, all 69 individuals scored as *Glb2-0/0* lacked the M_r 27,000 and M_r 26,000 polypeptides, while all those scored as *Glb2-+* contained these proteins. Likewise, the presence of the immunoreactive polypeptides of molecular mass larger than that of GLB2 is always correlated with the *Glb2-0/0* genotype (Fig. 2). The *Glb1*-specific protein processing intermediates proGLB1′ and GLB1′ (Kriz and Schwartz, 1986; Belanger and Kriz, 1989) are also prominent in these samples.

Northern Blot Analysis of *Glb* Null Variants

The above immunoblot analysis (Fig. 1b) indicated that the *Glb2* null phenotype is independent of the *Glb1* null phenotype. To determine if this was also the case at the mRNA level, northern blot analysis was performed to determine the relative amounts of *Glb1* and *Glb2* transcripts in embryos homozygous for either *Glb1-0* or *Glb2-0.* Total RNA was extracted from 27 DAP embryos of the inbred line Va26, *Glb1-0/0* embryos, and *Glb2-0/0* embryos. Samples were run in triplicate and then individually hybridized with ^{32}P-labeled probe from either *Glb1* cDNA, *Glb2* cDNA, or both (Fig. 3). As expected, normal levels of the *Glb1* transcript are present in *Glb2-0/0*

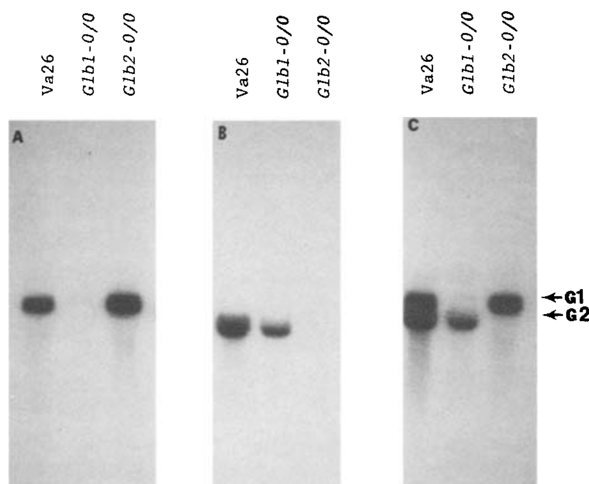

Fig. 3. Northern blot analysis of *Glb* transcripts from 27 DAP Va26, *Glb1-0/0*, and *Glb2-0/0* embryos. Ten micrograms of each sample was run in triplicate on a single gel and transferred to a nylon membrane. The membrane was cut into three parts and hybridized with radiolabeled inserts from pcGlb1S (A), pcGlb2 (B), or both pcGlb1S and pcGlb2 (C).

embryos (Fig. 3A), and *Glb2* transcripts are present in *Glb1-0/0* embryos but not in *Glb2-0/0* embryos (Fig. 3B). Use of both probes in a single hybridization experiment (Fig. 3C) demonstrates that *Glb1* and *Glb2* transcripts are readily distinguished by their electrophoretic mobilities. The apparent size of *Glb1* transcripts is 2.4 kb (Belanger and Kriz, 1989), and that of *Glb2* transcripts is 1.7 kb (Fig. 5). Although extremely low levels of *Glb1* transcripts are detectable in *Glb1-0/0* embryos [Figs. 3A and C (also see Belanger and Kriz, 1989)], no *Glb2* transcripts have been detected in *Glb2-0/0* embryos (Fig. 3 and additional data not shown). The absence of *Glb2* transcripts in *Glb2-0/0* embryos provides additional evidence that the immunoreactive bands with molecular mass slightly larger than that of GLB2 (Figs. 1b, 2) are unrelated to the *Glb2* gene.

Differential Expression of *Glb* Genes in Maize Embryos and Tissue Specificity of *Glb2* Transcripts

The relative amounts of *Glb1* and *Glb2* transcripts present during embryo development and germination were evaluated by northern blot analysis (Fig. 4). Total RNA was extracted from embryos at 15 to 36 DAP, as well as from embryos of dry mature kernels, kernels which had been imbibed overnight in water, and kernels which were allowed to germinate for 3 days. *Glb1*

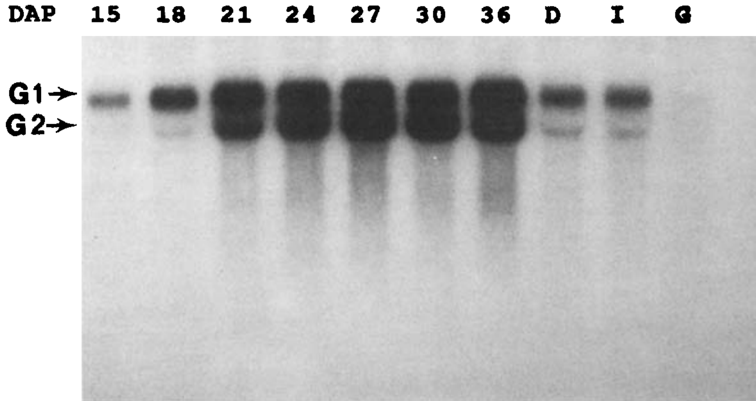

Fig. 4. Northern blot analysis of *Glb* transcripts in developing, mature, and germinating embryos. Total RNA was obtained from developing embryos of the inbred line Va26 at 15 to 36 DAP, from embryos of mature dry kernels (D), embryos of kernels imbibed for 24 hr in H_2O (I), and embryos of kernels allowed to germinate on moistened filter paper for 3 days (G). The samples were subjected to northern blot analysis in which radiolabeled inserts from both pcGlb1S and pcGlb2 were used as probe. Positions of *Glb1* and *Glb2* transcripts are indicated by G1 and G2, respectively.

transcripts are present from 15 to 36 DAP and persist at reduced, but significant, levels in embryos from dry and imbibed kernels. In contrast, low levels of *Glb2* transcripts are first detected at 18 DAP, after which they are present at levels similar to those of *Glb1* transcripts at 21 to 36 DAP. The amounts of *Glb2* transcripts in embryos from mature dry and imbibed kernels are greatly reduced relative to the levels of *Glb1* transcripts in these samples. Neither *Glb1* nor *Glb2* transcripts are detectable in embryos from germinating kernels. The presence of *Glb2* transcripts was investigated in several tissues from the maize inbred line Va26. As shown in Fig. 5, *Glb2* transcripts were detected only in developing embryos.

Characterization of a Second *Glb2* Null Allele

In a screen of maize germplasm for *Glb* variants by using SDS-PAGE and immunoblot analysis of embryo protein extracts, embryos of the inbred line H99 were found to lack both the GLB2 protein and the M_r 27,000 globulins (Figs. 6A and B, lane 3). An allelism test was performed by crossing a *Glb2-0/0* plant with an H99 plant, and 20 of the resultant F_1 embryos were examined by immunoblot analysis. All of these embryos lacked GLB2 and the M_r 27,000 globulins. Analysis of a representative F_1 embryo extract is shown in Fig. 6 (lane 4 in each panel). This demonstrates that the *Glb2* null trait present in the inbred line H99 is allelic to *Glb2-0*. The novel H99 allele

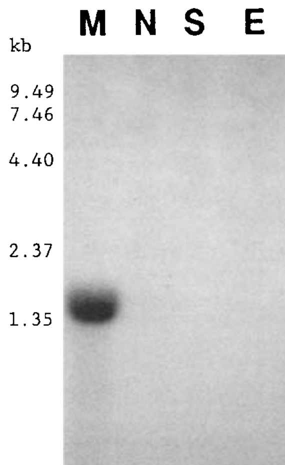

Fig. 5. Tissue specificity of *Glb2* transcripts. Ten micrograms of total RNA from 27 DAP embryos (M), 27 DAP endosperm (N), 7-day-old seedling (S), and immature unfertilized ears (E) from the inbred line Va26 were subjected to northern blot analysis in which the radiolabeled pcGlb2 insert was used as probe. Positions of RNA size markers are as indicated.

has been designated *Glb2-N1*. Like *Glb2-0*, *Glb2-N1* also appears to segregate as a Mendelian recessive trait. In a preliminary screen of 30 F_2 progeny which resulted from H99 initially being outcrossed to a *Glb2*-+/+ plant, 9 individuals (30%) exhibited the *Glb2* null phenotype (absence of GLB2 as well as the M_r 27,000 globulins) (data not shown).

There is a distinct difference, however, between *Glb2-0/0* and *Glb2-N1/N1* embryos with respect to the presence of the immunoreactive polypeptides of molecular mass larger than that of GLB2. These polypeptides,

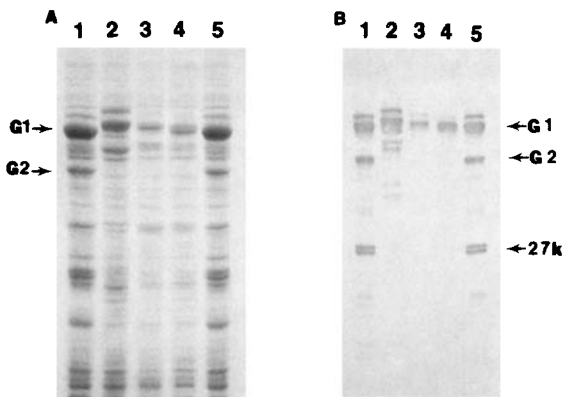

Fig. 6. Identification of a second *Glb2* null allele. Total protein extracts from individual embryos of different *Glb2* genotypes were subjected to SDS-PAGE (A) and immunoblot analysis in which antiserum to whole globulin was used as probe (B). Lanes 1 and 5, *Glb2*-+/+ (Va26); lane 2, *Glb2-0/0;* lane 3, *Glb2-N1/N1;* lane 4, *Glb2-0/N1.* G1, GLB1; G2, GLB2; 27K, M_r 27,000 globulins.

although consistently present in *Glb2-0/0* embryos (Fig. 6B, lane 2; see also Fig. 2), are absent in *Glb2-N1/N1* embryos (Fig. 6, lane 3). These polypeptides are also absent in F_1 embryos of the constitution *Glb2-0/N1* (Fig. 6, lane 4). Although the reason for these differences is not known, possible explanations for these observations are presented in the Discussion.

Southern Blot Analysis of DNA from Plants Homozygous for Different *Glb2* Alleles

Previous genetic analyses (Kriz, 1989) as well as those described above indicate that GLB2 is encoded by a single locus. In Southern blot analysis of maize DNA from plants homozygous for a functional *Glb2* allele, a *Glb2*-specific probe from the pcGlb2 cDNA clone hybridizes to a single *Eco*RI fragment of 1.8 kb (Fig. 7). DNAs from plants homozygous for either of the two *Glb2* null alleles, however, exhibit hybridization patterns distinct from each other. No 1.8-kb *Eco*RI fragment is present in DNA from *Glb2-0/0* plants, but a band of this size is present in *Glb2-N1/N1* plants. Both *Glb2-0/0* and *Glb2-N1/N1* plants possess *Eco*RI fragments of 7 to 7.5 kb in size which exhibit hybridization intensities weaker than that of the 1.8-kb fragment, and these fragments may represent gene sequences similar to, but distinct from, the *Glb2* structural gene. This analysis indicates that the defect in the

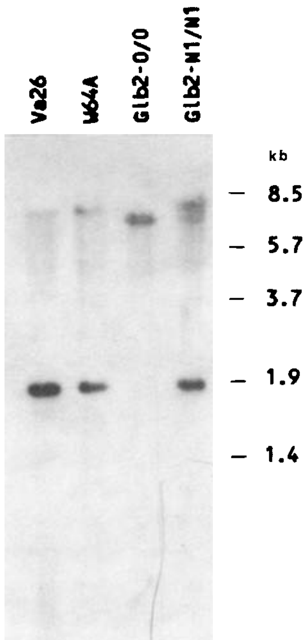

Fig. 7. Southern blot analysis of DNA from plants of different *Glb2* genotypes. DNA was cleaved with *Eco*RI, subjected to electrophoresis in a 0.8% agarose gel, and subjected to Southern blot analysis in which a radiolabeled fragment from pcGlb2 was used as probe. The inbred lines Va26 and W64A are homozygous for the *Glb2-+* allele. Positions of lambda size markers, generated by *Bst*EII cleavage, are as indicated in kilobases.

Glb2-0 allele may be due to some type of rearrangement, possibly a deletion, of the structural gene. The presence of a 1.8-kb *Eco*RI fragment in *Glb2-N1/N1* plants suggests that the null phenotype in this case is due to a different gene defect from that observed for *Glb2-0.*

DISCUSSION

We present here characteristics of the maize *Glb2* gene, which encodes the M_r 45,000 embryo-specific globulin GLB2. GLB2 is similar to the 7 S globulin (vicilin) class of proteins present in seeds of both dicots and monocots with respect to amino acid composition and accumulation profile (Kriz, 1989) and amino acid sequence (Wallace and Kriz, 1991). The present analysis demonstrates that *Glb2* expression, as determined by northern blot analysis, is similar to that of genes encoding other seed storage proteins in that *Glb2* transcripts are seed specific (Fig. 5) and present at high levels during most of seed development but at low levels in the mature seed (Fig. 4). This pattern of gene expression, which coincides with the period of cell expansion in the developing seed, is typical of genes encoding seed storage proteins (Higgins, 1984; Gatehouse *et al.,* 1986).

A unique feature of maize embryo globulins with respect to other seed storage globulins is that each of the major components, GLB1 and GLB2, is encoded by only a single gene (Schwartz, 1979; Kriz, 1989) which facilitates molecular analyses. In addition, the availability of allelic variants for both the *Glb1* and the *Glb2* genes allows for detailed analysis of plant gene structure and expression. The *Glb* genes are particularly suited to such studies since they are not essential for seed development, maturation, or germination; this is apparent from observations of *Glb1-0/0, Glb2-0/0,* and *Glb1-0/0 Glb2-0/0* plants, all of which are phenotypically normal except for the absence of specific seed globulins encoded by *Glb1* or *Glb2* (Fig. 1). Embryo globulins clearly serve a secondary role to endosperm zeins as storage proteins in the kernel.

As indicated above, allelic variants have been identified for both *Glb1* and *Glb2.* Although several electrophoretic variants (size alleles) have been described for *Glb1* [(Schwartz, 1979; Osterman, 1988) see also Fig. 1], the only *Glb2* variants identified to date are nulls. Embryos homozygous for either of the two *Glb2* null alleles *Glb2-0* and *Glb2-N1* are phenotypically similar in that they lack the GLB2 protein as well as the M_r 27,000 globulins (Figs. 2 and 6). Although the origin of the M_r 27,000 globulins has not been determined, the presence of these polypeptides is always correlated with the presence of GLB2. It is therefore likely that the M_r 27,000 globulins are related to GLB2, and it is possible that these smaller polypeptides are derived from GLB2 by proteolytic cleavage. This problem may be addressed

by investigating synthesis of *Glb2*-encoded polypeptides through *in vivo* radiolabeling experiments as previously described for analysis of GLB1 synthesis (Kriz and Schwartz, 1986).

Although embryos homozygous for either the *Glb2-0* or the *Glb2-N1* allele are similar with respect to the absence of GLB2 and the M_r 27,000 globulins, they differ with respect to the presence of immunoreactive bands of molecular mass larger than that of GLB2 (Fig. 6). These polypeptides were found in all *Glb2-0/0* embryos examined (over 100 individuals), but they were not present in any of the 50 *Glb2-N1/N1* individuals examined to date (data not shown). Several observations indicate that the presence or absence of these immunoreactive polypeptides, while a possible consequence of the *Glb2* null phenotype, is not due to homozygosity for either of the *Glb2* null alleles per se. First, these polypeptides are not related to the *Glb2* gene product in that *Glb2-0/0* embryos lack *Glb2*-specific transcripts (Fig. 3). Second, these polypeptides are apparently related to the *Glb1* proteins in that (a) they are not present in embryos of the constitution *Glb1-0/0 Glb2-0/0* (Fig. 1) and (b) these polypeptides are recognized by antiserum specific for *Glb1*-encoded proteins (data not shown). We previously demonstrated that *Glb1*-specific polypeptides of molecular mass lower than that of GLB1 are often present in normal maize embryos (Belanger and Kriz, 1989), and these presumably arise from proteolytic cleavage of the mature protein at certain sites as is the case for pea vicilin (Gatehouse *et al.*, 1983). The extent to which these cleavage products accumulate appears to be somewhat dependent on genetic background, independent of *Glb2* genotype, in that embryos of the inbred lines W64A and Va26, both of which are *Glb2-+/+*, contain different amounts of *Glb1*-related cleavage products (Belanger and Kriz, 1989). The occurrence of these *Glb1*-related polypeptides in *Glb2-0/0* embryos may be due to the absence of GLB2, and/or the M_r 27,000 globulins, in the cell which may somehow associate with GLB1 to prevent such cleavage. The absence of such cleavage products in both *Glb2-N1/N1* and *Glb2-0/N1* embryos indicates that absence of GLB2 and the M_r 27,000 globulins does not necessarily lead to accumulation of these products. The differences observed for *Glb2-0/0, Glb2-N1/N1,* and *Glb2-0/N1* embryos may be due to variability in genetic background other than *Glb2* genotype. This problem is currently being addressed by backcrossing the *Glb2-0* and *Glb2-N1* alleles into different inbred lines to determine the extent of genetic background effects on accumulation of the specific *Glb1* protein cleavage products.

Genetic analysis indicates that GLB2 is encoded by a single locus. This is confirmed by Southern blot analysis in which DNA from plants of the inbred lines Va26 and W64A, both of which are *Glb2-+/+,* exhibit a single *Glb2*-specific *Eco*RI fragment (Fig. 7). Southern blot analysis also reveals

that the two *Glb2* null alleles are genetically distinct as is evident from the different hybridization patterns observed for DNA from *Glb2-0/0* and *Glb2-N1/N1* plants. The absence of a 1.8-kb *Eco*RI fragment in *Glb2-0/0* plants suggests that the defect in this case involves some type of rearrangement, possibly a deletion, of the *Glb2* structural gene; such a situation has been described by Rao *et al.* (1989) for a gene encoding a major pea seed albumin protein. Plants homozygous for the *Glb2-N1* allele contain a 1.8-kb *Eco*RI fragment, indicating that the null phenotype in this case is not due to gross structural rearrangement of the *Glb2* gene. The defect in *Glb2-N1* may be due to minor nucleotide sequence variation, such as a translational frameshift mutation, as is the case for the *Glb1-0* null allele (F. C. Belanger and A.L.K., unpublished observations). Similar situations have been described for null alleles of genes encoding other seed proteins such as bean lectin (Voelker *et al.,* 1986), soybean glycinin (Scallon *et al.,* 1987), and soybean Kunitz trypsin inhibitor (Jofuku *et al.,* 1989). The nature of the 7- and 7.5-kb fragments, which exhibit weaker hybridization with the *Glb2* cDNA probe than the 1.8-kb fragment, has not been determined; these may represent rearrangements of the *Glb2* structural gene itself or they may be related sequences distinct from the *Glb2* gene. Efforts are under way to investigate inheritance of these higher molecular weight restriction fragments in progeny from appropriate crosses to determine if these particular fragments are genetically linked to the *Glb2* locus.

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