Comparison of the Effect of *Shrunken*-4, *Opaque*-2, *Opaque*-7, and *Floury*-2 Genes on the Zein Content of Maize During Endosperm Development[1]

C.-Y. TSAI and A. DALBY, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907

ABSTRACT

The starch-deficient mutant *shrunken*-4 (sh_4) reduces the zein content of maize (*Zea mays* L.) endosperm in a pattern distinct, in two ways, from *opaque*-2, *opaque*-7 and *floury*-2. First, zein accumulation does not start in sh_4 until 22 days after pollination, whereas other mutant and normal lines have synthesized approximately 30% of their final zein content by this time. Second, mature kernels of sh_4 contain only 10% of the normal content of zein per kernel; the other mutants contain 25 to 50% of the normal content. It is not known whether this is a direct effect of the sh_4 gene on zein synthesis, or a secondary consequence of defective starch metabolism.

Three mutant genes, *opaque*-2 (o_2), *floury*-2 (fl_2) and *opaque*-7 (o_7), of maize (*Zea mays* L.) are known which cause major alterations in the overall amino acid

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composition of the mature endosperm protein (1–3). These genes are important nutritionally because they effect increases in the proportions of lysine and tryptophan. In the homozygous state, $o_2/o_2/o_2$, $fl_2/fl_2/fl_2$, $o_7/o_7/o_7$, each mutant gene produces its effect primarily through a marked reduction in the amount of the low-lysine prolamine complex (zein) stored in the endosperm relative to a normal (+/+/+) control. Generally there is also an increase in the glutelin fraction (1,4–11). A number of other changes in enzyme and protein composition caused by these genes have also been reported (12–15). All three mutant genes produce a characteristic, visually opaque seed resulting from the soft, floury, texture of the endosperm, in contrast to the corresponding normal allele in which the endosperm is hard, horny, and translucent. Unlike o_2 and o_7, which are fully recessive, fl_2 shows a gene dosage effect in which there is a stepwise reduction in the zein level according to the number of doses (from 0 to 3) of fl_2 in the endosperm.[2]

Misra et al. (10), in a detailed study of the mutant *brittle*-2, noted that a number of other mutant genes affecting starch synthesis also increased the proportion of lysine in mature maize endosperm, both singly and in combination with o_2. They concluded that all these mutant genes behaved in a manner similar to o_2, o_7, and fl_2 by reducing the zein and increasing the level of non-zein protein fractions high in lysine.

We now report on a starch-deficient mutant, *shrunken*-4 (sh_4) which reduces zein in a manner distinct from o_2, o_7, and fl_2. Complete zein development curves for these three mutants are also presented.

MATERIALS AND METHODS

All maize samples were produced by self-pollination at the Purdue University Agronomy Farm, West Lafayette, Ind., during 1972 unless otherwise indicated. Inbreds W64A and W22, and their homozygous mutant versions, W64Ao_2, W64Afl_2, W22o_2 and W22o_7, were harvested at intervals from shortly after pollination until maturity. W64Ao_2 and W22o_7 were spontaneous mutants, whereas W64Afl_2 and W22o_2 were near isogenic (back-crossed six times to the recurrent parent) with the normal lines. Samples of homozygous *shrunken*-4 (sh_4) and its normal control (from a segregating ear) were harvested at 22 days post-pollination and at maturity. Kernels were cut from the ear, bulked, and frozen in Dry Ice in the field, and stored at –20° C.

For processing, approximately 10-g. samples of intact kernels were weighed, lyophilized to constant weight, ground in a Waring Blendor for about 30 sec., and a 0.5 g. sample powdered in a miniature ball mill (Wig-L-Bug, Crescent Dental Mfg. Co., Chicago, Ill.) for 5 min.

The zein content of the powder was determined by the method of Dalby (16), and nitrogen determined by the micro-Kjeldahl method (17).

RESULTS AND DISCUSSION

The pattern of zein accumulation in maize endosperm during development may be expressed in two ways (Fig. 1). On a dry weight basis there is apparently

[2]Dalby and Tsai, in preparation.

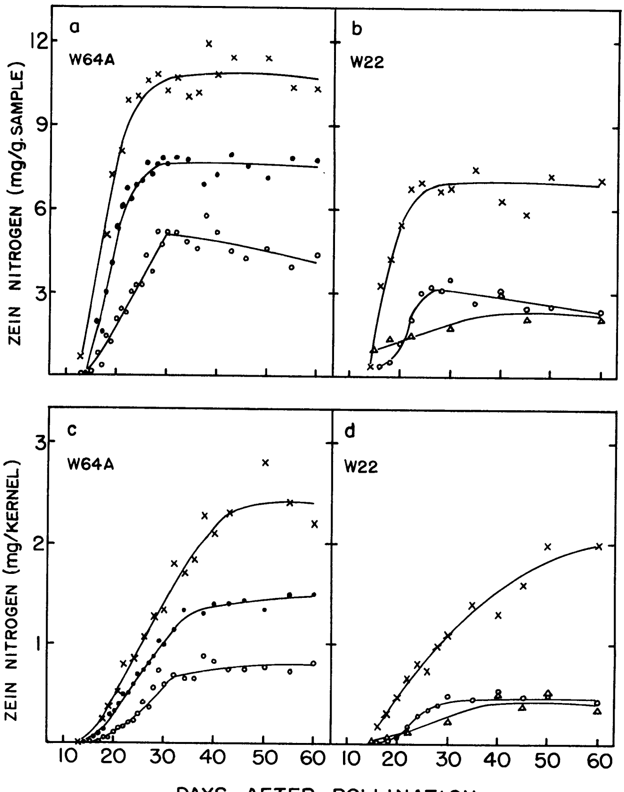

Fig. 1. Zein accumulation patterns in two maize inbred lines and their mutant versions: (a) and (c), W64A; (b) and (d) W22. Open circles, *opaque*-2; open triangles, *opaque*-7; solid circles, *floury*-2; crosses, normal control.

no increase in zein beyond 30 days post-pollination in either the normal inbreds, or their mutant sublines, o_2,fl_2, and o_7 (Fig. 1, a and b). For o_2 there appears to be a decrease in zein. Thus, after 30 days the increase in zein is matched (+, o_7,fl_2) or exceeded (o_2) by the dry weight increase (mostly starch). In contrast, when the changes in zein are related to the individual kernel, zein increases in the normal lines continue until much later in development, although mutants show little or no increase in zein after 30 days (Fig. 1, c and d). In all cases, zein is present in detectable amounts no later than 15 days after pollination. Of the three mutant genes, o_2 and o_7 have the lowest rate of zein accumulation during development, whereas fl_2 shows intermediate levels between o_2 and normal.

At 22 days after pollination, homozygous kernels of *shrunken*-4 (i.e., $sh_4/sh_4/sh_4$) contain no zein, in contrast to the normal which reached zein levels (6.9 mg. nitrogen per g. dry weight) comparable with W64A+ and W22+ (Fig. 1). The same result was obtained in material grown in other years. Further, all normal and mutant lines other than sh_4 have already accumulated approximately 30% of their final zein content by this time (Fig. 1).

At maturity sh_4 contains zein, but the amount present per unit weight (3.3 mg. nitrogen per g.) is only about 50% of that found in normal kernels (6.7 mg. nitrogen per g.). This last observation is in general agreement with Misra et al. (10) who note that at maturity, sh_4 and a number of other starch-modifying mutants have a lowered zein content. A 50% reduction in zein at maturity would seem to place sh_4 in the same category as o_2, o_7, and fl_2 in terms of zein synthesis. However, this is a misconception arising out of the method of expressing the results, i.e. on a weight basis. This method does not take account of alterations in other parameters, such as the smaller amount of starch in sh_4, which leads to a kernel dry weight of only about 30% of normal. If the amount of zein synthesis is based on the biological unit, i.e. the individual kernel or endosperm, the zein in sh_4 at maturity is reduced to only 10% of that in the normal (0.20 versus 2.04 mg. nitrogen per kernel).

Thus, sh_4 is distinctly different from o_2, o_7, and fl_2 for two reasons: a) the long delay before the onset of zein accumulation, and b) the extremely small amount of zein actually accumulated. Since abnormalities in phosphorylases (18) and the level of pyridoxal phosphate[3] have been reported these differences may reflect a secondary consequence of a defect in starch metabolism produced by sh_4. An alternative explanation would be that of a pleiotropic gene effect of sh_4 on both zein and starch production. Whatever the explanation may be, sh_4 appears to delay zein accumulation and reduce the zein levels in mature seed far more extensively than any previously reported gene.

[3]Burr and Nelson, personal communication.

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