Zein Accumulation in Phenotypically Modified Lines of *Opaque-2* Maize[1]

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ABSTRACT

Phenotypically modified (i.e., containing regions of horny endosperm) selections of maize (*Zea mays* L.) A545o_2 and B37o_2 previously shown to have distinctive developmental patterns of ribonuclease activity did not show zein accumulation patterns different from unmodified (i.e., completely floury) versions of inbreds W64Ao_2. In contrast, the zein pattern of modified B14o_2 was different from unmodified B14o_2. Thus, although phenotype, ribonuclease, and zein levels are all changed by *opaque*-2, it appears that these characteristics can be further modified independently.

Previously it was shown that seven of nine maize (*Zea mays* L.) inbreds carrying the mutant gene *opaque*-2 (o_2) had a common pattern of endosperm ribonuclease (RNase) activity during kernel development (1). This pattern was characterized by a divergence in RNase activity between the o_2 and normal versions of each inbred, which occurred early in development and persisted to maturity. For one of the seven inbreds (W64A), evidence was presented suggesting that the effect of o_2 on the rate of accumulation lasted until 16 days after pollination; thereafter the rate was the same as in normal (2). In contrast, two inbreds, A545 and B37, showed little or no divergence between their normal and o_2 versions until 22 and 25 days after pollination, respectively (1). This indicated that the o_2 gene was not affecting RNase accumulation until much later in the development of these inbreds. A545o_2 and B37o_2 were also unusual phenotypically, in that they did not have the fully floury-type endosperm considered characteristic of o_2, but contained regions of horny endosperm. This suggested a correlation between changes in phenotype and RNase development pattern.

It was important, therefore, to examine zein (prolamine) accumulation in A545 and B37 as another parameter regulated by o_2 (3). For comparison, another

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inbred (B14) having two phenotypically distinct o_2 versions (floury and nonfloury) was available, permitting a further attempt at correlating phenotype with zein content.

MATERIALS AND METHODS

Maize samples were obtained by controlled pollination at the Purdue University Agronomy Farm, West Lafayette, Ind. Four inbred lines, W64A, B37, A545, and B14, and their homozygous mutant versions, W64Ao_2 (floury), B37o_2 (modified), A545o_2 (modified), were employed. The designation in

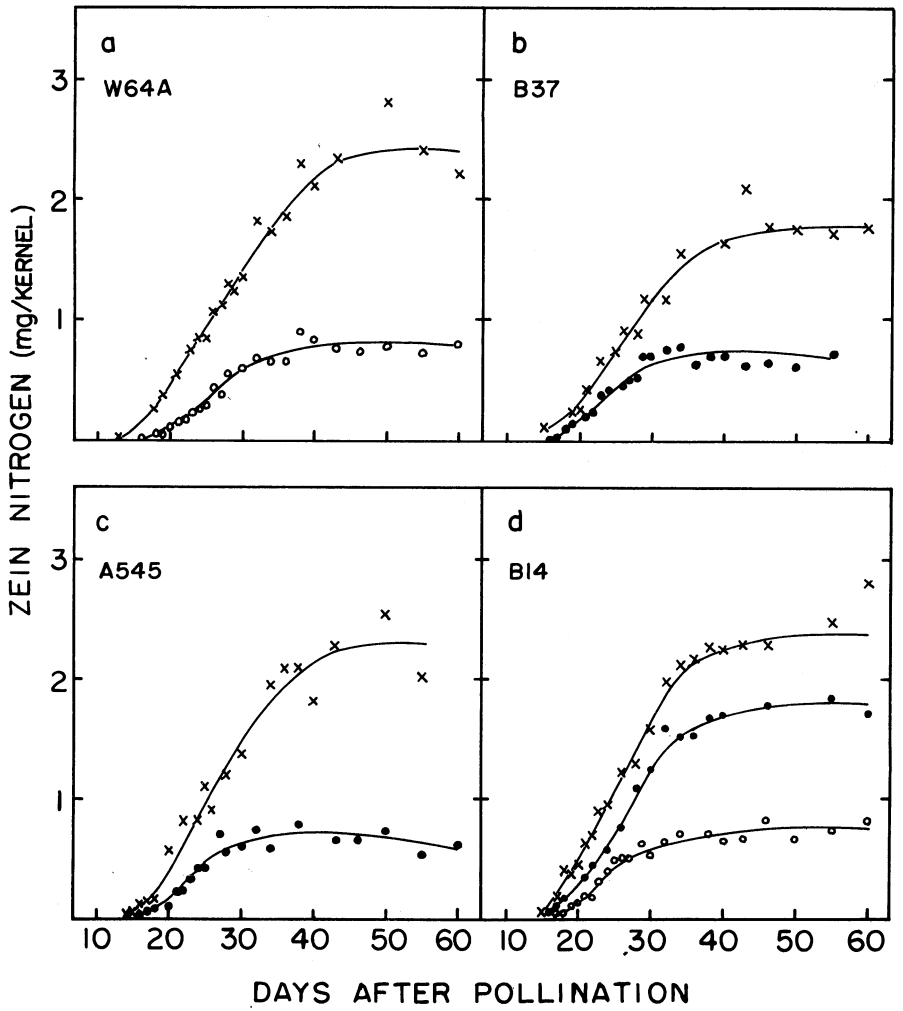

Fig. 1. Zein accumulation in normal (×), *opaque*-2 (open circles) and phenotypically modified *opaque*-2 (solid circles) versions of a) W64A, b) B37, c) A545, d) B14 maize inbreds.

parentheses refers to phenotype, "floury" being the fully opaque endosperm type and "modified" a mixed opaque and translucent endosperm, not fully normal. W64Ao_2 was a spontaneous mutant. B37o_2, A545o_2, and B14o_2 (floury) were near isogenic (back-crossed six times to the recurrent parent) with their

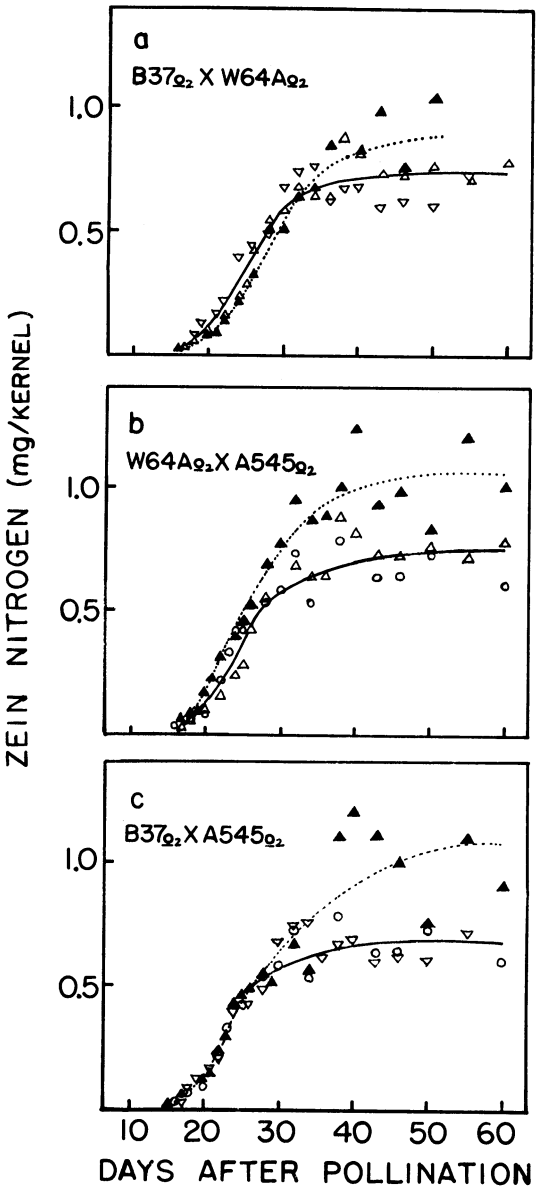

Fig. 2. Zein accumulation in W64Ao_2 (open, upright triangles), B73o_2 (open, inverted triangles), A545o_2 (open circles) and their crosses (solid triangles).

respective normal line. B14o_2 (modified) was a third back-cross. Each line was self-pollinated and harvested at intervals from shortly after pollination to maturity. The possibility of having complementation between W64Ao_2 and modified o_2 (B37 or A545) was investigated by sampling reciprocal crosses as indicated above.

Kernels were cut from the ears, bulked, frozen in dry ice in the field, and stored at −20° C. For processing, samples (approximately 10 g.) of whole, intact kernels were weighed, lyophilized to a constant weight, ground in a Waring Blendor for about 30 sec., and finally 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 (4) and nitrogen by a micro-Kjeldahl method (5). All values presented are the mean of duplicate determinations.

RESULTS

The accumulation of zein in the normal and o_2 versions of the modified inbreds A545 and B37 resembles that in the unmodified inbred W64A. In all cases the rate of zein accumulation is higher in the normal than in the o_2 version. Differences are readily detectable by about 16 days after pollination. Little or no increase in zein occurs after 30 days in any o_2 line, in contrast to about 40 days in the normal genotypes (Fig. 1, a,b,c).

Unlike A545 and B37, an o_2 selection of the inbred B14 having a modified phenotype shows a distinctly higher zein accumulation rate, and final zein level, than phenotypically floury B14o_2 (Fig. 1, d). The zein level is somewhat closer to normal than to the typical o_2.

In crosses between W64Ao_2, A545o_2, and B37o_2 there is no marked difference in zein accumulation rates between a cross and its inbred parents during approximately the first 30 days (Fig. 2). After 30 days the crosses tend toward a higher content of zein than their respective parental lines. The same result was obtained for the reciprocal crosses (not shown).

DISCUSSION

If the differences from the W64A pattern of ribonuclease development shown by phenotypically modified A545o_2 and B37o_2 and their normals (1) had carried over to the accumulation of zein, two results might have been anticipated. First, the rates of zein accumulation in A545o_2 and B37o_2 should be equal to those of their normal counterparts until some time during the fourth post-pollination week, followed by reduced rates resulting in final zein levels at maturity lower than their normal controls. Second, a possible complementation of gene activity in crosses between W64Ao_2 and the modified o_2 inbreds resulting in a prolongation of the period of activity of the o_2 gene (assuming that the 16 days' post-pollination cut-off time for o_2 activity previously suggested as applying to ribonuclease activity also applies to zein). Such complementation would have depended upon the particular dominance/recessive characteristics of the modifying gene(s) involved. Neither of these results was observed. The zein accumulation patterns in A545 and B37 were the same as in W64A and the final zein levels in the o_2 crosses were actually higher, rather than lower, relative to the parental types.

Thus, these results (in which a trend toward a normal phenotype *was not* accompanied by a zein content close to normal, coupled with a case (B14) in which a modified phenotype *was* accompanied by a change in the zein development pattern in o_2) suggest that the three parameters—phenotype, ribonuclease activity, zein level—altered by the o_2 gene are capable of being changed independently. This provides support for the belief that the current search for high-lysine (low zein) selections of o_2 lines having a normal, nonfloury phenotype is well founded.

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