

Effect of Maize (*Zea mays* L.) Endosperm Mutants on the Surface Relief of the Kernel Pericarp¹

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Summary. Relief studies on the outer surface of the kernel pericarp were conducted using interference microscopy. Comparisons involving pattern, cell relief height and cell width were made between dominant and recessive kernels at the waxy (*wx*), sugary (*su*₁), shrunken (*sh*₂) and opaque (*o*₂) loci. The pericarp of the dominant and recessive kernels at each locus was genetically identical. The effect of the alleles at the sugary locus was tested in two genetic backgrounds. At some loci, distinct differences in pattern, cell relief height and cell width were found between dominant and recessive kernels. Over all loci and kernel phenotypes, the means ranged from 1.56 to 2.86 μ for cell relief height and from 18.5 to 27.4 μ for cell width. Generally the recessive kernels had a greater relief height and width than the corresponding dominant kernels. The effect of the alleles at the sugary locus was altered by genetic background. Apparently, the alleles at these loci and genetic background can influence the relief of the outer surface of the kernel pericarp.

Introduction

By altering various biochemical pathways, endosperm mutants of maize produce many changes in kernel appearance and composition (Nelson 1967; Neuffer *et al.* 1968; Wassom and Hosenev 1973). Techniques have been developed to examine the surface relief of biological materials (Linskens 1966). The relief of leaf surfaces was found to be species-specific (Linskens 1966; Linskens and Kroes 1966) and thus could be used to distinguish species. The possibility exists that surface relief of vegetative and reproductive structures may distinguish genotypes within a species. Since endosperm mutants produce numerous changes in many aspects of the kernel, this study was undertaken to determine the effect of the alleles at the waxy (*wx*), sugary (*su*₁), shrunken (*sh*₂) and opaque (*o*₂) loci on the relief of the outer surface of the kernel pericarp. Comparisons were based on pattern, cell relief height and cell width.

Material and Methods

Dominant and recessive kernels were selected from one ear obtained by selfing one plant in each of the following populations: *Wx*(*K64* × *K55*) *wx*; *Su*₁(*Wf9* × *H55*) *su*₁; *Su*₁(*K64* × *K55*) *su*₁; *Sh*₂(*Wf9* × *H55*) *sh*₂; and *O*₂(*Wf9* × *H55*) *o*₂. The source of the dominant allele and the female parent in all populations is shown in parentheses. The sources of the recessive alleles were considered unrelated including the two recessive sugary sources. At the sugary locus, genetic backgrounds A and B are *Su*₁(*Wf9* × *H55*) *su*₁ and *Su*₁(*K64* × *K55*) *su*₁ respectively. All populations were grown under the same conditions and were selfed in 1973.

The techniques used to examine the surface relief of biological materials are described in an earlier publication (Linskens 1966). For this study, at least three kernels from each phenotype at each locus were selected. The kernels were washed in distilled water and thoroughly cleaned with acetone. Then replicas were made on the outer surface of the pericarp opposite the embryo using plastic film softened with acetone. After the film had hardened, it was removed. The replicas were then examined using an interference microscope at 280× in both air and water media. Photographs for pattern study and quantitative measurements were taken in either air or water medium or both depending on the proximity and clarity of the relief lines.

The cell relief height was measured from the photographs by two methods. Method 1 involved counting the number of lines from the margin to the apex of an individual cell. The cell relief height in microns was then determined by multiplying the number of lines counted by the appropriate line distance (air = 0.54 μ ; water = 1.5 μ). The major disadvantage of method 1 is that the cells had to be highly selected since the margin and apex had to be easily distinguished. Method 2 involved counting the number of lines and cells in a 250 μ line on the photographs. The mean cell relief height (CRH) in microns was then calculated using the formula $CRH = (A/2B)C$ where A = number of lines, B = number of cells and C = appropriate line distance. Generally, method 2 is more adaptable and accurate than method 1.

The mean cell width was determined by dividing the distance by the number of cells counted on a line approximately 250 μ on the photographs.

Results

Distinctly different relief patterns were found between dominant and recessive kernels at some loci. *Fig. 1* indicates the pattern differences between the dominant and recessive kernels at the shrunken locus. At this locus, pattern differences would be sufficient to distinguish kernel phenotype. *Fig. 2* indicates the

¹ Journal Series Paper No. 5375. Florida Agricultural Experiment Station.

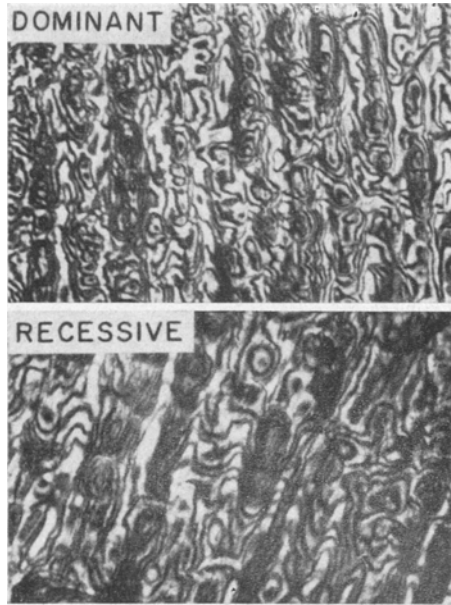


Fig. 1. Surface relief patterns of the dominant and recessive kernels at the shrunken locus in air (line distance = 0.54μ)

pattern differences between the dominant and recessive kernels at the opaque locus. In air, the patterns differed only slightly, but in water, distinct differences could be detected.

The quantitative measurements are presented in Table 1. In general, the cell relief height and width of the recessive kernels exceeded those of the dominant kernels. The only exception occurred at the sugary locus indicating that genetic background influenced the expression of the alleles at this locus. The effects of genetic background were further sug-

gested when comparisons were made between the dominant kernels which were somewhat related. As an example, the dominant kernels at the sugary locus background A had a higher cell relief height and a smaller cell width than the dominant kernels at the shrunken locus. At each locus, the source of the dominant allele was $Wf9 \times H55$. The relationship between the sources of the recessive alleles was unknown.

Discussion

The results of this study indicate that endosperm mutants can alter the relief of the outer surface of the pericarp. The pericarp is maternal tissue which forms a tough but flexible outer covering around the kernel. In this study, the pericarp of the dominant and recessive kernels within each locus were identical genetically and presumably in all other respects and, therefore, the observed differences must have been the result of relief patterns of the endosperm directly under the pericarp. These endosperm relief patterns were then reflected on the outer surface of the pericarp because of the flexibility of the pericarp. Differences in endosperm relief patterns have been associated with some endosperm mutants. A scanning electron microscope study comparing the endosperms of dominant and recessive kernels at the opaque locus has shown that patterns differ considerably and could be used to identify high lysine kernels (Wassom and Hosney 1973). Apparently, these endosperm mutants can alter the surface characteristics of the endosperm and these differences can be detected on the outer surface of the pericarp.

The results also indicate that genetic background can influence the relief of the outer surface of the pericarp. Here again, this must be related to the

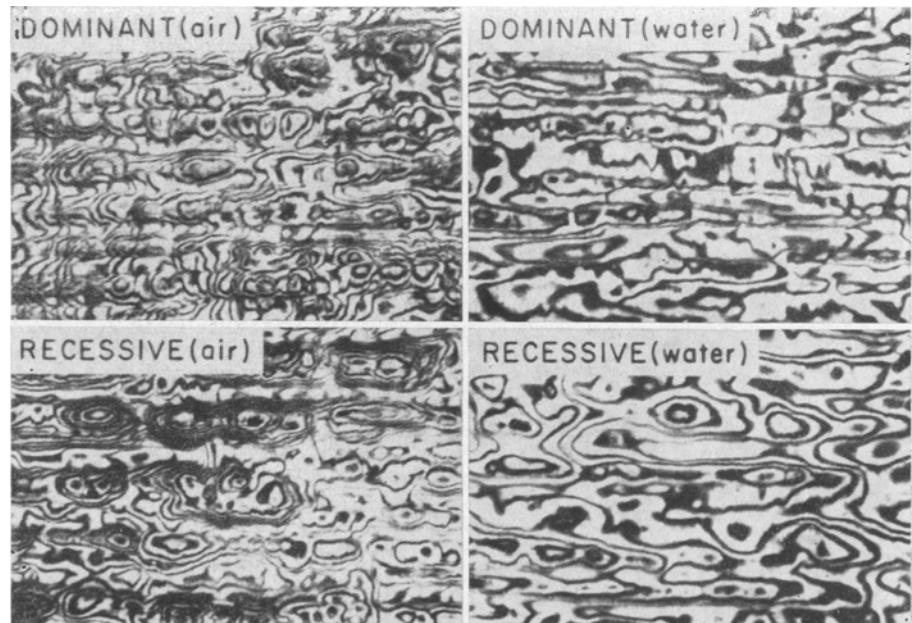


Fig. 2. Surface relief patterns of the dominant and recessive kernels at the opaque locus in air (line distance = 0.54μ) and water (line distance = 1.5μ)

Table 1. Influence of the endosperm mutants on the cell relief height and width

Locus	Genetic background	Kernel phenotype	Mean height (microns)		Width (microns)	
			Method 1 ^a	Method 2 ^a	Mean ^a	Range
Waxy (<i>wx</i>)		Dominant	— ^b	2.12	19.3	17.6–20.5
		Recessive	2.31	2.61	20.8	19.5–22.5
Sugary (<i>su</i> ₁)	A	Dominant	2.27	2.58	20.5	19.2–21.7
		Recessive	2.11	2.30	18.6	17.2–19.7
	B	Dominant	— ^b	1.56	18.5	17.6–19.4
		Recessive	1.99	2.20	19.1	18.0–20.3
Shrunken (<i>sh</i> ₂)		Dominant	— ^b	2.01	23.2	22.5–23.6
		Recessive	2.72	2.86	27.4	25.0–29.4
Opaque (<i>o</i> ₂)		Dominant	2.14	1.89	21.9	20.8–23.1
		Recessive	2.44	2.65	24.6	21.6–26.3

^a Each value represents the mean of at least 3 measurements.

^b Measurement impossible.

surface relief of the endosperm. In this study, differences at the sugary locus were found resulting from genetic background, but differences between the dominant kernels of slightly different genetic origin were also recognized. Apparently, the relief of the endosperm is influenced not only by the endosperm mutants studied but also by alleles at other loci. This would suggest that the relief of the outer surface of the pericarp might be used in taxonomy and also might represent a valuable tool in identifying cultivars within a species.

Acknowledgements

Sincere appreciation is expressed to H. S. Anspach for technical assistance.

Received April 16, 1974

Communicated by H. Stubbe

Literature

- Linskens, H. F.: Das Relief der Blattoberfläche. *Planta* (Berl.) **68**, 1–14 (1966).
- Linskens, H. F., Kroes, H.: Interference microscopy of the pattern of leaf surfaces. *Nature* **210**, 968–969 (1966).
- Nelson, O. E.: Biochemical genetics of higher plants. *Annu. Rev. Genet.* **1**, 245–268 (1967).
- Neuffer, M. G., Jones, L., Zuber, M. S.: The mutants of maize. *Crop Sci. Soc. Amer. Publ. Madison, Wisconsin* (1968).
- Wassom, C. E., Hoseney, R. C.: Selection of high lysine corn using a scanning electron microscope. *Crop Sci.* **13**, 462–463 (1973).

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