Theor. Appl. Genet. 50, 147-149 (1977)

Identification of Soybean Cultivars by the Surface Relief of the Seed Coat

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Summary. The relief of the outer surface of the seed coat of 16 diverse cultivars of soybeans, *Glycine max* (L.) Merrill, was compared on the basis of pattern, mound number and mound height in both air and water media. Significant differences among cultivars were found indicating that surface relief characteristics were cultivar-specific and could be used to describe and distinguish cultivars.

Key words: Soybean - Seed Coat - Surface Relief - Cultivar Description

Introduction

Generally, cultivar descriptions are based on vegetative and/or reproductive features which are difficult to measure either qualitatively or quantitatively. As a result, cultivars are difficult to distinguish unless they differ considerably in contrasting characteristics. Recently, a more sensitive method was suggested to identify maize cultivars (Pfahler et al. 1975). The surface relief characteristics of the outer surface of the kernel pericarp reflect relatively small differences in the genetic constitution of the kernel (Linskens et al. 1974; Pfahler et al. 1975). Therefore, the relief characteristics could be used to describe and distinguish cultivars. This method offers additional advantages in that single kernels can be individually tested and the kernel is undamaged by the testing procedure.

The use of this surface relief method to describe and distinguish cultivars in other cultivated species would be of significant value. The purpose of this study was to determine if differences in the relief of the outer surface of the seedcoat were present among 16 diverse cultivars of soybeans.

Materials and Methods

Surface relief studies were conducted on seeds from 16 cultivars listed in Table 1. The genetic relationships among the cultivars are either unknown or have been so altered by selection that no meaningful comparisons based on genetic affinity are possible.

At least three seeds from each cultivar were tested. The procedure involved submerging the seeds in chloroform for one minute and then cleaning them in 96% ethyl alcohol to remove the waxy cuticle. Plastic replicas were then made on the outer surface of the seed coat located over the apex or center of the cotyledon. Interference photomicrographs were taken at 148 × using both air (line distance = 0.54 µm) and water (line distance = 1.5 µm) media. Interference microscopy techniques for maize pericarp (Linskens et al. 1974; Pfahler et al. 1975) and surfaces of other biological materials (Linskens 1966) have been described.

From each photograph, quantitative measurements of various characteristics were made. Mound number was determined by counting the number of mounds in a standard distance. Mound height was measured by multiplying the number of interference lines by the appropriate line distance.

Appropriate statistical analyses were performed on the quantitative data. Minimum differences for significance were obtained from the revised Duncan's ranges using for p only the maximum number of means to be compared (Harter 1960).

Results

Considerable diversity in pattern was found among the cultivars tested. Figure 1 indicates the range of diversity in air medium where the line distance is smaller and the shape and regularity of the mounds are more obvious. Figure 2 presents the range of

Table 1. Surface relief measurements[1]

Cultivar	Mean mound number		Mean mound height (μm)	
	Air medium	Water medium	Air medium	Water medium
Improved pelican	23	21	1.63	3.53
Cobb	38	30	1.29	2.81
Hardee	28	17	1.79	3.62
Vicoja	28	25	1.36	2.41
Bragg	30	28	1.81	3.97
Forrest	24	18	1.46	2.88
Hutton	44	46	2.00	3.07
Pickett	45	32	1.73	3.22
Hampton 266A	33	38	1.58	3.28
Ransom	45	33	1.79	3.18
Hood	36	30	1.81	3.23
McNair 600	50	36	1.59	3.27
Otootan	18	18	0.82	1.72
Seminole	48	27	1.39	2.87
Biloxi	46	36	0.91	2.00
Acadian	41	33	1.47	2.37

[1] Minimum differences for significance at the 5 and 1 % level respectively: number in air medium = 15 and 20; number in water medium = 16 and 21; height in air medium = 0.49 and 0.63; and height in water medium = 1.08 and 1.41

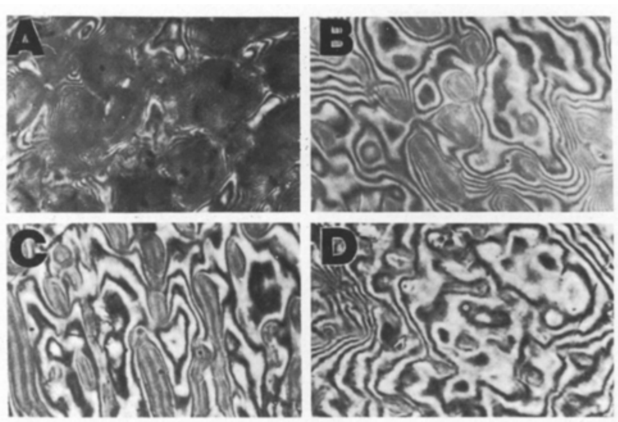

Fig. 1. Diversity of relief patterns observed among cultivars in air (line distance = 0.54 μm). A = 'Hutton'; B = 'Hardee'; C = 'Vicoja'; and D = 'Otootan'

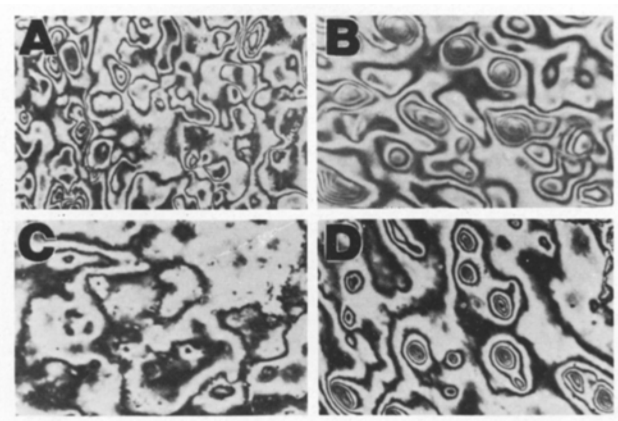

Fig. 2. Diversity of relief patterns observed among cultivars in water (line distance = 1.5 μm). A = Hutton; B = Ransom; C = Otootan; and D = Seminole

diversity in water medium where the line distance is greater and, as a result, the resolution of mound shape and regularity is correspondingly reduced. Substantial differences in pattern among cultivars were present when observed in the same medium. The effect of the medium on the general appearance and visual impact of the pattern can be seen by comparing specific cultivars such as 'Hutton' in air (Fig. 1A) and water (Fig. 2A). Apparently, the patterns of the cultivars differ when observed in the same medium but also the appearance of the pattern from the same cultivar is altered by the medium.

For all quantitative characteristics measured, highly significant differences among cultivar means were found (Table 1). Considerable variation among means was present so that many cultivars could be distinguished by one or a combination of these measurements.

Discussion

Using this method, a complete description of a soybean cultivar would consists of pattern, mound number and mound height in both air and water media. To distinguish cultivars, differences in one or more of these six categories would be required. This study indicated that a large number of patterns and considerable variability in the number and height of mounds were present. Therefore, the description of cultivars would be very specific and distinguishing even closely-related cultivars would be possible. The advantages of this system also include the testing of seeds individually and the absence of seed damage from the testing procedure.

In soybeans, the relationship between the surface relief pattern of the seed coat and the genotype of the seed coat or the embryo enclosed by the seed coat is not clear. In maize, kernels from the same ear exhibiting different endosperm mutant phenotypes differed in their pericarp surface relief patterns (Linskens et al. 1974). Since the pericarp is maternal tissue and the kernels had genetically identical pericarps, it was concluded that the surface relief pattern of the outer surface of the pericarp reflected variations in the surface relief of the endosperm. In maize, the pericarp is relatively thin and flexible and, as a result, could reflect the surface relief of the underlying endosperm. A second study with maize indicated that the surface relief pattern from cultivars not segregating for endosperm mutants varied considerably (Pfahler et al. 1975). Apparently in maize, the surface relief pattern can be altered by the relief of the underlying endosperm or by the genotype of the pericarp itself. In soybeans, the seed coat is maternal tissue which may or may not be genetically identical to the embryo enclosed by the seed coat. However since soybeans are highly self-pollinated, the seed coat and embryo of established cultivars generally have the same genotype. The seed coat proper has three distinct layers: epidermis, hypodermis and inner parenchyma (Carlson 1973). The weight and thickness of the seed coat and the amount and distribution of wall thickening in the cells of the layers suggest that the seed coat is quite rigid and stiff. Thus, the surface relief patterns reported in this study probably reflect the surface of the epidermis and not the lower seed coat layers, endosperm or cotyledons. The epidermis is composed of one layer of closely packed palisade cells which are elongated with their long axis perpendicular to the surface of the seed coat and have thickened, pitted walls in that part next to the surface of the seed coat (Carlson 1973). Thus, in soybeans, the differences in surface relief patterns result from the arrangement of these palisade cells. Presumably, the arrangement of these palisade cells is related to the genotype of the seed coat. Since the genotype of the seed coat is usually identical to that of the embryo, then the surface relief pattern is essentially associated with the genotype of the embryo.

The measurements obtained from the surface relief patterns in soybeans differed slightly from those made in maize (Linkskens et al. 1974; Pfahler et al. 1975) and other biological materials (Linskens 1966). In soybeans, the limits of individual cells could not be accurately defined from the patterns and, as a result, the general term, mounds was used to describe areas with increased elevations. These mounds could be individual cells or cell groupings. Thus, the results are reported in mound number per standard distance and mound height. In maize and other biological material, the limits of each cell could be readily identified and, as a result, the results were reported in cell width and cell height.

Acknowledgement

Journal Series No. 366, Florida Agricultural Experiment Station. Thanks are extended to Dr. Kuell Hinson for supplying seeds of the cultivars tested in this study.

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Received January 25, 1977
Communicated by H.F. Linskens

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