4. A leaf growth pattern index for differentiating strains of maize.

For the past few years we have been testing several methods of differentiating such a complex function as leaf growth in several races of maize. One such method that may prove to be valuable for this purpose and also for differentiation of inbred lines is based on transforming the successive length/width ratio of the leaves of maize to a relative percentage index value. The procedure of transformation goes stepwise as follows:

1) Plot in succession log length of leaf in the abscissa axis against log width in the ordinate axis, for each leaf (or mean values of leaf positions for several plants selected from the most frequent leaf number class in the population), on squared paper.

2) Draw lines that go in order from the first leaf point on the plane between both axes, to the second, and from this one to the third, and so on, ending by uniting the point for the last leaf with the first one.

3) Fix an arbitrary reference point on each axis and draw a line perpendicular to each axis at each point. Points may be selected so that the area limited by the coordinate system will be divided into four quadrants about equal in area. The two reference lines will make the four characterizing quadrants: I - upper left, indexing short, wide leaves; II - upper right, indexing long, wide leaves; III - lower left, indexing short, narrow leaves, and IV - lower right, indexing long, narrow leaves.

4) Determine by addition of squares or with an Amsler planimeter the area of the irregular shaped figure obtained at the end of step 2. Determine next the area of the sections of this figure that fall within each of the four quadrants, and express them in percent of the total area.

As an example, a comparison between single crosses and their S_1 line parents, is shown in the next table:

Pedigree	Quadrant			
	I	II	III	IV
C.P. -117	14.37	36.11	35.92	13.61
C.P. -117 x NS 54 -223	11.67	57.81	10.42	20.10
NS 54 -223	9.15	56.64	6.36	27.84
P.C. -79	13.66	81.49	0.58	4.25
P.C. -79 x NS 54 -52	3.28	70.79	3.50	22.43
NS 54 -52	10.49	56.00	21.87	11.63
HIM -40	11.94	71.97	0.25	15.83
HIM -40 x P.C. -37	0.0	90.66	0.0	9.34
P.C. -37	26.99	52.91	20.09	0.01

Among several conclusions that may be drawn from the comparisons shown above, referring to the II Quadrant, where most of the leaves from the middle of the plant fall, we may see that in the first single cross, the line NS 54 -223 is dominant over C.P. -117 in determining long, wide leaves. In the second single cross, an intermediate value is found between the indexes for both parents. In the third single cross, HIM -40 x P.C. -37, there is a higher value 90.66 than the index for either parent, pointing to marked heterosis for length and width of leaf.

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1. Anthranilic acid incorporation in Bf-1 and normal seedling leaves.

Bf-1 seedlings and anthers are known to accumulate several substances that fluoresce blue in ultraviolet light (Proc. Nat. Acad. Sci. 37: 645-649 and MGCNL 24: 12). One of these substances was identified as anthranilic acid (AA) and two others were found to have microbiological activity as AA. In order to obtain evidence on the relationship of AA to the other fluorescent AA-containing substances, radiocarbon labeled AA was employed. Normal bf-1/bf-1 and mutant Bf-1/Bf-1 seedling leaf slices were incubated in pH 6.5 phosphate buffer containing 2 microM uniformly labeled (biosynthesized) AA containing ca. 2×10^6 cpm. Parallel normal and mutant leaf slices heated at 100° C for 5 minutes in buffer before incubation with AA were used as controls. After 6 hours incubation the leaf slices were ground, centrifuged, and the supernatants taken for paper chromatography in butanol-acetic acid solvent. Fluorescent spots were marked and some chromatograms cut into strips for direct counting and radioautograms prepared from others. It was found that (a) normal (boiled or unheated) seedling leaves did not convert AA to the two major blue fluorescent substances as indicated by fluorescence or radioactivity, and (b) mutant (unboiled but not boiled) seedling leaf slices incorporated AA into one of the major fluorescent materials and probably the other. Thus, the mutant accumulated AA and AA-like materials, and also contains a thermolabile system for conversion of AA to AA-like substances; normal leaves do neither. Unless the AA-converting system is adaptive over a longer time than 6 hours, it appears that the Bf-1 gene does not operate simply by causing the accumulation of AA which is convertible to AA-complexes by processes common to both normal and mutant seedling leaves.

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