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Cytohistological Abnormalities Associated with the Gene "Pigmy" (Py-1) in the Primary Root Tip of Corn (Zea Mays L.)

Michael Byer
University of Minnesota

MICHAEL BYER[1]
University of Minnesota, Minneapolis

BOTANY

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INTRODUCTION

The stunted appearance of maize seedlings homozygous recessive for the gene "pigmy" (py-1) has suggested the present cytohistological study. The outward appearance of such seedlings is short, with a stout primary root and shoot, the mesocotyl being extremely short. To date, so far as the writer is aware, the only work which deals with the expression of this gene is Cotton and Loan's paper (1955), which describes the effects of temperature on the growth rates of pigmy root tips and those of the normal sibs.

MATERIALS AND METHODS

The material used in this investigation was inbred line A188 seed segregating for the gene py-1.[2] The seed was taken from experimental stocks of the Laboratory of Plant Morphology, Department of Botany, at the University of Minnesota. The gene has been introduced into these stocks by backcrossing to the inbred line for six generations.

In the first series of experiments, seedlings were germinated in flats containing a sand-loam mixture in the University of Minnesota greenhouse. Sampling was begun on the fifth day after planting and continued over a three day period. The root tips were killed in "Craf" (Sass 1951:16), embedded in paraffin, and longitudinal serial sections were made. These were stained in safranin and fast green.

In the second series of experiments, the seeds were dusted with Arasan and planted without preliminary soaking. These seeds were

[1]The writer wishes to express his appreciation to Dr. E. C. Abbe for his suggestions and guidance, to Miss Agnes Hansen for her assistance in the preparation of materials and photographs, and Mr. Robert McCleester for his suggestions concerning the growing of seedlings.
[2]The homozygous recessive does not set seed.

germinated in a moist chamber attached by rubber bands to slabs of porous fiber board, according to a method suggested by R. McLeester. This method of growing the seedlings resulted in good development of root hairs and thus made it possible to delimit the mature region of the root apex in longitudinal section. Sampling was begun on the second day after planting and continued over a four-day period. Every twenty-four hours, all primary root tips of the pigmies over 1.5 cm in length were taken, as were all normal primary root tips which had attained a length of 3.0 cm or more. Approximately one-half of these root tips were killed in "Craf", longitudinal serial sections made, and the sections stained with haematoxylin. The remainder were squashed with aceto-carmine, and the preparations were made permanent by McClintock's method (McClintock 1929).

The first set of slides was examined for general histological expressions of the gene, and the longitudinal extent of necrotic files of cells was noted in the pigmy.

In the sections of the second set, the study of histological expression was continued. Five median longitudinal root tip sections of the pigmy and five of the normal sibs were measured lengthwise from the apical initials to the beginning of the root hair zone, and the total width of the root tips was measured at the beginning of the root hair zone. The average length and width of root tips of the two phenotypes was then calculated.

A second analysis involved a measurement of the width of the procambium as compared to other tissues at the beginning of the root hair zone in five root tips of each phenotype, with respect to the width of these tissues in micra and to the number of files of cells involved in each tissue.

Finally, a representative sample of procortical cells half way between the initials and the beginning of the root hair zone was measured in the same sections for which the procambium analysis was made in order to obtain an estimate of the average dimensions of these cells in each phenotype.

Mitoses observed in the first set of sections prompted the investigation of mitoses in the root-tip squashes. In three root-tip squashes

OBSERVATIONS

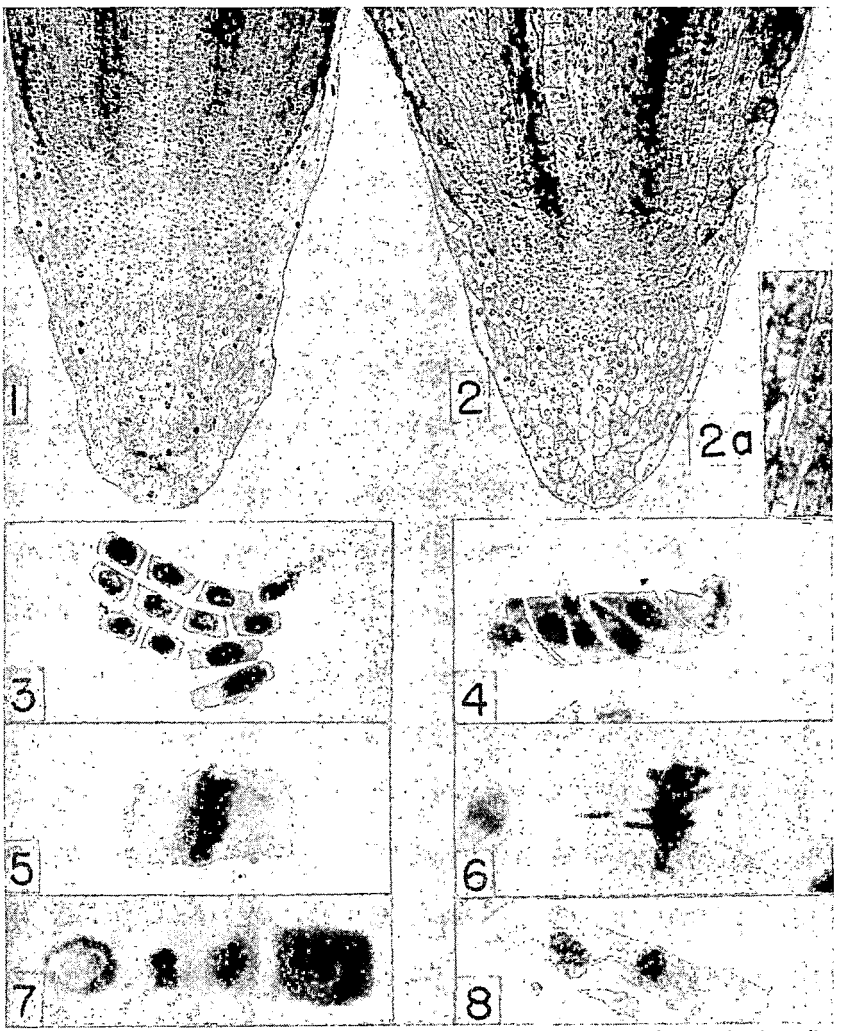

Fig. 1. Longitudinal median section of a normal corn root tip. **Fig. 2.** Longitudinal median section of a pigmy corn root tip. **Fig. 2a.** Cavities between cell files in pigmy root tip. **Fig. 3.** Procortical cells in normal root tip. **Fig. 4.** Procortical cells in pigmy root tip. **Fig. 5.** Metaphase in normal root tip. **Fig. 6.** Pigmy metaphase with lagging chromosome. **Fig. 7.** Telophase in normal root tip. **Fig. 8.** Pigmy telophase with bridge configuration. (Scale: Figs. 1, 2, 85X; Fig. 2a, 185X; 3, 4, 360X; 5, 6, 7, 8, 866X.)

each, of both the pigmy and the normal sibs, every cell was examined and the normal and abnormal metaphases and anaphases were counted. The prevalent types of mitotic abnormalities were recorded. The results for the two phenotypes were then compared numerically. In addition, it was noted that abnormal telophases were present.

Shape and size of the root apex: The general appearance of the primary root tip of pigmy maize seedlings is strikingly different from that of the normal primary root tip. The apical cone, in the specimens observed, was consistently much flatter and broader in the pigmies than in the normal sibs. The pigmy root tip (Fig. 2) is comparatively stout in appearance and broader than the normal sib (Fig. 1). Table 1-a showing the dimensions of the five root apices measured, serves to illustrate these facts numerically.

The most startling difference appears in the width, for the average pigmy root tip is over twice as wide as the normal sib. A smaller difference appears in the length, the pigmy being approximately four-fifths as long as the normal, although a difference so slight as this could conceivably result from sampling error. The ratio of length to width shows the pigmy root tips to be on the average only about 38% as long in relation to their own width as are the normals.

Histology and histogenesis—qualitative: Some expressions of the pigmy gene stand out immediately upon comparison of the root tip sections. These are: (1) The comparatively confused arrangement of cells, and (2) the presence of longitudinal gaps among cell files.

Figures 1 and 2 show the arrangement of cells in the two types of maize. In the normal root tips, cells are produced by the initials in orderly files of nearly uniform width which, in the ground meristem region, flare proximally in a parabolic fashion. In the promeristematic region, they become straight files parallel to the longitudinal axis of the root tip and closely approximating one another. The transverse walls of the cells in these files are nearly always perpendicular to the longitudinal axis or essentially so, and the cells have a rectangular appearance.

In the pigmy root tips, however, differences are already apparent in the apical initial cells. These cells are not rectangular but often

have four sides of unequal dimensions. This shape is preserved throughout the meristematic region of the root, and transverse cell walls appear throughout the meristematic regions at angles ranging from perpendicular to the longitudinal axis of the root tip to nearly or quite parallel to it. Characteristically, the cross walls of a series of cells in a file are oriented at approximately the same angle, but walls adjacent to this series change direction abruptly. Moreover, although individual files of cells are distinct, there is no orderly arrangement of such files in a parallel fashion with respect to one another nor to the longitudinal axis of the root tip. The files seem to wind in a sinuous fashion, changing direction and varying in width. Files in a single longitudinal section seem, in general, to appear from behind another file, to increase in width, and then to decrease in width and to disappear behind yet another file. This would suggest that in three-dimensional aspect these files may be arranged in a spiral fashion. A group of procortical cells from the normal and another from the pigmy root tips is shown in Figures 3 and 4 respectively.

In Figures 2 and 2a, the gaps between the files of cells are noteworthy. The writer conceives of two possible hypotheses for the origin of such gaps. One hypothesis is that the mode of growth causes intercellular dislocation, involving a widening of intercellular spaces between cell files. The nature of some of the gaps indicates that they were probably formed in this manner. These cavities would be schizogenous. A second explanation is suggested by the files of dead and dying cells, or necrotic strips, seen in some of the sections although not visible in Figures 2 and 2a. Such necrotic strips may extend the length of several to as many as forty adjacent cells of a file. They appear anywhere within the meristematic regions. However, incipient necrotic files were observed in very young meristematic tissues only 30-40 micra in a proximal direction from the apical initials as well as in the older ground meristem and in differentiating tissues. The varying degrees of darker staining of the cells, the dense and structureless appearance of the protoplasts, and the fragmentation of cell walls, all suggest disintegration of the cells. Necrotic strips, followed by gradual digestion and resorption of the cells involved, probably account for the remainder of the gaps. Gaps thus formed would by lysigenous.

Histology and histogenesis—quantitative: Two quantitative determinations undertaken on the cells themselves were a determination both of the ratio of the procambium to other tissues at the beginning of the root hair zone in the two phenotypes, and of the dimensions of procortical cells midway between the apical initials and the root hair zone.

The procambium was chosen for the first study, since it seemed to be the least affected by the pigmy gene, while the procortex seemed most affected. The results of this study are given in Tables 1-b and c.

TABLE 1.—*Dimensions of primary root tip and comparison of tissue dimensions in pigmy and in normal maize.**

	NORMAL			PIGMY			PIGMY/ NORMAL
	max.	avg.	min.	max.	avg.	min.	
a) Dimensions of primary root tip							
Length in micra	3598	2778	2148	2339	2263	2094	
Width in micra	555	437	394	1253	938	627	
Length/width		6.36			2.41		
b) Width of tissues in root tip in numbers of files of cells							
No. cell files in procambium	29	23.6	19	37	27.6	24	1.17
No. cell files, other tissues	22	18.8	15	33	27.4	24	1.46
No. cell files, total	46	42.4	39	63	55.0	50	1.30
c) Width of tissues in root tip in micra							
Procambium	295	236	172	439	384	320	1.63
Other tissues	242	227	217	716	625	541	2.75
Total	520	463	414	1107	1009	898	2.18

*Based on a sample of five median longitudinal sections of each phenotype.

It is evident from these tables that the pigmy root tips contain more files of cells than do the normal sibs. The procambium of the pigmy contains, on the average, about 15-20% more files of cells than the normal root tip. In the remainder of the tissues, however, the number of files in the pigmy root tip is nearly 50% greater. This suggests that the number of files is increased by the pigmy gene in

all tissues of the root tip, but that this influence is less marked in the procambium than in other tissues.

A more dramatic difference is seen in the measured width of the meristematic tissues. The procambium in the pigmies analyzed was on the average over one and one-half times as wide as that in the normal. Yet the difference in the width of the other tissues is even greater, indicating that the pigmy gene increases the width of all tissue regions, but has somewhat less influence on the procambium than on the other tissues taken as a whole.

One may infer, then, that the pigmy gene increases the width of all tissues with respect both to the width of the individual cells and to the number of files of cells. These two expressions, however, seemingly occur independently of one another and exert varying degrees of influence in different tissue regions.

TABLE 2.—*Dimensions of procortical cells and incidence of abnormal mitoses in the primary root tips of pigmy and normal maize.**

	NORMAL	PIGMY
a) Dimensions of procortical cells		
Average length in micra	13.10	12.76
Average width in micra	15.23	18.49
Width/length	1.16	1.45
b) Abnormal mitoses, metaphases		
Total normal	66	109
Total abnormal	1	9
Percent abnormal	1.49	6.78
c) Abnormal mitoses, anaphases		
Total normal	27	63
Total abnormal	1	4
Percent abnormal	2.15	6.49

*The dimensions of procortical cells are based on a sample of five median longitudinal sections of root tips of each phenotype; the calculations for abnormal mitoses are based upon all the metaphases and anaphases observed in squashes of three root tips of each phenotype.

The results of the study of the relative widths of the procortex in the two phenotypes are listed in Table 2-a. The cell lengths shown in the table are not notably different in the two phenotypes. The pigmy cells, however, are shown to be roughly 21% wider than the normal cells. Barring too great a sampling error, these data support

the original working hypothesis that procortical cells of the pigmy root tip are relatively shorter and wider than comparable cells in the normal sibs. This difference is not as great as it was at first thought to be, however, and the generally wider appearance of the procortical cells in the pigmy may be in part an illusion caused by the arrangement of the files and the oblique walls.

Abnormal mitoses.[3] Abnormal cell divisions observed in the pigmy root tips were of two main types. These were: (1) Metaphases and anaphases with lagging chromosomes (Figs. 5, 6), and (2) anaphases and telophases with chromosome bridges (Figs. 7, 8), as well as telophase nuclei with long chromosomes protruding from them suggesting former bridges or lagging chromosomes.

Squashes of three normal and three pigmy root tips yielded the results shown in Tables 2-b and 2-c. It is evident that the incidence of abnormal mitoses is much higher in the pigmy root tips than in the normal sibs. It should be pointed out that one anaphase with a bridge configuration, and one metaphase with a lagging chromosome, were observed in the normal material. It seems possible, therefore, that the pigmy gene has a partial expression in the heterozygous condition, although the heterozygotes are not distinguishable from the genotypically normal individuals on the basis of external morphology or of root tip histology. Only a much larger sample would indicate the true proportions of abnormal mitoses in all three genotypes.

While no numerical analysis of abnormal telophases was made, it should be pointed out that, while there was one questionable lagging chromosome in all telophases observed in the normal material, several abnormal telophases were found in each of the pigmy root tips.

The abnormal mitoses suggest a cause of the necrotic files of cells. It seems feasible that such mitoses, which would produce daughter nuclei with a genetic imbalance, could be responsible. The deficiencies in such nuclei might be great enough to cause almost immediate deterioration of the cells. On the other hand, they might be small enough to allow the cells in which such nuclei occur to

[3]This work was a continuation of studies begun by Mr. Ijaz Hussain, formerly at the Department of Botany, University of Minnesota.

divide a number of times, or else of such a nature that they would not be lethal until the cells began to mature. The deficient cells are perhaps capable of drawing for a time upon their own reserves of essential compounds, or upon the metabolic products of neighboring cells. Such a series of cells which could survive for a time might ultimately die, resulting in a necrotic strip.

In view of the rarity of recorded naturally occurring abnormal mitoses reported in the literature, it seems noteworthy that they should occur in this material. Their occurence, and the possibility that subsequent divisions of the deficient cells followed by ultimate death of all daughter cells occurs, suggest that the pigmy gene may cause chromosomal deficiencies and inequalities in meiosis, which are carried over into somatic tissue and are preserved for a time in a manner described by McClintock (1936). No definite statement as to the causes of these abnormalities can be made at this time, however.

SUMMARY

The observed expressions of the pigmy gene in the primary root tip of maize may be summarized as follows:

1) There is no overall change in the gross arrangement of tissues in the primary root tip of the pigmy individuals. The meristematic regions still occupy the same relative positions with respect to one another as they do in the normals.

2) The arrangement of the cells in files is distributed, the files no longer being vertically oriented but rather twisted and bent in various directions, and it is possible that they are spirally arranged. Tissue tensions involved in this peculiar habit of growth are a possible cause of schizogenous cavities.

3) The transverse cell walls in the meristematic tissues lie at various angles oblique to the longitudinal axis of the root tip. This pattern of cytokinesis could be responsible for the spiral or twisted pattern of file arrangement.

4) The pigmy root tips are broader and possibly shorter than the normal root tips. This is probably associated with (a) an increase in the number of files of cells, and (b) an increase in the width of the individual cells. These expressions differ in intensity in the different tissue regions, and are apparently independent of one another.

5) Lagging chromosomes and chromosome bridges occur frequently in mitosis in the pigmy root tips. It is suggested that, if the genetically unbalanced cells thus produced can survive for a time and produce daughter cells with the same abnormalities, then the ultimate death of all these daughter cells may result in the necrotic strips observed. The final deterioration and readsorption of the necrotic cells may result in lysigenous cavities.

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