Cell division and cell elongation in the developing leaves of *Zea Mays*

T. C. JAIN

SKN College of Agriculture, Jobner (Jaipur) Raj.

Received 25th August, 1971.

Growth of leaves or any other organ of a plant is a result of cell elongation, cell division or both. Much work has been done to investigate the morphological aspects of leaf growth in graminaceous crops, but even today very little is known about the regions of cell elongation and cell division of growing leaves. Epidermis increases in area as a result of continued division and enlargement of epidermal cells which is a reflection of continuous changes in internal tissues of young plants. But how long and at which portion of leaf this division and enlargement takes place has not yet been fully investigated. The problem is more difficult in the graminaceous crops as the extreme base of leaf is the point of attachment of leaf sheath to the stem and not to the leaf blade. What is the basis of growth in leaf sheath and leaf blade ? Where is the region of elongation and division of cells, and how long are they active are important to understand the growth in its real sense ?

Ashby and Wangerann (1950) reported that enlargement of *Ipomea* leaves took place through cell division from 0.5 to 2.33 cm^2 and thereafter the growth of leaf was entirely due to cell enlargement. Jones (1956) also suggested that division took place only at a small portion of leaf and for a short time. But Brown (Milthorpe, 1956) did not agree to this and stressed that in his preliminary investigations on lupin leaves, division went on for a longer period. Wangermann taking part in discussion of Jone's paper also stressed that cell division does continue even after expansion commences. More recent workers however, indicated that the division continue for a longer period than suggested by previous workers and the expansion process goes on simultaneously for a considerable time (Sunderland, 1960 ; Milthorpe and Newton, 1963 ; Maksymowych, 1963). Morphological analysis of growth of individual maize leaves has already been published by the author (Jain, 1970).

MATERIAL AND METHODS

The investigations were conducted at the University of Louvain (Belgium) under two different sets of experiments:— (i) Preliminary investigations to locate the region of elongation and division of cells in leaf blade by horizontal markings (ii) By studying the transverse sections of leaf sheaths under microscope. In the first case plants germinated on 9th July, 1967, were measured for their growth of internodes and leaves in the green house for the age of 4, 5, 8, 11, 16, 19, 22, 30, 36, and 47 days after germination. The growth was measured by making horizontal marks by pencils having no chlorophyll solvent on the stem (leaf sheath) and leaf blades. The horizontal markings were made at definite intervals to obtain the definite growing region for further detailed studies. The details of the pots, type of soil and the green house environment have already been published (Jain, 1970).

The results of these preliminary investigations indicated that there was no meristematic activity in the leaf blade as well as the visible leaf sheaths. The leaf blade was increasing in its length mainly by pushing from down side with no visible changes in tissues of lamina. Study of the young leaf sheaths was therefore made to locate the region of elongation and division of cells by cutting transverse sections.

Transverse sections of leaf sheaths:

Transverse sections of different young leaf sheaths (9 days old, 4 visible leaves) were studied. The lamina was carefully removed and the portion of leaf encircling stem was separately taken out carefully. The details of the transverse sections studied are given as follows—

Leaf sheath No. based on leaf position	*Number of sections*	*Transverse section number and the specific portion of leaf sheath cut*
1	Two	1. Base lower half and 2. upper half
2	Two	3. base lower half and 4. upper half
3	Three	5. base 1 cm., 6. the middle and 7. the top
4	Four	8. base 1 cm., 9. base 1–2 cm., 10. between 2 and 4 cm. and 11. rest of the portion
5	Three	12. the base 1 cm., 13. base 1–2 cm. and 14. rest of the portion

The base indicates the part of the leaf sheaths near the point the sheath is attached to the stem, and the top the leaf sheath near the point the sheath attached to the lamina.

The stained sections (Hematoxyline and Fast green) were compared by taking the photographs at difinite point. The first vein let from the side of the main vein (left or right) was taken for comparison. As the photographs were not clear to indicate the precise details of the sections, comments were made simultaneously looking to the sections under microscope.

RESULTS

I. *Studies made by horizontal markings* :

The length of internodes was recorded from the position of leaf blade on the shoot as the stem is covered by sheath and was not pissible to locate without injuring the plants. Practically this was a measure of difference between the bases of two consecutive leaf blades.

The first three internodes come out at the age of 4, 5 and 9 days respectively whereas internodes 4th and 5th were visible only at the age of 16 and 30 days (table 1). The elongation took place only at the joint of sheath and leaf blade and only in the last two or three internodes as evidenced by horizontal makings. But it was interesting to note that there was no much diffeence between the visible sheaths elongating and sheaths inactive. The fault was then traced out. These marking observations gave an idea that the stem was elongating by the elongation of internodes at the junction of two. Gut actually the marks were made on the two different sheaths and the elongation was the result of some activity at its base (the sheath part attached to the stem).

TABLE 1.—Length of internodes at the various stages of crop growth in green house

Age in days after germination	*Internodes.....length in mm.*										
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
4	12										
5	21	23									
6	28	27									
9	29	30	10								
10	–	32	19								
11	–	33	28								
12	–	–	32								
16	–	–	35	18							
19	–	–	–	50							
22	–	–	–	77							
30	–	–	–	89	35						
36	–	–	–	91	108	23	18				
43	–	–	–	–	122	89	50	57	20		
47	–	–	–	–	–	109	56	73	59	43	23

The growth of internodes was mainly caused through increase in length of last two internodes with very small or no activity in the older internodes. The growth continued till the formation of ligule at the joint of leaf sheath and blade.

II. *Transverse sections of leaf sheaths* :

Fourteen transverse sections were prepared from a young maize plant as indicated earlier. These sections were compared by taking the microscopic photographs. The meristematic activity was judged by the relative size of cells, and nucleus and the degree of differentiation was estimated by counting the number of sieve tubes and vessels. The comparison of various sections is indicated in table 2.

Figure 1 A, B & C indicates the changes in meristematic activity of leaf sheath 3 and figure 2 A, B, C and D that of leaf sheath 4. The meristematic activity in the same leaf sheath was maximum at its base and decreased gradually towards the joint of leaf sheath and leaf blade. Figure 1D is not taken from the veinlet, but it is the transverce section of the youngest tissue of the youngest leaf sheath 5th, section no. 12). The activity of nucleus and the chromosomes in this section is clearly visible which is a sign of active meristematic activity.

It is also evident in table 2 that the levels of section 9 and 13 were also active and these were just the further ends of the sections 8 and 12 respectively. There was no meristematic activity in leaf sheath 1 and 2 and very little only at the base in sheath 3. This suggests that the growing cycle of leaf 1 and 2 is already completed.

TABLE 2—Meristematic activity in different parts of leaf sheaths of young maize plant (9 days old, 4 visible leaves)

Section No.	*Portion of leaf sheath*	*No. of sieve-tubes*	*No. of vessels*	*Collenchymatous tissues*	*Relative size of*		*Degree of Meristematic activity*
					Cell	*Nucleus*	
1	Leaf 1, ½ base	5	4	—	Fully exp.	V. small	No more
2	Leaf 1, ½ top	3–4	3	—	do.	do.	do.
3	Leaf 2, ½ base	4–5	1	—	do.	do.	V. little
4	Leaf 2, ½ top	5–6	Young	Young	do.	do.	No more
5	Leaf 3, base 1 cm.	3	—	—	Comp. small	Small	Little
6	Leaf 3, middle	6	6	+	do.	V. small	V. little
7	Leaf 3, top	7–8	8	+	Expanded	do.	No more
8	Leaf 4, base 1 cm.	—	—	—	V. small	Biggest	Most active
9	Leaf 4, base 1–2 cm.	1	—	—	Small	Big	Active
10	Leaf 4, middle	1	—	—	Comp. small	Mediam	Less active
11	Leaf 4, top	3	—	+	do.	Small	V. little
12	Leaf 5, base 1 cm.	1	—	—	V. small	V. big	Most active
13	Leaf 5, middle	—	—	—	Comp. small	Big	Active merist.
14	Leaf 5, top	1–2	—	—	Small	Small	Little

exp. = Expanded.
Comp. = Comparatively.
V. = Very.
Marist. = Meristematic.

DISCUSSION

The study of transverse sections of leaf sheaths indicated that there was no meristematic activity in leaf sheaths 1 and 2 and very little in leaf sheath 3 on the 9th day after germination. Similar observations were recorded by the author (Jain, 1970) while measuring the length of leaf blades by horizontal markings. The growth of leaves 1, 2 and 3 was completed in 4, 5 and 9 days after germination respectively. Whereas, the growing cycle of leaves 4 and 5 started after 4 and 7 days (visible leaf blade) and continued till 12 and 17 days respectively. Thus, the active part of the leaf is the leaf sheath and particularly the portion very near to the attachment with stem. On the basis of these results the following hypothesis could be given regarding the regions of elongation and division of cells in maize leaves.

HYPOTHESIS

The primordia coming out of growing apex gives rise to some active cells. These cells multiply and elongate the region at the base of the sheath so that *first phase* may be the ' multiplication of dividing cells '. This was indicated by the higher meristematic region in the base of visible leaf sheath 4 than the youngest leaf 5. These cells at the top of the region elongates and become bigger cells ; simultaneous multiplication of cells still took place at the base, as the cells are highly meristematic. Thus the *second phase is* division and elongation phase. The young cells now continue to elongate pushing the old and mature cells. The cells forming leaf blades are relatively mature, as the activity of division and elongation took place near the junction of stem and leaf sheath. So the *third phase* is ' elongation dominating ' as the elongation is mainly responsible for the growth of leaves during this period. The *fourth and last* phase is probably the ' elongation ' as there was growth even when the cell were not in a stage of multiplication. Moreover the cells at the base too increases in size with time. This is a qualitative hypothesis based on the results in the present investigations. The quantitative data can be collected regarding the length and duration of different phases.

The morphological growth hypothesis given above resembles the four different phases of growth given by Dale (1964) based on the estimation of volume of cells (fresh wt./No. of cells) in *Phaseolus vulgaris*. His four stages are (1) Division commences, (2) Exponential phase of division commences (3) Emergence unfolding and (4) Division ceases. However the division phase appeared to continue longer than reported by Dale in the present investigations. Dale

(1964) mentioned that division ceases when the leaf covers 17 per cent of its total length. Little meristematic activity was observed in the present investigations even at the base of 3rd leaf sheath which has completed about 2/3rd of its total blade length. The leaf 4th sheath was under most active meristematic conditions at its base, which too has completed about a little less than half of its total blade length. Thus the division continued longer than reported by Dale for *Phaseolus vulgaris*. The value of Dale was also smaller than indicated by Milthorpe and Newton (1960) for lupin. The variation in the results may be because of the fact that the visible elongation region is not responsible for growth and the results may be different if the data are based on the study of a part of leaf blade. This may also be due to differences in growth habits of various species.

SUMMARY AND CONCLUSION

Investigations were carried out at the Institute of Agronomy, University of Louvain (Belgium) in the year 1967-68 to locate the region of elongation and division of cells in growing leaves of *Zea mays*. The results of green house experiments are summarised as follows :

(1) It was observed by horizontal markings that the leaf blade was pushed from its sheath and there was very little activity in the blade itself.

(2) In the study of transverse sections of leaf sheaths of young maize plants (9 days old), meristematic activity was maximum near the junction of stem and leaf sheath and was reducing towards more mature parts of leaf sheaths and blade.

(3) An hypothesis was given indicating the four different phases of leaf growth namely :—(1) Multiplication of dividing cells or elongation of multiplying region, (2) Division of cells with simultaneous elongation in the upper mature cells, (3) Elongation of cells with little activity of division in the basal cells and (4) Elongation of remaining active cells.

ACKNOWLEDGEMENT

The investigations are a part of the thesis submitted to the University of Louvain, Belgium for the Degree of " Docteur en Science Agronomique " in the year 1968. The author wishes to express his gratitute to Prof. Labrun for his valuable guidance and suggestions

during the course of these investigations. Thanks are also due to Dr. J. Desloover of the laboratory and Dr. P. Bronckers of the Laboratory of Tropical Phytotechnique (Prof. Opsomer) for their help in preparation and interpretation of the microscopic photographs.

REFERENCES

*Ashby, E and Wangermann, E. *New Phytol.* 49 : 189-92, 1950.

Dale, J. E. Leaf growth in *Phaseolus vulgaris*. I Growth of the first pair of leaves under constant conditions. *Ann Bot.* 28 : 579-90, 1964.

Jain, T. C. Morphological aspects of leaf growth in *Zea mays*. *Israel J. Bot.* 19 : 42, 1970.

Jones, H. Morphological aspects of leaf expansion especially in relation to change in leaf form. The growth of leaves (Milthorpe, 1956) : 93-106, 1956.

Maksymowych, R. Cell division and cell elongation in leaf development. *Xanthium pennsylvanicum*, *Am. J. Bot.* 50 : 891-901, 1963.

Milthorpe, F. L. The growth of leaves 3rd Easter School of Ag. Science University of Nottingham. Butterworths, London, 1956.

Milthorpe, F. L., and Newton, P. Studies on the expansion of leaf surface. 3 The influence of radiation on cell division and cell expansion. *J. Exptl. Bot.* 14 : 483-95, 1963.

Sunderland, N. Cell division and cell expansion in the growth of leaf. *J. Exptl. Bot.* 11 : 68-80, 1960.

6—K 7996 (10/71)

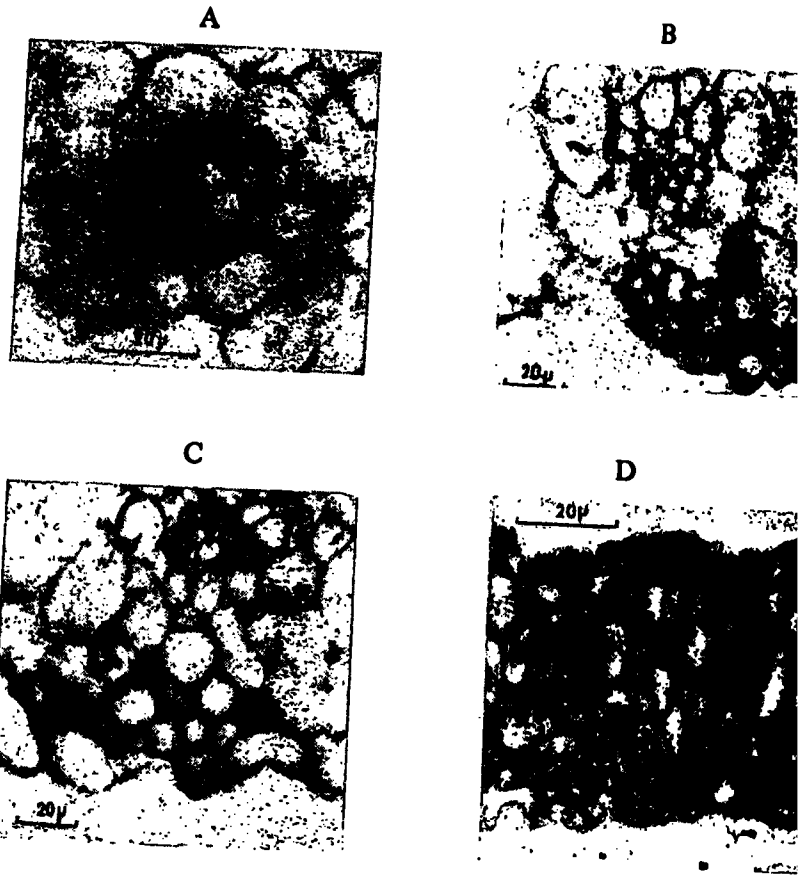

Plate 1. A, B, C, and D, are section no. 5, 6, 7, and 12 respectively in Table 4.

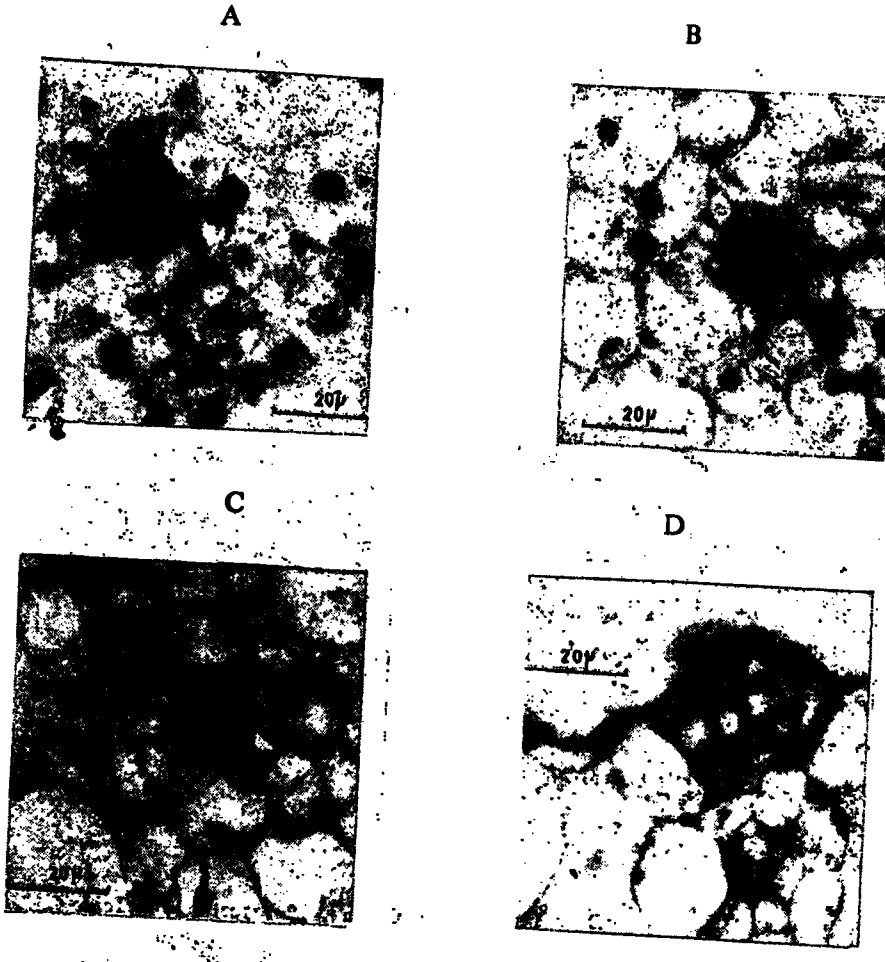

Plate 2. A, B C, and D are section no. 8, 9, 10 and 11 respectly in Table 4