Studies in the Physiology of Leaf Growth

II. Growth and Structure of the First Leaf of Rye when cultivated in Isolation or attached to the Intact Plant

BY

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With six Figures in the Text

INTRODUCTION

FEW workers have studied the processes of leaf growth and differentiation in the Gramineae. Apart from some early observations of Douliot the main contributions on this subject were made by Rösler (1928) and Kliem (1936), who described the mode of origin of the leaf at the growing-point in Avena and Triticum. The external morphology of the shoot apex in various grasses and cereals was studied by Sharman (1942), who distinguished three main types of apex. The growth and development of the shoot in Sinocalamus has been described by Hsü (1944). Percival (1921) has given a fairly detailed description of the structure of the mature leaf in Triticum.

The purpose of the present study was to follow the process of differentiation in the first leaf of rye both when attached to an intact plant and when cultured in isolation on sucrose-mineral agar, as previously described (de Ropp, 1945). In particular an answer was sought to the following questions: (i) Is the limited growth of the first leaf attached to an isolated stem tip due predominantly to an increase in cell number or in cell size? (ii) How does the structure of the first leaf grown in isolation compare with its structure when grown attached to the plant? (iii) How is growth and differentiation distributed in isolated and attached leaves?

MATERIALS AND METHODS

Stem tips excised from rye embryos were cultured at a constant temperature of 25° C. in 50-ml. flasks containing sucrose-mineral agar. Intact rye grains were grown in 100-ml. flasks on the same medium without the sucrose. Material for histological study was fixed for 24 hours in Bouin's solution and embedded in paraffin wax (m.p. 52° C.). Sections were usually cut to 8 μ and stained by means of Newton's gentian violet. Drawings were prepared by projecting the microscope image on to a sheet of paper and drawing in the cells.

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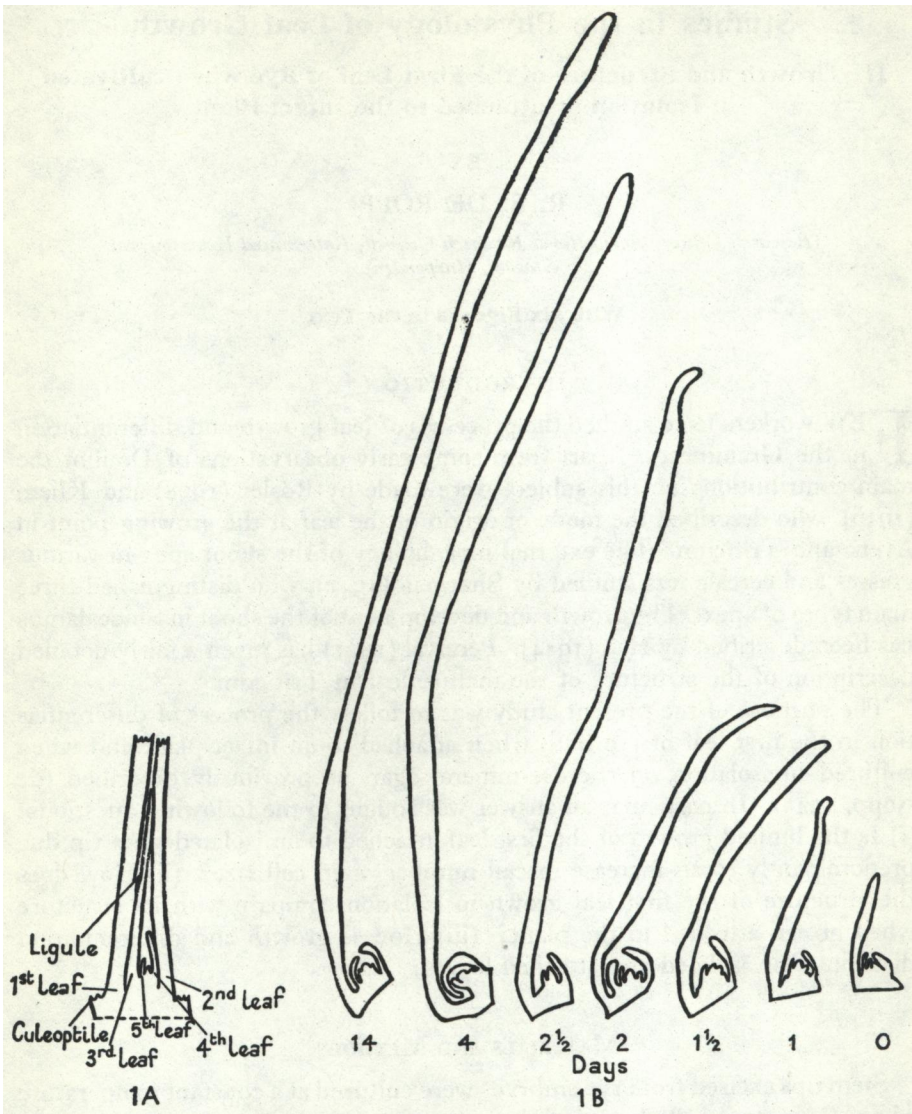
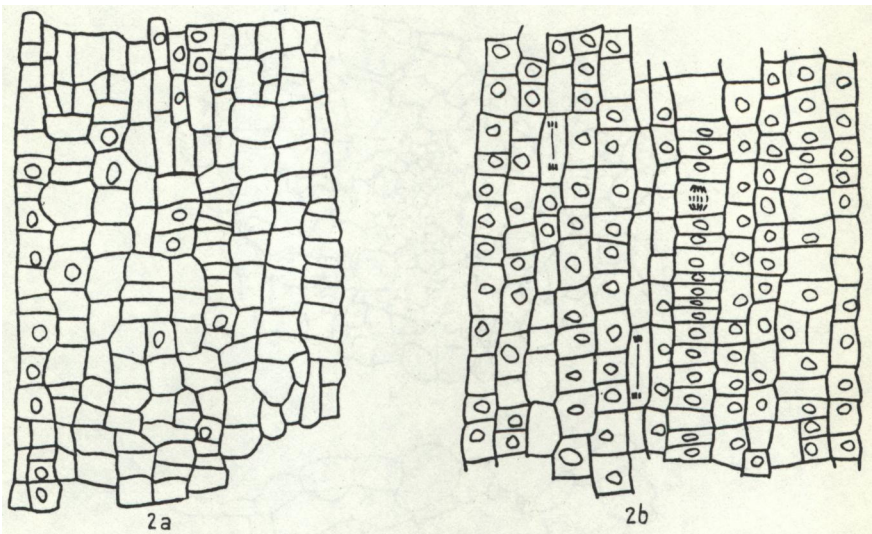
The distribution of growth in the first leaf was studied by carefully stripping the coleoptile from the embryo prior to germination and marking off the



FIGS. 1A and 1B. 1A. Growth during first 14 days of isolated stem tips cultured on nutrient agar, showing limited expansion of the first leaf and lack of development of younger leaves. (Median longitudinal sections.) (× 30.) 1B. Median longitudinal section of 3-day-old intact embryo, showing development of younger leaves. First and second leaves truncated and coleoptile removed. (× 14.)

exposed leaf primordium into four equal segments. Marking was carried out under a binocular microscope, a single hair dipped in a mixture of vaseline and lamp-black being used for the purpose. These marks had to be renewed

at intervals as growth proceeded. Each segment marked off measured from 0·2 to 0·3 mm., the total length of the primordium being about 1 mm. The distance of these points from the point of attachment of the leaf was determined at daily intervals after germination by means of a travelling microscope. It was found that the above treatment, when performed carefully, did not interfere with leaf growth. Any specimens showing abnormal growth were rejected.



FIGS. 2*a* and 2*b*. Longitudinal sections through the base of the 1½-day leaf. 2*a*. Leaf attached to isolated stem tip. 2*b*. Leaf attached to intact plant. (× 220.)

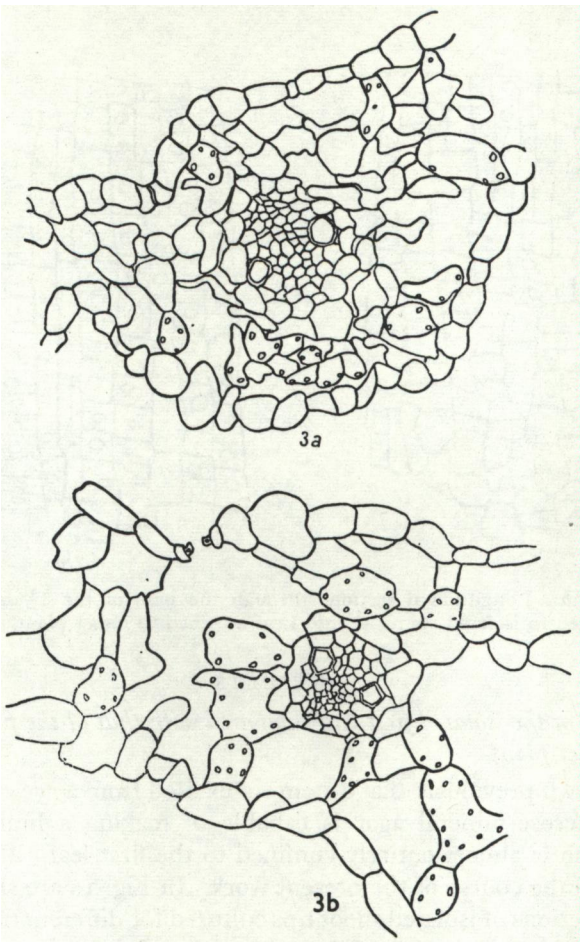
RESULTS

(i) *Structure of the isolated first leaf compared with that of the first leaf attached to the intact plant.*

It was shown previously that a stem tip excised from a rye embryo and cultured on sucrose-mineral agar is capable of making a limited amount of growth which is almost entirely confined to the first leaf. This finding was confirmed in the course of the present work. In Fig. 1A are shown a number of median sections of isolated shoot tips cultured for different times on sucrose-mineral agar. A comparison of the appearance of these sections with that of a 3-day-old intact embryo (Fig. 1B) shows the extent to which the growth of younger leaves has been inhibited in the detached stems.

Sections of isolated stem tips cultured on nutrient agar were examined to discover whether the limited growth of the first leaf primordium was entirely due to an expansion of already existing cells or whether any increase in cell number had also taken place. Fig. 2*a* shows a drawing of the basal region of the first leaf on an isolated stem tip after 1½ days' growth. No mitotic figures were visible in the section and the cells were no longer of the meristematic type. The same region of the first leaf on an intact plant is shown in Fig. 2*b*.

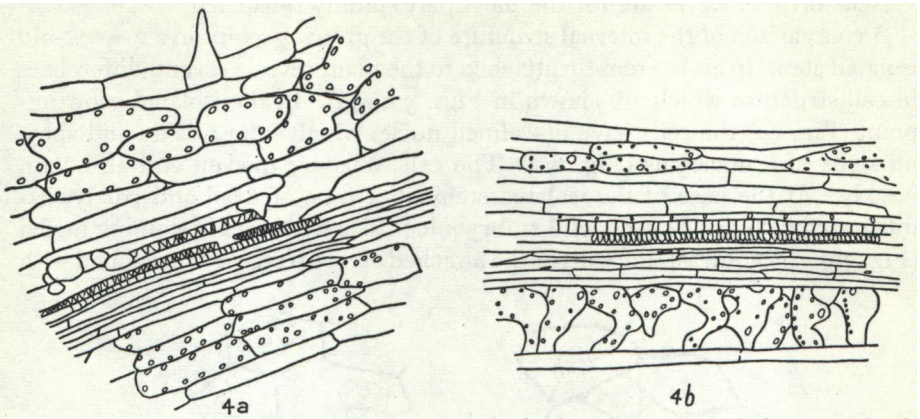
Here the signs of active cell division were obvious. The whole basal region of the leaf was meristematic. Many mitotic figures were visible; the cells were small, laid down in regular series, with dense unvacuolated cytoplasm and deeply staining nuclei. Since a careful examination of many sections of isolated first leaves failed to reveal any signs of recent cell division, it was concluded



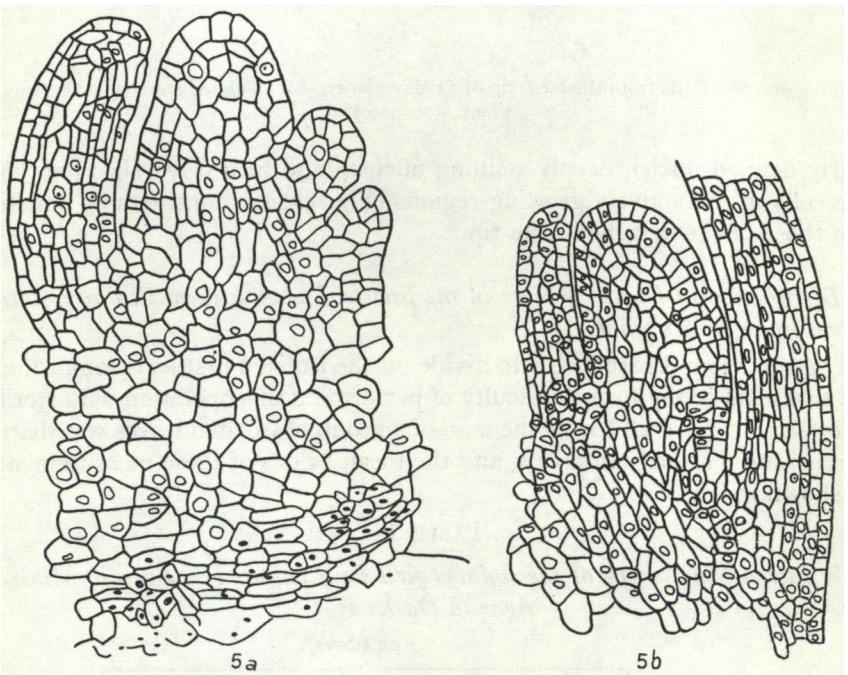
FIGS. 3*a* and 3*b*. Transverse sections through the lamina of a 14-day leaf of rye. 3*a*. Leaf attached to isolated stem tip. 3*b*. Leaf attached to intact plant. (× 200.)

that the limited amount of growth made by these leaves was due entirely to an increase in size of the already existing cells. Cell measurements showed that the length ratio of an embryonic epidermal cell to a mature epidermal cell was about 1 : 15. The length ratio of the embryonic leaf primordium to the fully grown leaf cultured in isolation was about 1 : 18. Cell elongation would thus appear to be sufficient to account for the increase in length of the leaf. This absence of cell division in the isolated stem tip of rye contrasts with the

behaviour of the isolated stem tips of *Stellaria media* described by White (1933), who succeeded in tracing the actual course of cell division in some of the stem tips he studied.



FIGS. 4*a* and 4*b*. Longitudinal sections through the lamina of a 14-day leaf of rye. 4*a*. Leaf attached to isolated stem tip. 4*b*. Leaf attached to intact plant. (× 150.)

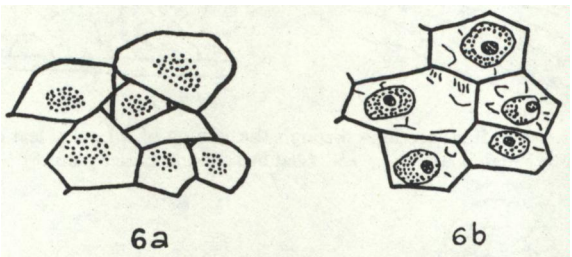


FIGS. 5*a* and 5*b*. Median longitudinal sections through the stem tip of 14-day-old rye plants. 5*a*. Isolated stem tip. 5*b*. Intact plant. (× 200.)

Transverse and longitudinal sections of the isolated and attached leaf (Figs. 3*a*, *b*, and 4*a*, *b*) did not reveal any obvious differences in structure in the mature leaf tissues. The appearance of vascular bundles in cross-section

(Fig. 3*a* and *b*) was that of a typical monocotyledonous leaf. The cells of the leaf parenchyma and of the epidermis are slightly smaller in the isolated leaf than they are in the attached leaf. As may be seen from Fig. 4*a* and *b*, the vessels in these leaves are for the most part spirally thickened.

A comparison of the internal structure of the growing-point in a 2-week-old isolated stem tip and a stem tip attached to the plant reveals several differences in cell structure which are shown in Figs. 5 and 6. In the isolated growing-point (Fig. 5*a*) the cells have ill-defined nuclei which stain weakly and show no signs of a nucleolus (Fig. 6*a*). The cell walls are uneven and also stain weakly. At the base of the isolated stem tip a zone of dead and partly disintegrated cells can be seen, still embryonic in size with deeply staining nuclei (Fig. 5*a*). By contrast, the cells of the attached stem tip are well developed with



FIGS. 6*a*. and 6*b*. Cells from the stem tip of 14-day plants. 6*a*. Isolated stem tip. 6*b*. Intact plant. (× 600.)

clearly defined nuclei, deeply staining nucleoli and firm cell walls (Fig. 6*b*). The cells in the actively growing regions (Fig. 5*b*) are considerably smaller than those of the detached stem tip.

(ii) *Distribution of length increase in the first leaf when attached to an isolated stem tip or intact plant.*

It was not found practicable to divide off the isolated first leaf primordium into segments owing to the difficulty of performing this operation with sterile materials. The overall length increase of a group of isolated leaves was therefore measured at daily intervals, and the mean values of these measurements are shown in Table I.

TABLE I

Daily Increase in Length of the isolated first Leaf cultured on Sucrose-Mineral Agar in Darkness

	Age (days).								
	0	1	2	3	4	5	6	7	8
Length (mm.)	1·1	2·0	5·0	9·8	14·0	16·0	17·2	18·5	18·5

These figures agree with those given in an earlier paper showing that growth in length of the first leaf is completed under these conditions within 7 days from the beginning of growth.

Leaf primordia which had been marked off into four approximately equal segments by the method previously described were grown both in darkness and in intermittent light (12 hours of light alternating with 12 hours of darkness). The length of each segment of the leaf was measured daily, and the values obtained are given in Table II.

TABLE II

Growth in Length (mm.) of the First Leaf attached to the Intact Grain cultured in Light and Darkness

	Age (days).								
Segment.	0	1	2	3	4	5	6	7	8
IN LIGHT									
Tip (4)	0·3	0·9	3	3	3	3	3	3	3
(3)	0·3	0·8	5	7	7	7	7	7	7
(2)	0·3	0·9	3	13	14	15	15	15	15
Base (1)	0·3	0·5	3	24	50	63	71	77	77
Total	1·2	3·1	14	47	74	88	96	102	102
IN DARKNESS									
Tip (4)	0·3	1·0	3	4	4	4	4	4	4
(3)	0·2	0·4	1	4	9	9	9	9	9
(2)	0·3	0·5	1	5	17	84	91	95	95
Base (1)	0·3	0·3	1	11	32	72	117	131	132
Total	1·1	2.2	6	24	62	169	221	239	240

These figures show clearly the extent to which elongation in rye is concentrated towards the basal portion of the leaf. The upper segment appeared from sections to receive no further increment of cells during its period of growth. Its increase in length was apparently entirely due to elongation and differentiation of cells already present in the embryonic primordium. The nearer a segment was situated towards the base of the leaf the larger was the increase in the number of its cells. Sections showed that the meristematic region of the leaf was situated slightly above its point of attachment. Differentiation of the tissue began at the tip of the leaf and progressed towards the base. The absence of mitotic figures from sections of leaves which were more than 3 days old suggested that the total number of cells in the leaf had already been completed by the third day after germination. Subsequent growth resulted not from the production of new cells but from the progressive elongation and differentiation of cells already laid down. It appears, therefore, that the ultimate size of the leaf is largely regulated by the activity of the basal meristem during the first 3 days of growth.

The figures given in Table II also provide information about the effect of light on the elongation of the leaf. Elongation was most rapid in leaves grown in the light up till the third day after germination. Subsequently, the leaves

grown in darkness elongated more rapidly and attained a final length of more than twice that of the leaf cultured in the light. This finding has a bearing on the view, already suggested, that leaf growth during the first 3 days after germination is due mainly to an increase in cell number, whereas subsequently it is due almost entirely to an increase in cell length. It would be during the phase of cell elongation that an exposure to light might be expected to reduce growth in length owing to its effect on the hormone mechanism controlling cell elongation.

No sign of a leaf sheath could be seen in leaves attached to an isolated stem tip. In the leaves attached to the whole plant the formation of the sheath was indicated by the differentiation of the ligule during the second day after germination. The ligule resulted from a group of periclinal divisions in certain cells of the inner epidermis. As soon as it could be clearly distinguished from the lamina the leaf sheath was also marked off into four approximately equal segments and the length of these segments measured at daily intervals. These values are shown in Table III.

TABLE III

Growth in Length (mm.) of the Leaf Sheath of the First Leaf of Rye attached to the Intact Plant

	In Light Age (days)					In Darkness Age (days)				
Segment.	3	4	5	6	7	3	4	5	6	7
Tip (1)	0·7	2	4	5	5	1	4	6	11	11
(2)	0·8	1	4	7	7	1	3	6	10	13
(3)	0·7	1	3	6	9	1	2	8	14	17
Base (4)	0·8	1	3	6	7	1	2	6	9	10
Total	3·0	5	14	24	28	4	11	26	44	51

It will be seen that the distribution of growth in the leaf sheath differs considerably from its distribution in the leaf as a whole. An examination of sections suggested that practically all the cells that go to form the leaf sheath are laid down by the third day after germination, and that further elongation of this organ results in the main from the elongation of cells already present.

For purposes of comparison the distribution of growth in the coleoptile was also studied by marking this organ while still in the embryo into four equal segments and measuring their length after growth had been completed. As can be seen from Table IV the lower half of the coleoptile contributed most to the final length of the organ, but the distribution of growth was unlike that observed in the leaf. No localized basal meristem could be observed in the coleoptile, though some cell divisions did occur during the early stages of coleoptile growth. These findings agree fairly closely with those recorded for the Avena coleoptile by Avery and Burkholder (1936).

TABLE IV

Growth in Length (mm.) of the Coleoptile of Rye in Darkness

	Age of coleoptile.	
Segment.	0 days.	7 days.
Tip (4)	0·4 mm.	6 mm.
(3)	0·4 mm.	21 mm.
(2)	0·4 mm.	27 mm.
Base (1)	0·4 mm.	12 mm.
Total	1·6 mm.	66 mm.

DISCUSSION

It appears from the findings described in this paper that the very limited growth made by the first leaf in rye when attached to an isolated stem tip is due to the failure of the basal meristem to produce new cells. The amount of possible growth of the primordium is therefore limited by the number of cells already present within it. The activity of the basal meristem is responsible for producing by far the greater number of the cells composing the normal mature leaf; restriction of its function must therefore result in a considerable reduction in leaf size. It has always been observed in these experiments that when isolated stem tips developed roots, young leaves attached to the stem tip began to develop. It seems that the roots in some way activate the basal meristem of the leaf, and that in their absence no new cells can be laid down. The nature of this root effect has still to be investigated.

Regarding the mode of growth of the first leaf attached to the plant, these investigations indicate that nearly all the cells are laid down within the first 3 days after germination. The meristem then ceases to be active and further growth of the leaf results from the elongation and differentiation of cells already formed. Differentiation proceeds from the tip towards the base, the ligule and sheath being differentiated last. In the coleoptile no clearly defined meristem could be observed, and the growth of this organ seemed to be mainly due to the elongation of cells already present, though some cell divisions were also observed. The similarity of the distribution of growth in the coleoptile and leaf sheath is of interest and suggests that the coleoptile might be looked upon as a modified leaf sheath.

SUMMARY

The growth of the first leaf attached to the isolated stem tip of rye was compared with that of the first leaf attached to the intact plant.

Growth of the isolated first leaf was shown to be due to the development of cells already present. In the absence of roots, cell division in the basal meristem of the leaf did not occur.

Growth of the attached first leaf depended mainly on the activity of the basal meristem during the first 3 days after germination.

Differentiation in the attached leaf began at the tip and proceeded towards the base.

No clearly defined meristem was observed in the coleoptile and the growth of this organ seemed mainly due to the elongation of existing cells.

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