REGENERATION IN SPLACHNUM AMPULLACEUM (L.) HEDW.[1]

KRAFT E. VON MALTZAHN AND MARY M. MACNUTT

Abstract

Successive leaves isolated from the gametophore exhibit differences in the number of regenerates obtained per organ. Starting from the base, successive leaves show an increasing number of regenerates per leaf. This pattern is similar in leaves isolated from young and older gametophores, although the younger ones show a much greater absolute number of regenerates.

No correlation exists between the number of cells per organ and the number of regenerates. Although the size of the wound surface plays an important part in the number of regenerates produced, this factor cannot determine the regenerative pattern between successive leaves.

Correlative factors are shown to exist in individual leaves and the distribution of regeneration in the leaf is determined also by these factors.

It is concluded that the age of the organ determines the regular pattern of protonematal regeneration of successive organs.

Introduction

Mosses exhibit generally great potentialities for regeneration. Experimentally these can be realized by means of isolating parts of the whole. This indicates that correlative factors between parts of the whole gametophore inhibit regeneration and that a disturbance of natural correlations, e.g. by means of the isolation of parts, will remove this inhibition. The regenerative behavior of different parts of the gametophore may therefore be an indication of the distribution of correlative factors in the plant.

Not every cell, however, shows the same potentiality of regeneration after isolation. The regenerative behavior will not only depend on correlative factors, but also on the developmental state of the part. The present study is concerned with the problem of the distribution of regenerative potentialities and their respective realization in the moss gametophore.

Materials and Methods

Throughout the present study the male strain of *Splachnum ampullaceum* was used. The plant material has its origin from the Cambridge (England) Collection. Leaves were isolated from the gametophore; those directly at the apex were too small for dissection and were not included in the present observations. The isolated leaves were grown under sterile conditions on Beijerinck's inorganic medium with 0.1% sucrose and 1% agar at pH 6 and under daylight fluorescent lamps. Each datum represents the average obtained from 10 to 12 plants.

Results

Comparing the behavior of successive leaves isolated along the axis of the gametophore, it was found in each case that the relative number of regenerates between successive leaves did not change with time, although the absolute number per leaf increased with time. Regeneration of successive leaves

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may therefore be compared after the same absolute time interval. Leaves were isolated and the number of regenerates was generally counted 4 days after planting.

The results of a typical experiment are described in Fig. 1. Starting from the base, successive leaves show an increasing number of regenerates per leaf.

In a number of similar experiments, however, considerable variation in the absolute number of regenerates was found to occur. Since the age of the gametophores was not considered in the above experiments, it seemed to be necessary to test whether such a variation may be due to differences in the ages of the gametophores.

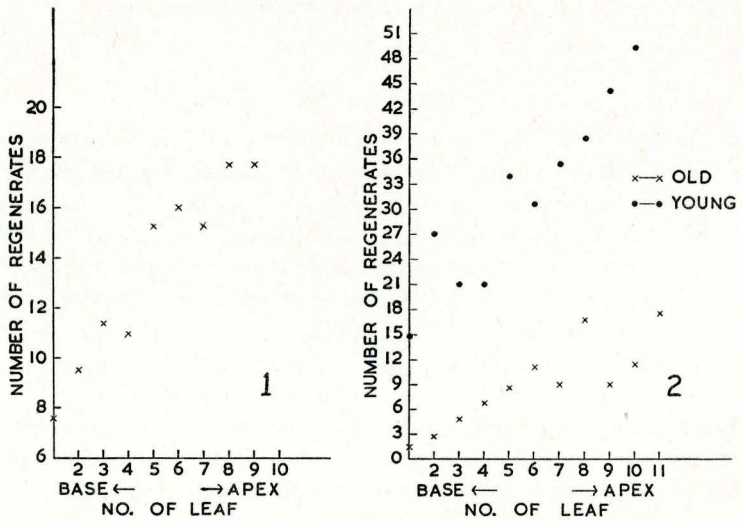

FIG. 1. Protonematal regeneration from successive leaves.
FIG. 2. Protonematal regeneration from successive leaves from young and older gametophores.

Successive leaves were isolated from young and older gametophores and their regenerative behavior compared. Fig. 2 gives the results of this experiment. In each case there is an increase in the number of protonematal regenerates per leaf from the base to the tip of the gametophore. The absolute number of regenerates of the same leaf is rather different, though, depending on the age of the gametophore. Leaves from younger gametophores show consistently a much higher number of regenerates compared to leaves from older plants.

It may be suggested that differences in the number of regenerates in successive leaves are due to differences in the number of cells per leaf from which regeneration may occur. To test this factor leaf size and cell size of successive leaves were determined. Cell size of comparable regions was approximately the same between successive leaves. Leaf size, expressed in terms of leaf length and width, showed considerable differences. This is represented in Figs. 3 and 4. Particularly, successive leaves at the base show differences

in mature size. Since cell size was approximately the same, differences in the size of successively formed mature leaves must be due to the number of cells per leaf.

Since the curve for the number of protonematal regenerates per successive leaves does not show any similarity to the curve for the size of successive leaves, the size of successive leaves cannot account for the pattern of protonematal regeneration from successive leaves.

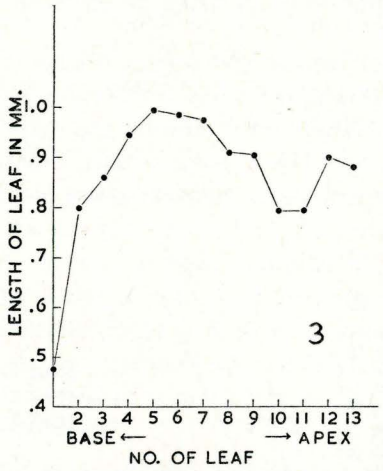

FIG. 3. Length of successive leaves.

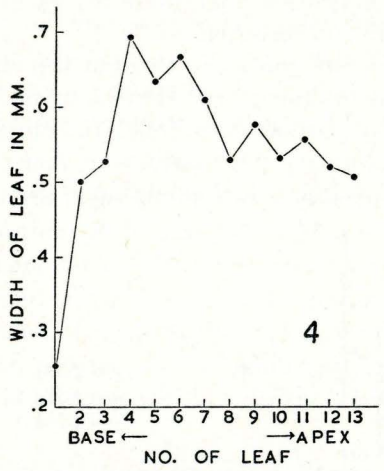

FIG. 4. Width of successive leaves

It is also important to investigate the influence of the size of the wound surface on regeneration. For this question the regenerative behavior of whole isolated leaves was compared with that of leaves cut into halves along the longitudinal axis and those in which two cuts were performed at right angles to the longitudinal axis dividing the leaf into three parts. The following values were obtained:

	Number of protonematal regenerates per leaf
Whole leaf	31.5
Leaf cut into two halves (along longitudinal axis)	42.6
Leaf cut into three equal parts (proximal, middle, distal)	49.7

Evidently the size of the wound surface plays an important part in determining the number of regenerates per leaf. Since the wound surfaces between successive leaves which have been isolated are of approximately the same size, differences in the regenerative behavior of successive leaves cannot be due to differences in the size of the wound surface.

Not only correlative influences between successive leaves have to be considered but also correlative influences between different parts of the same leaf. Whole leaves were marked into three zones (I distal, II middle, and III proximal). The regenerative behavior of these zones in whole leaves is compared with that in which these zones are isolated. Figs. 5 and 6 give the results of these experiments. Zone II shows the greatest amount of regeneration, while both Zones I and III lag behind Zone II. This would be an indication that these leaves do not show polarity in terms of their regenerative behavior.

When the zones are isolated the zonal behavior changes. Pieces corresponding to Zones I and III pick up and show approximately the same number of regenerates as does Zone II. Zone II apparently inhibits the realization of regenerative potentialities of Zones I and III to some extent. Once the zones are isolated, this inhibition is removed and activation takes place.

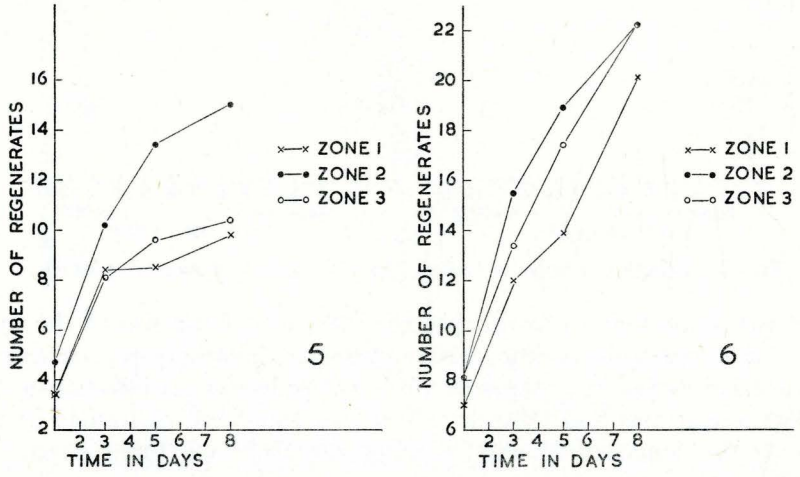

FIG. 5. Protonematal regeneration from three zones of whole leaves.
FIG. 6. Protonematal regeneration from three isolated zones of leaf corresponding to the zones of Fig. 5.

Discussion

Correlative factors are responsible for the inhibition of regeneration in whole intact plants and for the distribution of regeneration in whole detached leaves. The distribution of regeneration in successive leaves, on the other hand, does not seem to be a function of the position of the leaf in the whole gametophore or mature size of the leaf, but rather of the developmental stage or age of the organ. This conclusion is further supported by the fact that differences in the age of the whole gametophore will have a considerable influence on the regenerative behavior of comparable isolated parts.

Working with isolated leaves of the gametophore of *Funaria hygrometrica*, Bopp (1) did not find a progressive decrease of regenerative powers of the leaves from the tip to the base of the gametophore. The leaves located toward the middle of the stem gave the smallest number of regenerates.

In his material, therefore, primarily position of the leaf in the gametophore, not age, determined the number of regenerates per leaf. Gemmell (2) found also a second maximum in regeneration, in similar experiments with detached leaves of *Atrichum undulatum*, so that in this material position also and not only age seems to be important. Leaves of *Splachnum ampullaceum* seem to show strictly age dependence in their regenerative behavior in contrast to both *Funaria* and *Atrichium*.

Porzer (5) studied the stainability of leaves of foliose Hepaticae with alkaline dyes. He found a gradient in stainability from very young to the older leaves, indicating a change in terms of certain cellular conditions with an increase in the age of the organ. Similar results were obtained by Kressin (3) (see Strugger (6)).

The development of regenerative protonemata in the present material was not confined to one particular portion of the leaf, although the middle region showed a higher number of regenerates. This was found to be due to correlative factors, since after isolation of the distal, middle, and proximal regions, the distal and proximal ones caught up with the middle zone in the number of regenerates, suggesting the presence of an inhibitory influence on distal and proximal parts by the middle zone, which is removed by means of isolation.

Meyer (4) studied the regenerative behavior of leaves of *Physcomitrium turbinatum*. Protonematal regeneration was not confined to any particular part of the leaf. Bopp (1) showed the distal end to be the predominant location of regeneration in detached leaves of *Funaria*, the proximal region in those of *Tortula muralis*. In *Funaria*, isolation of the regions led to an increase in regeneration. He explained this observation also by means of a removal of inhibitory influences brought about by isolation. Different mosses apparently show quite a different behavior.

Predominant region of leaf in which regeneration takes place:	
Distal	*Funaria hygrometrica* (after Bopp)
	Atrichum undulatum (after Gemmell)
Middle	*Splachnum ampullaceum*
Proximal	*Tortula muralis* (after Bopp)
Region not defined	*Physcomitrium turbinatum* (after Meyer)

In each case in which a predominant region for regeneration is present, isolation of the regions leads to an increase in the number of protonematal regenerates in the inhibited zones due to a removal of the inhibitory influences.

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