Acta Biologica Szeged. *27* (1—4), pp. 117—126 (1981)

EFFECT OF SHORT PERIODS OF LIGHT AND DARKNESS ON THE HISTOLOGICAL STRUCTURE OF BEAN, MUSTARD AND PEA

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(Received September 16, 1980)

Summary

Dry matter production and histological structure of bean, mustard and pea grown under a short-period (30—15 min) and under a 16—8 hour period alterations of light and darkness were compared. The short-period treatment caused a considerable diminution of dry matter of the bean, a slight diminution of that of the mustard while in the case of pea hardly any change occured. The leaves of bean and mustard grown under the short-period treatment became thinner and larger than those in 16—8 hour period; in first line the reduction of mezophyll can be attributed to the decrease of spongy parenchyma. The stems of the bean were thinner, the ration of phloem and xylem was lower, that of the pith higher. In the stems of the mustard the opposite tendency was observed.

Introduction

It was first observed by BROWN and ESCOMBE (1905) that photosynthetic effectivity in higher plants can be highly (more than 100 per cent) increased by photoperiodic treatment. Subsequently EMERSON and ARNOLD (1935) established that exploitation of light energy per unit in the photosynthesis of Chlorella is very high when very short (about 10 μs) flashings are interrupted by longer (about 10 ms) dark periods. The striking importance of very short (0.01—100 s) light-dark periods on growth and CO_2 assimilation was confirmed by RABINOWITCH (1956), WITTINGHAM and BROWN (1958), WITTINGHAM and BISHOP (1961) on algae and by POLLARD (1969) on *Populus grandidentata.*

The first detailed description of the effect of longer light-dark periods on growth and flowering of higher plants are found in the classical works of GARNER and ALLARD (1920, 1927, 1931). They changed the length of light and darkness from 5 s to 12 h; using several species it was established that

a) Each species requires different periods of light and darkness for vegetative growth and flowering respectively: plants requiering long phoroperiods, plants requiering short periods and plants indifferent in this respect can be distinguished.

b) In enhancing growth two maxima were observed: 5 s and 12 h. Later BONDS (1955) observed a third maximum in the case of tomato at 4—4 h and in the case of Xanthium at 1—1 h period.

c) In general, the 1—15 min periods considerably inhibit growth.

GREGORY and PEARSE (1937) observed that a short periods cause partial closing of stomata of *Pelargonium zonale*. The slit between the guard cells is smallest at about a 1 minute period. The same was observed on the stomata of *Cucumis sativus* by PORTHSMOUTH (1937). Parallel to the decrease of the slit on the 22 day dry matters weight (a single cucumber plant) was the fourth area of the leaves the half as compared to plants grown under a 12—12 h period. Considerable reduction in dry matter, height and leave-area under 1—15 min periods was established also by BEHREND (1950), BONDE (1955), FOGG (1968), RAJAN et al. (1971).

Effect of inequal periodicity in light and darkness was investigated by HORVÁTH and MIHALIK (1977, 1978), HORVÁTH, TAKÁCS and MIHALIK (1979). 4 hours light and 2 hours dark cycles caused a 20 per cent increase, 2 hours light and 1 hour dark a 20 per cent decrease in dry matter as compared to a control of 16—8 h period.

According to above data the different light-dark cycles has great importance in the utilization of light energy. However, there are no data (except ALSUBAI and HORVÁTH 1980) about the effect of light-dark cycles on histological structure. To supplement this deficiency is the aim of this paper.

Materials and Methods

The investigated plants were: bean, *Phaseolus vulgaris* L. cv. Cheroque; pea, *Pisum sativum* L. cv. Kelvedons wonder; and mustard, *Sinapis alba* L.

In the phytotron (HORVÁTH, 1972) the plants got an irradiation of 35 Wm^{-2} in the 400—700 nm interval from light tubes F_{29}. The light-dark periods applicated were 30—15 min and 16—8 h respectively.

In the clima-boxes the temperature of air was 22—25 °C, the relative humuidity 50—75 per cent, the CO_2 concentration 0.03 per cent.

The plants were grown on a mixture of perlit and san 1:1 (volume) in Knop solution for six weeks. Water capacity of the substrate was daily adjusted to 70 per cent with distilled water.

The plants were harvested and investigated after six weeks growth. Dry weight was measured after fixation at 105 °C and drying at 70 °C.

For histological investigation in the case of bean the primordial and the first tripartite leaves and the stem between them, in the case of mustard and pea the fourth and fifth leaves and the internode beneath them were used. The epidermis was investigated in macerated preparates. Leaves and stems were cut after embedding in colloidine. The tissues of leaves were stained with Ehrlich's acidic haematoxyline, the stem cross sections with malachit green vesuvin double staining procedure.

In the leaves the number of epidermis cells and stomata per mm^2 and the thickness of the mesophyll and the palisade parenchyma were established. Cross sections of stems were drawned on paper with the aid of a projecting microscope; the ratio of the different histological regions was established by weighing the cut-out paper scraps.

Results

Changes in morphological characteristics

The short period (30—15 min) cycle (as compared to the 16—8 h cycle) differently influences the different plants. The bean's leave appears sooner, bean and mustard become higher, the area of the leaves become larger (Table 1). The leave of the pea reacted only slightly on the treatment.

Accumulation of dry matter and its distribution in the organs

This also shows differences in the different plants. As the result of short-period treatment dry weight is considerably decreased in bean, a little did it in mustard and

Table 1. Effect of the lenght of light-dark cycles on the number of leaves, area of leaves and height of plants.

	Bean		Mustard		Pea	
	1/2—1/4 (h)	16—8 (h)	1/2—1/4 (h)	16—8 (h)	1/2—1/4 (h)	16—8 (h)
Number of leaves per plant	6.6	4.6	11.5	12.0	10.6	11.0
Area of leaves cm^2 per plant	321.6	285.3	118.9	89.7	137.3	136.2
Height of plant cm	28.9	22.1	63.3	52.9	25.7	26.8

hardly changed in pea. In the case of pea a slight increase in dry matter might be attributed to the flowering enhancing effect of the short periods (Table 2).

Changes of thickness of the mesophyll

Short periods diminished in all the three plants the thickness of leaves. This change is the smallest in pea, in bean it attains 20—30 per cent. Decrease in thickness can be attributed in first line to the diminishing of the spongy parenchyma (Table 3). Absolute thickness of the palisade parenchyma changed only slightly but its relative share in the mesophyll increased (Plate I).

Table 2. Effect of the length of light-dark cycles on dry matter (mg) one plant

	Bean		Mustard		Pea	
	1/2—1/4 (h)	16—8 (h)	1/2—1/4 (h)	16—8 (h)	1/2—1/4 (h)	16—8 (h)
Root	205.5	390	228	252	219	246.5
Stem	390	580	712	734	323	323
Leave	388	664	312	353	368	351
Fruit	25	117	—	—	478	322
Total	1009	1751	1251.5	1390	1388	1242

Table 3. Effect of the length of light-dark cycles on the thickness of mesophyll

	Bean (primordial leave)		Bean (tripartite leave)		Mustard		Pea	
	1/2—1/4 (h)	16—8 (h)	1/2—1/4 (h)	16—8 (h)	1/2—1/4 (h)	16—8 (h)	1/2—1/4 (h)	16—8 (h)
Thickness of mesophyll (μ)	102.5	138	90	113.5	100	121	146	148
Thickness of Spongy parenchyma (μ)	58.4	86.9	45.9	59.0	53	79.9	87.6	94.7

Changes in the structure of the epidermis

This characteristic was differently influenced by 0.5—0.25 hour light-dark cycle in the different plants and depended of the position of the leaves as well. The number of epidermis cells and stomata per unit area was increased on bean (primordial leave) and mustard and decreased on bean (tripartite leave) and pea. The changes in the dimension of epidermis cells (at 3rd and 5th leaves) is, in general, greater on the adaxial than on the abaxial surface (Table 4, Plate II).

Table 4. Effect of the length of light-dark cycles on the epidermis of leaves

	Bean (primordial leave)		Bean (tripartite leave)		Mustard		Pea	
	1/2—1/4	16—8	1/2—1/4	16—8	1/2—1/4	16—8	1/2—1/4	16—8
Adaxial surface number of stomata per mm²	29.4	23.9	—	—	189	130	129	145
Adaxial surface number of epidermis cells per mm²	424	418	389	496	872	699	438	481
Abaxial surface number of stomata per mm²	264	219	198	237	331	257	157	175
Abaxial surface number of epidermis cells per mm²	950	841	797	992	875	756	352	390

Histological features of the stem

The stem of mustard and that of bean were more elongated under the short-period treatment. The elongated stem is thinner in case of bean and thicker in case of mustard. Short-period treatment decreased the relative participation of sclerenchyme, cortex, phloem and xylem and increased that of pith (Table 5, Plate III).

In case of mustard increase of phloem and xylem was dominant, the ration of sclerenchyme and pith decreased (Plates II and III). In case of pea, being rather unsensitive to the treatment, no significant differences were established between the two cycles (Table 5).

Plate I

1. Bean *(Phaseolus vulgaris)*, cross section of tripartite leave; light-dark cycle 16—8 h (x230)
2. Bean, cross section of tripartite leave; light-dark cycle 0.5—0.25 h (x230)
3. Pea *(Pisum sativum)*, cross section of leave; light-dark cycle 16—8 h (x230)
4. Pea, cross section of leave; light-dark cycle 0.5—0.25 h (x230)
5. Mustard *(Sinapis alba)*, cross section of leave; light-dark cycle 16—8 h (x230)
6. Mustard, cross section of leave; light-dark cycle 0.5—0.25 h (x230)

Plate I

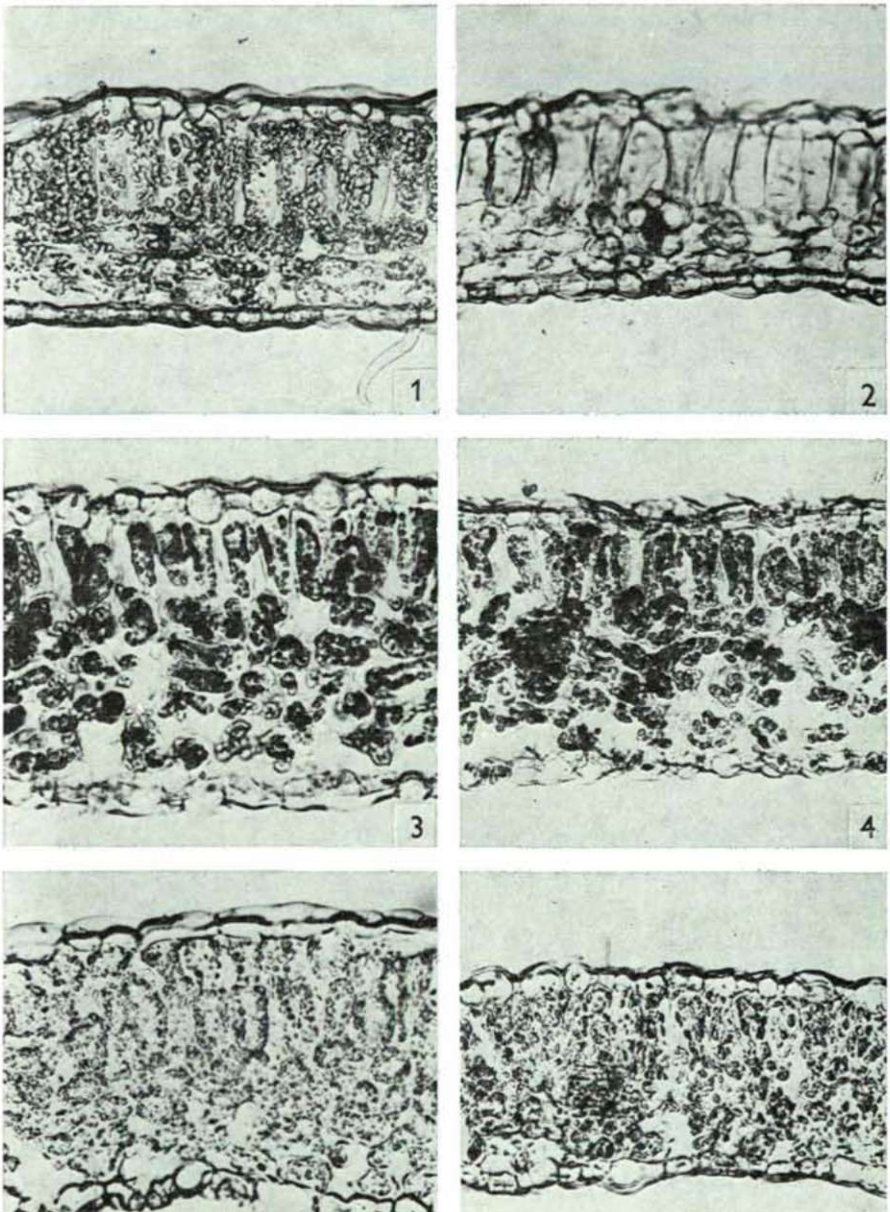


Table 5. Effect of the length of light-dark cycles on the proportion of the different tissues

	Bean		Mustard		Pea	
	1/2—1/4	16—8	1/2—1/4	16—8	1/2—1/4	16—8
Area of the stem cross section mm²	7.1	9.4	12.1	10.0	2.7	2.8
Cortex per cent	19.6	21.2	20.4	16.8	61.8	55.1
Sclerenchyma per cent	3.4	3.6	1.1	1.5	5.2	4.2
Phloem per cent	9.1	11.3	3.2	2.9	7.5	8.9
Xylem per cent	13.9	15.2	17.0	15.2	13.2	12.5
Pith per cent	54.0	47.2	58.4	63.8	11.8	13.0

Discussion

Authors' investigations demonstrated the essential significance of the different light-dark periods on growth, dry matter accumulation and histological structure of the plants. It was also established, in accordance with GARNER and ALLARD (1931), GREGORY and PEARSE (1937), PORTSMOUTH (1937), BONDE (1955) etc., that different plants react differently on cyclic treatments. In authors' experiments the 30—15 min periods influenced most sensitively the bean, less the mustard and least the pea.

GARNER and ALLARD (1931) explained different reactivity of plants by their short-day, long-day properties and their indifferency in this respect. But some of the data of GARNER and ALLARD can hardly be interpreted with the photoperiodic demand. E.g. the long-day plants *Rudbeckia bicolor* and *Delphinium ajacis* flower the quickest and accumulate maximal dry matter at 5—5 s cycle. On the other hand their experiences showd that there are cycles with similar effect on different plants: the short-day plant *Cosmos sulphureus* and the indifferent *Fagopyrum vulgare* shows similar growth and dry matter production at 5—5 s, 1—1 min and 12—12 h periods.

General decreasing effect on dry matter accumulation of the 1—15 min light-dark cycles was observed also by GREGORY and PEARSE (1937), PORTSMOUTH (1937), BONDE (1955), FOGG (1968), RAJAN et al. (1971) etc.

It seems, according to literary data and to authors' results, that the "favourable" cycles occur in the intervals of 1—15 s, 1—4 h and 12—16 h respectively.

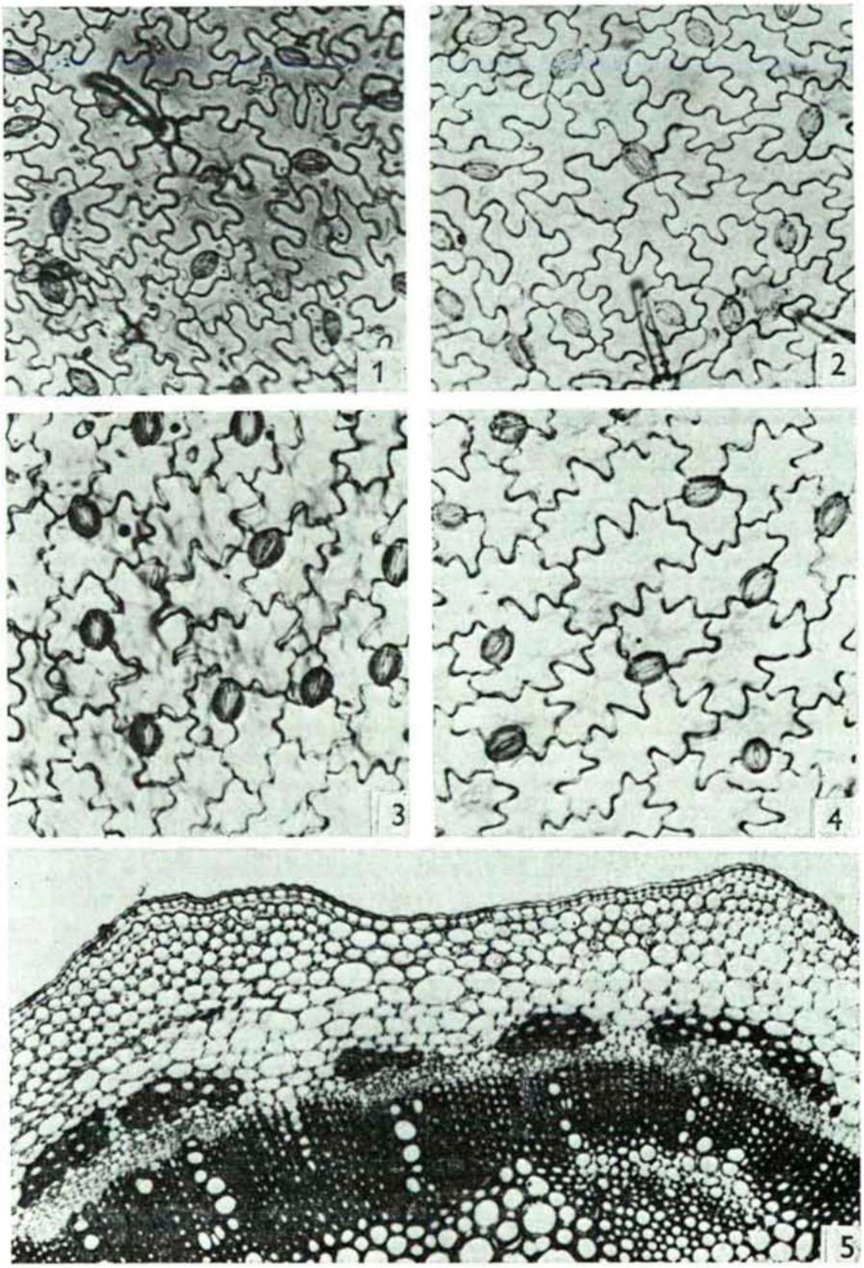
Former investigations of TAKÁCS and HORVÁTH (unpublished) showed that the excellent dry matter production of bean (cv. Aranka) at 4—2 h cycles considerably diminished at the 2—1h and 1—0.5 h cycles (Fig. 1).

As it could be expected, in recent experiments at 0.5—0.25 h periods the thinnest leaves, the lowest dry matter accumulation and the greater histological changes were observed in the case of bean.

Plate II

1. Bean, tripartite leave, abaxial epidermis; light-dark cycle 16—8 h (x230)
2. Bean, tripartite leave, abaxial epidermis:, light-dark cycle 0.5—0.25 h (x230)
3. Pea, adaxial epidermis; light-dark cycle 16—8 h (x230)
4. Pea, adaxial epidermis; light-dark cycle 0.5—0.25 h (x230)
5. Mustard, stem cross section; light-dark cycle 16—8 h (x100)

Plate II



It may be supposed that one of the favourable cycles of the pea is about one hour. On the other hand the unfavourable effect of a cycle about 1—15 min were demonstrated on several algae and higher plants, although there might exist plants (pea?) which are insensitive in this respect.

The mechanism of the effect of light-dark cycles, especially of short (1—15 min) cycles is unknown. On the basis of investigations about changes in pigment caused by short cycles (MARÓTI et al. 1980) it may be supposed that the light-dark cycles exert their activity through the light-dependent electron and proton transport. The specific differences are correlated with the membraneous systems of the chloroplasts.

The great variability of the spongy parenchyma and its reduction are elicited by short-period cycles (Plate III, Fig. 1). It is supposed (MARÓTI, 1976) that in the spongy parenchyme the noncyclic while in the palisade parechyme the cyclic phosphorilation predominate.

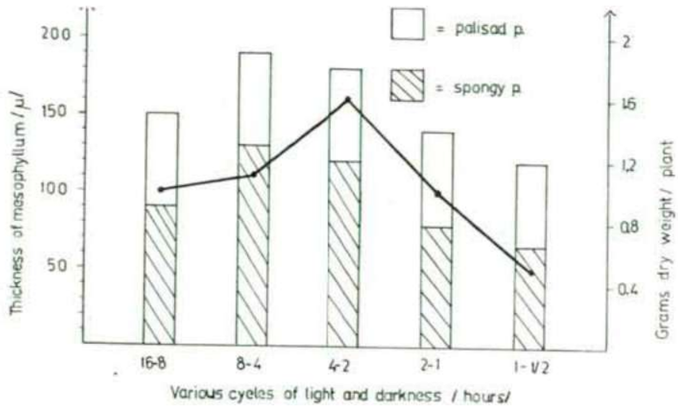


Fig. 1. Effect of the length of light-dark cycles on the ratio of spongy-parenchyma and palisade parenchyma and on the accumulation of dry matter in bean (cv. Aranka).

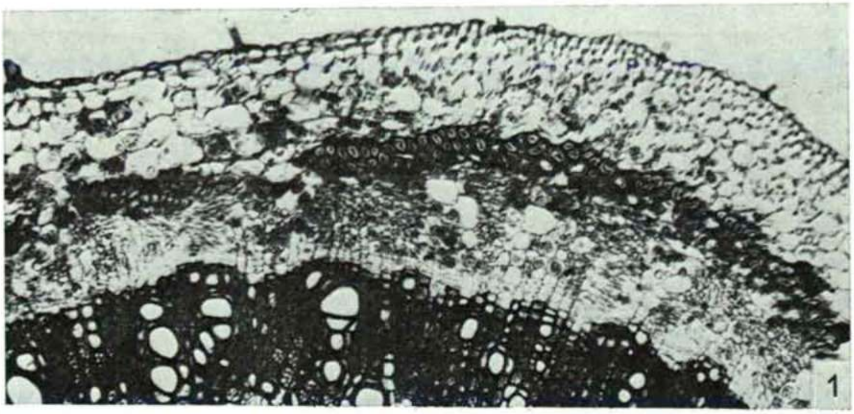
An important role can be attributed to the spongy parenchyma in the production of the primary and intermediary products of the photochemical reaction. The great variability of the spongy parenchyma makes possible the adaptation of the plants to different light-conditions.

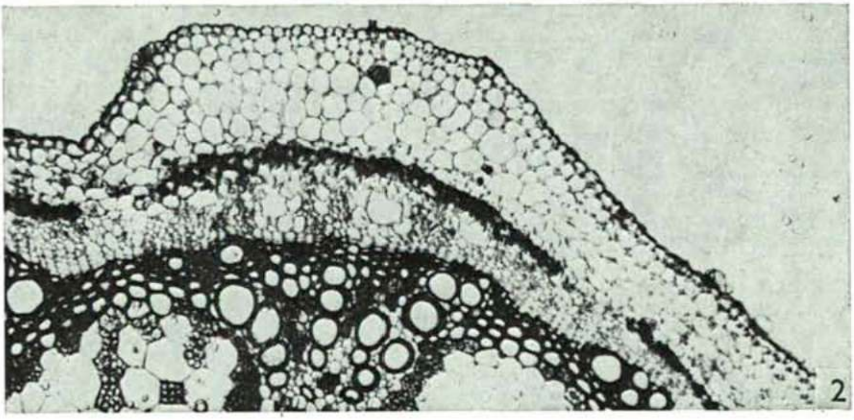
The opposite reaction of the histological struture of the stem in the bean and in the mustard may be contributed to the tendency of leave producing in the dwarf bean and to the tendency of stem producing in the mustard. The mechanism regulating the ratio of the different tissues in organs is unknown.

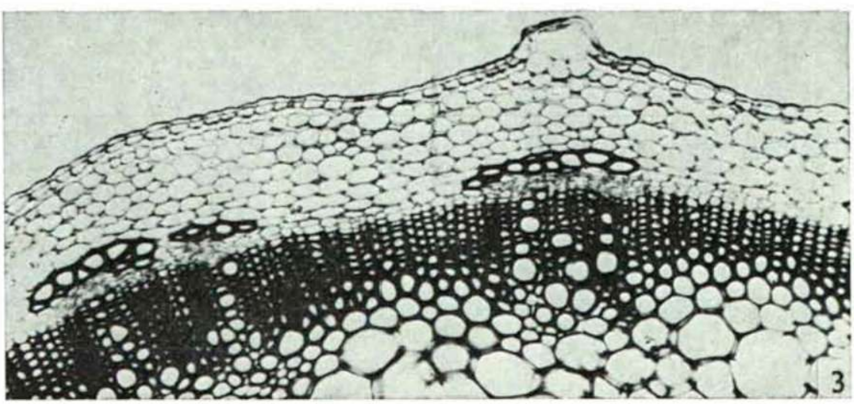
Plate III

1. Bean, stem cross section; light-dark cycle 16—8 h (x100)
2. Bean, stem cross section; light-dark cycle 0.5—0.25 h (x100)
3. Mustard, stem cross section; light-dark cycle 0.0—0.25 h (x100)

Plate III







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