The Development and Distribution of Chlorophyll in Roots of Flowering Plants grown in the Light.

1. Introduction.

The effect of light on roots has been studied chiefly in relation to growth - comparisons have been made between the rate of growth in light, in darkness and in sudden changes from one to the other. Leitch in her experiments on the influence of temperature on the rate of growth in Pisum, carried out as a control some experiments on the influence of light and came to the conclusion that, at least in the case of the experiments she was performing, light exerts no influence on the rate of growth.(1)

Other observers have studied the heliotropic response of roots. On the subject of the development and distribution of Chlorophyll, however, few references could be found. Dr. Arber mentions the occurrence of Chlorophyll in the roots of water plants grown under natural conditions. "Aquatic roots", she states, "often exercise another function, which is more remote from those generally assumed in the case of terrestial plants - namely, that of assimilation; their colour is sometimes quite conspicuously green".(2) Noack in his work on the relation between plastids and chondriosomes used Elodea because-"Die häufig auftretenden grünen Adventivwurzeln erwiesen sich als ganz verzüglich geeignet zur Lebenduntersuchung der Plastiden im Vegetationspunkt."(3) The present account deals with the development and distribution of chlorophyll in roots, under various experimental conditions.

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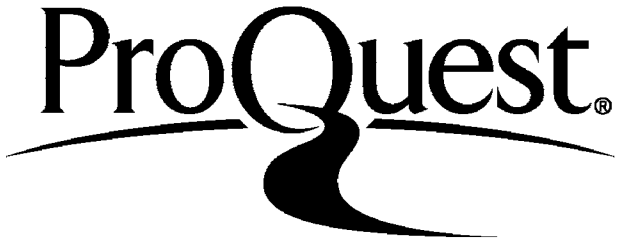
(1) Leitch I. A.B. "Some experiments on the influences of Temperature on the Rate of Growth in Pisum sativum." A.B. 1916.

(2) Arber A. "Water Plants" p.207.

(3) Noack K.L. "Untersuchungen über die Individualität der Plastiden bei Phanerogamen." Zeit. f. Bot. 1921.



II. Experimental Methods and Results.

A. Distribution of Chlorophyll in illuminated roots of different species.

1. Method Employed.

Seedlings of sixteen different species were used in this experiment. The seeds were germinated and the seedlings allowed to grow in darkness for about two days (except for a few of the species which were "earth-grown") and then planted out with their roots passing through holes in a sheet of cork, the cork being supported by four thin glass rods on the rim of a beaker, which was filled with water.

The beakers containing the various seedlings were then arranged around a 200 c.p. lamp, in a deep white sink. The electric bulb was suspended in a large glass jar, which was weighted down with lead, and the sink was filled with water until the water was almost on a level with the rims of the beakers. This arrangement served both to prevent the temperature from becoming too high and to render the illumination as intense and uniform as possible.

The roots were kept well aerated by means of a stream of water passing into each beaker from the tap by way of a piece of rubber tubing, from which the water dripped into a small funnel fixed through the centre of the sheet of cork carrying the seedlings. An overflow from the sink was arranged to prevent the water level becoming too high and so swamping the seedlings.

By such means it was possible to keep the seedlings growing in a healthy condition and free from algae for six weeks, if required.

Controls were grown in the dark.

Experimental Results

After about a fortnight's growth in the light, transverse sections were made of the roots of all the species and 13 out of the 16 species were found to have developed chlorophyll. The distribution of the chlorophyll in the roots, as given below, was constant for any given species.

(a) In stele only - none in cortex.

Vicia Faba L.)	) In various parenchymatous cells throughout
Rumex sp.)	the stele.
Acer pseudoplatinus L.	In 2 rows of cells above the protoxylem groups.
Aesculus Hippocastanum L.	In the medullary rays and in outer parenchymatous cells of pericycle.

(b) In stele and in cortex.

Pisum sativum L.(7 vars)	Very distinct green zone in centre of root. Chloroplasts in the parenchymatous cells of all the stele tissues. Also in cells of endodermis and inner cortex.(Plate I, fig.1.). The different varieties all showed the same distribution the only difference observable being one of intensity.
Vicia sativa L.	Chiefly in the cells of the inner and middle cortex but also in the outer parenchymatous cells of the stele.
Zea Mays L.	In pith: in cortex sharply limited to one layer outside endodermis.(Plate I.fig 3.)

(c) In cortex but not within the stele.

Triticum vulgare Vill.	Sharply limited to one layer outside endedermis (c.f.Zea Mays L. In Triticum there is no pith, the primary xylem reaching right to centre). (Plate II, Fig 4.)
Ranunculus Ficaria L. (Fibrous roots)	Sharply limited to one layer outside endodermis (c.f. tuberous roots below)
Hordeum vulgare L.	Limited to about 3 layers outside endodermis.
Scilla nutans. Sm.	In middle cortex of thick contractile root.(Plate I.Fig.2.)
Helianthus annuus L.	Throughout cortex.
Bellis perennis L.	Throughout cortex. (Plate II, Fig 5.)

(d) No Chlorophyll developed.

Ranunculus Ficaria L. (Tuberous roots)	
Ricinus communis L.	

Fagopyrum esculentum Moench

Allium Cepa.L.
(Conditions of experiment unfavourable to growth of this species)

Longitudinal sections showed that in all species except Triticum and Hordeum, the chlorophyll was developed throughout the main roots to within 1-2 cm. of the apex, the greenness decreasing in intensity from the base to the apex. In Triticum and Hordeum, however, the chlorophyll was developed only in the first 1.5 - 2 cm of the base of some of the roots and not at all in others.

No chlorophyll was developed in the controls grown in the dark.

B. Effect of various conditions on the development of chlorophyll in the roots of Pisum sativum.

1. Roots illuminated, shoots in darkness.

It was thought possible that the development of chlorophyll in the roots might be conditioned by the formation of substances during assimilation, therefore, with this in mind, the general method described above (A.1.) was employed but with the shoots in the dark, a small light-proof shade being placed on the cork sheet.

The seedlings so grown showed only a very slight development of chlorophyll compared with those grown for the same length of time with both shoots and roots in the light.

2. Shoots illuminated, roots in darkness.

In order that the roots should be in darkness, the beaker was placed inside a blackened tin, while the shoots were exposed to the light of the 200 c.p. lamp.

No chlorophyll was developed in these roots except just at the base, complete exclusion of light from which was difficult.

3. Delayed illumination of seedlings.

Other seedlings were grown for a fortnight in the dark and then transferred to the light, both root and shoot being illuminated.

These seedlings even after a month's illumination showed scareely any development of chlorophyll in their roots, although the leaves became green and the stem faintly so.

This experiment then, like Experiment B1.above, suggests that the formation of chlorophyll in the roots is favoured by conditions advantageous to assimilation in the shoot.

4. Removal of Cotyledons and Epicotyl

The cotyledons and epicotyl were removed from some of the vigorous seedlings growing in the light.

The effect of this operation was that the main roots of these seedlings ceased to grow and became, after a few days, green to their tips. Later, new lateral rootlets were formed in which chlorophyll did not extend to the tip.

In this case, the production of chlorophyll in the root tip was probably due to the maturing of the root tip tissue.

C. The effect of culture solutions on the development of chlorophyll in roots.

1. Method Employed.

The same general method as described above was employed, but the roots of the seedlings were placed in :-

(i) .2% cane sugar solution.

(ii) .2% potassium nitrate solution.

Controls were grown in tap water.

In this case no means of aeration of the r oots was devised but the solutions were changed daily. The species used in this experiment were :- Pisum sativum L. Triticum vulgare Vill. Hordeum vulgare L. Zea Mays L. Vicia Faba L.

After some weeks growth in these two solutions the roots of the seedlings all showed a similar distribution of chlorophyll to those grown in tap water.

D. Roots grown in damp air.

It was thought possible that the development of chlorophyll in illuminated roots might be dependent on the degree of aeration, which must always be relatively poor in roots growing immersed in a solution. Some seedlings of Pisum sativum L. were therefore grown in specimen tubes with a thin strip of damp filter paper dipping into water down one side of the tube.

After a period of illumination the distribution of chlorophyll in the roots was investigated and found to be the same as in those grown entirely immersed in water, so that the poor aeration of roots growing in solution did not appear to be a relevant factor.

E. Examination of material fixed for plastids & chondriosomes.

In the course of the work the possibility suggested itself that the cultures might provide material which would throw some light on the vexed question of the relation between plastids and chondriosomes. It has been suggested by some workers e.g. Guillermond (i) that plastids have their origin in the chondriosomes which normally seem to occur in embryonic cells i.e. that the chondriosomes of mature cells are potential plastids which have not developed. By others e.g. Mottier (ii) it is held that in embryonic cells there are two distinct classes of bodies plastid initials (primordia) which develop into the plastids of the mature cell and chondriosomes which do not give rise to plastids but which maintain their characteristics in the mature cells, the function of which is still a matter of conjecture. It was thought possible that as the tissues of the roots became green on exposure to light, some evidence of the transition from chondriosomes to plastids might be obtained.

(i) Guillermond A. "Sur l'origine Mitochondriale des plastids" Ann.Sci.Nat.10 ser Tm.1. 1919

(ii) Mottier D.M. "Chondriosomes and the primordia of chloroplasts & leucoplasts" A.B.1919

A detailed study of the distribution of plastids and chondriosomes was therefore made in Pisum roots, both of those grown in the light and in the dark. The fixatives used were :-

(1) 20 vol. of 10% solution of ordinary commercial formalin together with 80 vol of 3% solution of Potassium bichromate as recommended by N.H. Cowdry for fixing chondriosomes.(i)

(2) Weak Chromo-acetic acid solution.

The former of these s hould preserve the plastids and chondriosomes, the latter, the plastids only. The stains used were:

(1) Iron Alum haematoxylin

(2) Acid Fuchsin and Picric acid.

A study of the fixed and stained material soon showed that the hope that the material would be specially favourable for observing transition stages between chondriosomes and plastids would not be fulfilled, for well developed plastids were present in all cells of the cortex as well as the green zone (Plate I. Fig 1.). If anything, the colourless plastids in the cortex (Plate II, Fig.7) were larger than those of the green zone.(Plate II Fig.6). In short, the illumination of the roots caused the development of chlorophyll but apparently did not affect the distribution of the plastids. In the controls grown in the dark, a similar distribution of plastids was found. In this case, however, all the plastids appeared to be smaller (Plate II, Figs.8& 9.)

In the material fixed with bichromate-formalin mixture, Chondriosomes were observable in the form of small granular or rod-shaped bodies occurring together in the same cell (Plate III, Fig Fig 10.) much as described and figured by Noack (ii). Chondriosomes appeared to be present together with plastids in all the parenchymatous cells of the mature root tissue (Plate II, Figs.6.7.8.9., Plate III, fig 10). In a number of cells the chondriosomes seemed especially numerous in the n eighbourhood of

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(i) N.H. Cowdry "A comparison of Mitochondria in Plants and Animal cells. Biol. Bull. V ol. 33.

(ii) Noack K.L. "Untersuchungen über die Individualität der Plastiden bei Phanerogamen". Zeit. f. Bot. 1921

the nucleus as shown in the micro-photograph.(Plate III, fig 10) Guillermond noticed a migration of the chondriosomes to and from the nucleus in living material. (1)

In the embryonic tissues of the root there appeared to be present some bodies of intermediate size as well as the undoubted plastids and c hondriosomes.

No distinct chondriosomes could be made out in corresponding material fixed in weak chromo-acetic acid solution although the plastids were preserved.

Discussion of Results.

These experiments seem to show that most, if not all, roots have the potential capability of developing chlorophyll. In which cells of the root the chlorophyll is developed seems to depend on the species. In this respect the work of D.G.Scott is of interest.(2) She has recorded the difference in distribution of chlorophyll in the young shoots of woody plants. She examined 24 species and found that the distribution varied considerably and that in these shoots chlorophyll was often developed in situations where light could not penetrate to any great extent e.g. in the medullary rays and in the pith. Two of the species examined by her were used in this experiment - namely, Aesculus Hippocastanum L. "Chlorophyll developed in cortex, scantily in the medullary rays and in the medullary cells bordering on the protoxylem" and Acer Pseudoplatinus L. "Chlorophyll developed in the cortex, medullary rays (especially in the very broad ones) and in the medullary cells bordering on the protoxylem." The shoots of the species used in the present experiment showed, when cut, a similar distribution of chlorophyll to that of the roots, except that there was in all cases, in addition, a development of chlorophyll in at least the outer layers of the cortex.

(1)Guillermond A. "Observations vitales sur le chondriome des végétaux .... Rev.Gen.Bot. 31. 1919

(2) Scott D.C. "On the Distribution of Chlorophyll in the Young Shoot of Woody Plants". A.B. 1907.

The reason for the development of chlorophyll in some cells and not in others, is not at all clear. It certainly cannot be explained as due to the presence of undersized or deformed plastids in the colourless cells, as has been found to be the case with many variegated plants.(1) In Pisum, at any rate, the plastids in the outer cortex, appear to be particularly large and well formed. (Plate II, Fig.7)

The fact that the plastids, whether green or colourless, are larger in the light grown parts is of interest. This may be due to the direct effect of light or to better nutrition owing to assimilation or to some other indirect effect of light. Experiments on etiolated shoots fit in with such explanations as these. That the amount of nutritive material available may influence the development of chlorophyll is suggested by the work of Coupin(2) upon the development of chlorophyll in etiolated seedlings. Coupin found that when etiolated seedlings were exposed to diffuse light the time of exposure to light required for the production of chlorophyll was least in those regions, e.g. cotyledons, in which nutritive material was most abundant. It appears unlikely, however, that the development of chlorophyll only in certain sharply delimited regions of the root as occurs in some species can be due to such a cause. On the other hand it is possible that, rather than light exercising a positive effect encouraging the development of larger plastids and chlorophyll in some cells, the effect is a negative one due to darkness, i.e. in the dark substances may be produced which have a depressant effect.

The development of chlorophyll in the root seems to be closely correlated with the development of chlorophyll in the stem, for in the case of those seedlings whose shoots were covered up while their roots were exposed to the light after being in the dark for 14 days, there was only a very slight development of chlorophyll in both stem and root even after a month's subsequent exposure to the light, although the leaves developed the normal amount.

(1) Randolph L.F. "Cytology of Chlorophyll Types in Maize.B.G.1912.
(2) Coupin H. "Sur la production de la chlorophylle par les végétaux exposés à une lumiere discontinue".Compt.Rend.Acad.Sci. Paris 170.1920

The presence of both plastids and chondriosomes in all cells throughout the tissues, even in the root tips, is note-worthy. It was, however, almost impossible, to decide whether there were any structures of intermediate size present or not. In the older cells the structures were clearly divisible into these two classes, but in the root tip this could not be said to be the case, as there were certainly some granules which might have been said to have been either large chondriosomes or else plastids which had just undergone division. However, in tracing the cell contents from the root tip to the mature tissues, there did not appear to be any striking increase in number of plastids and corresponding decrease in number of chondriosomes as would be expected if Guillermond's view of the origin of plastids from chondriosomes were correct.(1)

Summary of Results.

1. It was found that chlorophyll was developed in the roots of 13 out of 16 species grown with their roots exposed to the light of a 200 candle-power electric lamp. Moreover, it was found that the distribution of chlorophyll in the roots was constant for any given species but varied very considerably from species to species; in some cases the green zone was sharply delimited (e.g. in Triticum vulgare VIII) and formed a very striking feature in transverse sections.
2. The experiments on the effect of various conditions on the development of chlorophyll in the roots of Pisum sativum, suggested that the formation of chlorophyll in the roots was favoured by conditions advantageous to assimilation in the shoot.
3. The effect of culture solutions on the development of chlorophyll in the roots was a negative one, the development and distribution of the chlorophyll being similar to that of roots grown in tap water.
4. Seedlings grown in damp air showed the same distribution of chlorophyll as those grown in water, so that the poor aeration of roots growing in solution did not appear to be a relevant factor in the distribution of chlorophyll.

(1) Guillermond A. "Sur l'Origine mitochondriale des plastids" Ann. Sci. Nat. 1919

5. In an examination of fixed material, well developed plastids were found to be present in all the cells of the cortex as well as in the green zone i.e. the effect of illumination of the root was to cause the development of chlorophyll in some cells, and not to affect the distribution of the plastids.

6. Chondriosomes appeared to be present together with the plastids in all the cells of both the light and dark grown roots.

My thanks are due to Prof. W. Neilson Jones for suggesting this s ubject to me and for his valuable advice and criticism throughout the course of the experiments.

In the course of writing the above account my attention has been called to a notice, in "Botanical Abstracts", of a paper by A. Siebert (1) entitled "Ergrünungsfähigkeit v on Wurzeln" which might possibly have some bearing on this subject. Unfortunately, up to the present, I have been unable to obtain a a copy of this paper.

Doris Powell

Bedford College.

April. 1924

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(1) Siebert A. "Ergrünungsfähigkeit von Wurzeln" Kiel 1920
Bot. Abs. Vol.12. No.1. Jan. 1923. p.116.

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6. Guillermond A. "Observations vitales sur le chondriome des végétaux et recherches sur l'origine des chromoplastids et le mond de formation des pigments xanthophylliens et carotiniens." Rev. Gen. Bot. 31. 1919

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Explanation of Plates.

(Plate I, Figs - 1, 2, 3. Plate II. Figs 4 and 5.
Chloroplasts shown diagramatically in black)

Plate I.

Fig. I. T.S. of root of Pisum sativum L. grown in light, showing distribution of plastids. Fresh material. Colourless plastids in outline.

Fig. 2. T.S. of contractile root of Scilla nutans Sm. grown in light showing distribution of chloroplasts. Fresh material

Fig. 3. T.S. of root of Zea Mays, grown in light, showing distribution of chloroplasts. Fresh material.

Plate II

Fig. 4. T.S. of root of Triticum vulgare Vill. grown in light showing distribution of chloroplasts. Fresh material.

Fig. 5. T.S. of root of Bellis perennis L grown in light, showing distribution of chloroplasts. Fresh material.

(Figs. 6 - 9. Drawn with the camera lucida from prepared slides, fixed formalin-potassium bichromate. Stained with iron alum haematoxylin.)

Fig. 6. Cell from "green region" of root of Pisum sativum L grown in light showing nucleus, chloroplasts and chondriosomes.

Fig. 7. Cell from outer cortex of root of Pisum sativum L, grown in light showing nucleus, plastids, some with starch inclusions, and chondriosomes.

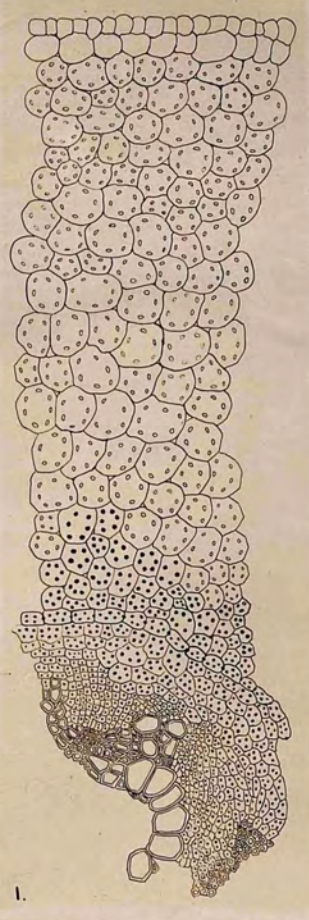
Fig. 8. Cell from region corresponding to that of Fig 6. of root of Pisum sativum L. grown in dark showing nucleus, plastids and chondriosomes.

Fig. 9. Cell from region corresponding to that of Fig.7. of root Pisum sativum L. grown in dark showing nucleus, plastids and chondriosomes.

Plate III

Fig.10. Micro-photograph of cell from root of Pisum sativum L. grown in light showing the nucleus, plastids and chondriosomes (fixed formalin-potassium bichromate - stained iron alum haematoxylin).

Plate I



1.

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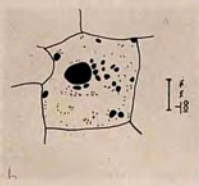
Plate II

4.

5.



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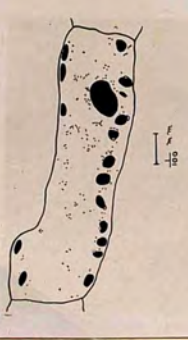

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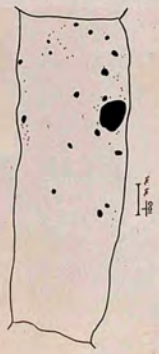

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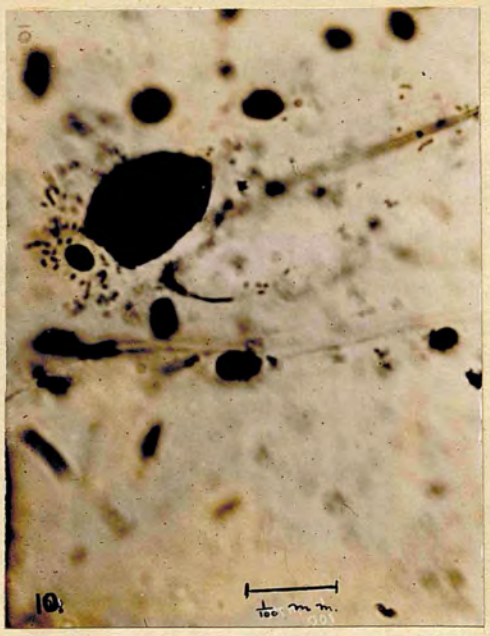
Plate III



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