McGINNIS

Regeneration in the cotyledons of Lupinus Albus

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REGENERATION IN THE COTYLEDONS OF LUPINUS ALBUS

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REGENERATION IN COTYLEDONS

OF

LUPINUS ALBUS.

The term regeneration has been used by different botanists with very different meanings. Most later writers however define it as the replacement of parts that have been removed, and thus make it broad enough to include all the different phenomena that have been regarded as regeneration.

This replacement may be accomplished in three ways, all of which may be illustrated by regeneration in roots. Simon (:04) and Nemec (:05) have shown that if a very small part of the root-tip (about .75 millimeters) be removed, a callus will form over the whole cut surface and from this the tip will regenerate in its original form. Pfeffer and Nemec have restricted the word regeneration to include only this kind of replacement. According to them regeneration is the replacement in its original form and with its original functions, of an organ or part that has been removed. If the root-tip be cut off farther back (more than .75 m.m.) it is replaced in a somewhat different manner. It may take place by the development from the callus on the cut surface, of several roots, or it may take place by new growth arising from meristematic cells present in the periblem. The third method of regeneration, as illustrated by roots is by the development of primordia already present in the periblem into lateral roots, after the removal of more than .75 m.m. of the tip.
This third method of accomplishing the replacement of lost parts is the commonest one in higher plants. Besides in roots it may be seen in cuttings of the stem of the willow, poplar, etc. which regenerate both roots and shoots in this way. McCallum has made some interesting investigations of regeneration which takes place by the development of primordia, in seedlings of the common scarlet runner bean. If the epicotyl be cut off, the two primordia, in the axils of the cotyledons, which otherwise would not develop, may take the place of the epicotyl and grow to be the size of the normal plant.

Regeneration in the restricted sense of Pfeffer and Nemec while not as general as regeneration by development of primordia is seen occasionally in the higher plants in other members besides roots and is common in the lower plants. Goebel (:02) found that when certain fern leaves are split at the apex that each half completes itself in the same manner that roots complete themselves when a very small portion of the tip is removed.

Regeneration in the second manner discussed above for roots, that is by the new growth arising from meristematic cells, or from cells already differentiated, is not unusual in the higher plants. This (together with the development of primordia already organized) occurs in cuttings of the willow and poplar (Vöchting: 78). Many leaves produce new roots and shoots from parenchyma in the neighborhood of the vascular bundles. (Goebel: 02, Winkler: 03). If parts of the thallus of Marchantia be cut off transversely, they will be replaced by the multiplication of cells which are apparently mature and differentiated, at the cut surface or back of it. This phenomenon of regeneration is
the one with which this paper is particularly concerned.

While the subject of regeneration is a very old one, and a great many experiments have been carried on concerning it, with both animals and plants, these experiments have failed almost entirely to answer the questions that arise in regard to the internal factors which influence regeneration, and our knowledge concerning the external factors, which influence it, is very uncertain.

The present experiments were undertaken with the object in view of contributing something definite to our knowledge with regard to the influence of external factors upon regeneration. The readiness with which roots form, under the conditions of my experiments, upon the isolated cotyledons of Lupinus albus made these favorable objects for the study of this phase of regeneration.

Blooscizewski, as early as 1876 experimented with Lupinus luteus and reported in connection with some other experiments that roots had regenerated on cotyledons from which the hypocotyl had been removed. Von Rabe (05) while conducting some experiments for testing the resistance to drying, of seeds in various stages of germination, incidentally observed that when cotyledons of Lupinus coeruleus, albus and luteus were removed at the right time from the partially decayed seedlings, they remained green and healthy for about six weeks, and during that time, developed callus and roots. Smith (07) experimenting with Lupinus lutens and augustifolius obtained only negative results. Smith gives an extensive table, designed to report all the known cases of
regeneration in isolated cotyledons. He fails to list Lupinus albus. Miss Kupfer is the only investigator (besides von Rabe) who so far as I know has reported root formation on the isolated cotyledons of Lupinus albus. These records however are without reference to the conditions under which such regeneration takes place, and are very incomplete and indefinite.

**Methods and Material.**

In all of my experiments seeds of Lupinus albus of as nearly uniform size as possible were used. After soaking twenty-four hours the seed coats were removed, and the plant axes together with a little less than one third of the cotyledons were cut off. (See Fig.5) Any variation in the above procedure will be mentioned in the account of the special experiment in which the variation was made. The cotyledons were thus separated from the plant and all primordia lying near the axes were removed. The cotyledons were then placed with cut surfaces down, in moist sand, in Koch's moist chambers. The two cotyledons from the same seed were placed opposite to each other in order to observe any difference in their behavior. The dishes were then placed in the greenhouse under favorable conditions of light and moisture. Here I was not able to keep the temperature as nearly uniform during an experiment as could be desired. However all of each series was exposed to the same variations of temperatures, and hence can be directly compared. The sand was weighed and the desired percent of moisture added and thoroughly distributed by stirring. The percent of moisture was kept constant by bringing the dish of cotyledons up to its original weight after
the loss of moisture by evaporation. The moisture added was distributed over the surface by means of an atomizer.

**General Observations.**

When the hypocotyl is cut off in the manner described above, a thick callus forms over the surface of the wound. This callus is most abundant at the cut end of the prominent vascular bundle or midrib. (Fig.1) In some cases smaller protuberances are found at the cut ends of branches of the midrib. For convenience I shall refer to the cut surface of the cotyledon as the base, and the cut ends of the vascular bundles on this surface as the basal ends. The other ends of the vascular bundles I shall speak of as the apical ends. The larger callus at the basal end of the midrib grew larger as the cotyledons grew older, becoming in some cases as much as three millimeters in diameter. Those cotyledons which in addition to having the hypocotyl removed, had the sides cut off parallel to the midrib (Fig.2) developed a scant callus on the cut lateral surfaces but there were no knoblike growths of callus produced at the cut apical ends of the branches of the mid-rib.

The new roots which form after the removal of the plant axis develop from the callus at the basal end of the midrib, or they make their way through the tissues of the cotyledons and break forth farther up, but always along the course of the mid-rib. I have never seen any roots form more than three millimeters above the base. My observation has been that those roots which regenerate early— in from ten to twenty days— almost universally come from the midrib. The roots which develop from
the midrib may emerge either from the dorsal or ventral side of the cotyledon. I shall not attempt to explain why those roots which are formed early develop from the callus and those which are formed later develop from the midrib, but it is clear that the newly formed callus possesses the power to regenerate roots to a greater degree than the parenchyma of the vascular bundle, and that later the callus loses this power. After this any roots which are produced come from the parenchyma.

Another general observation in these experiments is the excessive growth of the cotyledons. These isolated cotyledons grow to be even larger than those which develop on the plant. Those which regenerate roots grow considerably larger than those which do not. The average size of ten cotyledons, after soaking, is 13.7 X 10.6 millimeters; of ten, after producing roots, is 27.3 X 20.3 millimeters, and of ten of the same lot which did not produce roots is 20.5 X 18 millimeters. In the very limited anatomical studies which I made of the cotyledons, there was no evidence of growth by multiplication of cells, except in the region mentioned above, the parenchyma of the vascular bundle. This excessive growth of the cotyledon then, is probably due to the enlargement of the cells already formed. Since photosynthesis is active, it is not remarkable that the products of this activity, used only for the work of the cotyledon, and having no outlet except to the regenerating root, should stimulate growth. Those cotyledons which regenerate roots may become larger than those which do not, on account of their greater water content.

Most earlier investigators of regeneration in cotyledons have mentioned the increase in size. Smith and Van Tiegham
have made some actual measurements, but do not discuss the manner in which this growth takes place.

My experiments and their results may be considered under seven headings, as follows: (1) the effect of moisture, (2) the effect of temperature, (3) the effect of light and darkness, (4) the effect of the size and character of the wound, (5) the effect of cutting off different proportions of the cotyledons, (6) the influence of the age of the cotyledon on the ability to regenerate, (7) the formation of shoots.

The Effect of Moisture.

Frequently the young leaves on trees are frozen during a late frost, and afterward drop off. After the removal of leaves in this way the dormant buds in the axils of the leaves develop at once while ordinarily they would not until the next year. Wiesner attributes this stimulation to development of the dormant buds, to the greater amount of moisture that they are supplied with, in the absence of the transpiring leaves. Klebs (:03) and Vöchting both regard the amount of moisture as an important factor in regeneration, while McCallum (:05) is able to induce regeneration in Phaseolus while the plant is evidently suffering for lack of moisture. My experiments, while they do not show that moisture is the factor which induces regeneration, do show that the amount of moisture has a marked effect on the rate of regeneration and upon the per cent of cotyledons which regenerate, as may be seen by a perusal of Table I.
### Table I

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Wt. of moisture</th>
<th>Wt. of sand</th>
<th>Percent of moisture</th>
<th>No. of cotyledons</th>
<th>No. of roots at first observation</th>
<th>No. of roots at last observation</th>
<th>Percent of roots</th>
<th>Date of setting up experiment</th>
<th>Date of first appearance of roots</th>
<th>Date of last observation</th>
<th>No. of days to form roots</th>
<th>No. of days between first and last observations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel A</td>
<td>18° - 25°C</td>
<td>5.5 g.</td>
<td>564</td>
<td>.98</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>Nov. 13</td>
<td>Nov. 25</td>
<td>Jan. 6</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>Vessel B</td>
<td></td>
<td>17</td>
<td>658</td>
<td>2.3</td>
<td>30</td>
<td>1</td>
<td>5</td>
<td>16 ½</td>
<td>Nov. 25</td>
<td></td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>Vessel C</td>
<td></td>
<td>18</td>
<td>291</td>
<td>5.8</td>
<td>57</td>
<td>1</td>
<td>17</td>
<td>28.2</td>
<td>Nov. 25</td>
<td></td>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>Vessel D</td>
<td></td>
<td>15</td>
<td>673.5</td>
<td>1.0</td>
<td>30</td>
<td>2</td>
<td>5</td>
<td>16 ½</td>
<td>Nov. 25</td>
<td></td>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>Vessel E</td>
<td></td>
<td>38</td>
<td>210</td>
<td>15.3</td>
<td>30</td>
<td>2</td>
<td>4</td>
<td>12.2</td>
<td>Jan. 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel F</td>
<td></td>
<td>33</td>
<td>143.5</td>
<td>2.2</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Jan. 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel A</td>
<td>20° - 25°C</td>
<td>10 g.</td>
<td>533 g</td>
<td>1.8</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>Jan. 7</td>
<td>Jan. 20</td>
<td>Mar. 2</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>Vessel B</td>
<td></td>
<td>12.5</td>
<td>510.7</td>
<td>2.3</td>
<td>30</td>
<td>2</td>
<td>15</td>
<td>50</td>
<td>Jan. 20</td>
<td></td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>Vessel C</td>
<td></td>
<td>23</td>
<td>599.5</td>
<td>3.7</td>
<td>30</td>
<td>5</td>
<td>24</td>
<td>80</td>
<td>Jan. 20</td>
<td></td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td><strong>Experiment III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel A</td>
<td>19° - 22°C</td>
<td>6 g.</td>
<td>632 g</td>
<td>.9</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>Apr. 21</td>
<td>Apr. 30</td>
<td>May 22</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Vessel B</td>
<td></td>
<td>21.5</td>
<td>570.5</td>
<td>3.6</td>
<td>28</td>
<td>2</td>
<td>11</td>
<td>39.2</td>
<td>May 2</td>
<td></td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Vessel C</td>
<td></td>
<td>34</td>
<td>718</td>
<td>4.2</td>
<td>30</td>
<td>9</td>
<td>20</td>
<td>66 ½</td>
<td>May 2</td>
<td></td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Vessel D</td>
<td></td>
<td>69</td>
<td>665</td>
<td>9.4</td>
<td>26</td>
<td>6</td>
<td>16</td>
<td>61.5</td>
<td>May 2</td>
<td></td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Vessel E</td>
<td></td>
<td>60</td>
<td>400.5</td>
<td>13</td>
<td>30</td>
<td>2</td>
<td>5</td>
<td>16 ½</td>
<td>Apr. 30</td>
<td></td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>
The experiments in regard to the effect of moisture were set up in the usual way except that each vessel in a series was supplied with a different per cent of moisture. So far as I was able to judge this was the only variation in conditions in the different vessels of a series, and any variation in the rate of regeneration or the per cent of cotyledons which regenerate may be attributed to the moisture content.

An examination of Table I shows in experiments I and III, that there is a certain optimum amount of moisture which is most favorable for regeneration of roots in Lupinus albus. Below this amount the number of roots diminishes with the moisture until a minimum amount of moisture is reached, which we can conclude is something above .9 per cent, since no roots were produced in sand with that moisture content. Above the optimum the number of roots decreases with the increase of moisture until a maximum is reached, above which no roots will form. In Experiment I this is shown to be between 22 per cent and 15.3 per cent. Apparently from my experiments the maximum, optimum and minimum amounts of moisture are not the same for all temperatures.

A further examination of Table I reveals the remarkable difference in the rate of regeneration between different cotyledons in the same dish. Some cotyledons produced roots in thirteen days, while the latest ones in the same dish required fifty-four days to regenerate and intermediate ones formed roots in eighteen, twenty-three and forty-nine days. Since this variation cannot be due to difference in external conditions it must be due to some internal factor. Another observation that may be
mentioned in this connection is the similarity in behavior between the two cotyledons from the same seed. In the majority of cases if one of these cotyledons formed roots, the other one did, or if one remained negative, so did the other, and in those cases which gave positive results, the two cotyledons belonging to the same seed usually formed roots in the same length of time. Smith reports similar results for the cotyledons of Cucurbita.

**Effect of Temperature.**

That temperature is an important factor in regeneration has long been recognized. The earliest experimenters on this subject of regeneration noticed that animals regenerate lost parts more readily in warm temperatures than in cold, but I have seen no accounts of experiments dealing with the relation between temperature and plant regeneration.

My experiments concerning the effect of temperature on regeneration were carried on under conditions that were somewhat adverse, and hence the results are not as complete as could be desired. I was not able to keep a constant green-house temperature as the records for Experiment I and II, in Table I show. Late in the year a dark room was provided in which the temperature was fairly constant, remaining at twenty-one degrees C. most of the time. Once during the course of an experiment the temperature went up to 22° and once it fell to 19°. This dark room proved to be a very favorable place for the development of roots. Another difficulty was that I was unable to find a suitable thermoregulator for the conditions of my experiment.

In spite of these difficulties however I have been able to
establish as some facts with certainty, in regard to the effect of temperature in the regeneration of roots.

Experiment IV.

A layer of moist sand was put in a fruit jar, laid on its side. In this sand thirty cotyledons were placed, in the manner already described. The jar was tightly sealed so that it would not admit water, and immersed in water in a rectangular tank with glass sides. The tank was covered with wool at the ends to prevent loss of heat. A thermoregulator kept the temperature constant between 30° and 31° C. for a short time. A stirrer which kept the water in motion equalized the temperature. The jar containing the cotyledons was aerated by two glass tubes which entered through a rubber stopper in the lid and extended up through the water to the air. In the five days during which this constant temperature was maintained, the cotyledons rotted. I concluded from this that a constant temperature of 30° C. was above the maximum for regeneration of roots in Lupinus albus.

Experiment V.

In running water I found that the temperature remained pretty constantly between 17° and 18° C. An experiment was set up in a glass fruit jar in a similar manner to the one described above. After ten days the cotyledons showed only very slight indication of callus and were beginning to rot without forming roots. It is clear from this that 18° is below the minimum temperature for the regeneration of roots.

Table I shows an important fact in regard to temperature, namely that a temperature varying between 22° and 25° C. is much more favorable to regeneration, than the lower temperatures,
recorded for Experiments I and III. Experiment III was carried on in the dark room with the constant temperature. The objection might be made that Experiment III was not comparable on this account to the other experiments which were carried on in the light. However for reasons which I will discuss in connection with the effect of light and darkness, I think I am justified in making the comparison. Moreover I have kept numerous records of experiments carried on in light which show the same thing, but not for such long series as is recorded for Experiment III. In order to show the effect of temperature clearly I have chosen for tabulation, dishes from different series all cultivated in the light. These dishes have practically the same moisture content, and the other conditions are uniform with the exception of the temperature. (See Table II).

A study of Table II shows plainly that in an average temperature of 19°, only 13 1/3 per cent of the cotyledons produced roots, while in an average temperature of 22°, eighty per cent produced roots, and in a temperature of 20°, forty-three and one-third per cent formed roots.

It is evident then from the above data that the per cent of regeneration is greater, up to certain limits in higher temperatures.

Table II shows also that the rate of regeneration increases with the temperature. In connection with Experiment IV, we can say that this is true up to certain limits. I have not been able to try for any considerable time any temperatures between 22°C, which was most favorable for root formation, and 30° in which no roots formed.
<table>
<thead>
<tr>
<th>Temperature</th>
<th>Date of setting up</th>
<th>Wt. of moisture</th>
<th>Wt. of sand</th>
<th>Percent of moisture</th>
<th>No. of cotyledons</th>
<th>No. of roots at first appearance</th>
<th>No. of roots at last appearance</th>
<th>Percent of roots</th>
<th>Date of first observation</th>
<th>Date of last observation</th>
<th>No. of days to form roots</th>
<th>No. of days between first and last observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 - 22°C. Average temperature about 19°C.</td>
<td>Mar. 23</td>
<td>29 g.</td>
<td>789.5</td>
<td>3.6</td>
<td>30</td>
<td>1</td>
<td>4</td>
<td>13 1/3</td>
<td>Apr. 15</td>
<td>May 22 (Rotten)</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>20° - 25°C. Average temperature about 22°C.</td>
<td>Jan. 1</td>
<td>23 g.</td>
<td>599.5</td>
<td>3.7</td>
<td>30</td>
<td>5</td>
<td>24</td>
<td>80</td>
<td>Jan. 20</td>
<td>Mar. 2</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>15° - 25°C. Average temperature about 20°C.</td>
<td>Feb. 3</td>
<td>14</td>
<td>409</td>
<td>3.3</td>
<td>30</td>
<td>1</td>
<td>13</td>
<td>43 1/3</td>
<td>Feb. 24</td>
<td>Mar. 16</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>
Effect of Light and Dark.

Since it is generally accepted that darkness is more favorable to the growth of roots than light, it was thought that the same thing might be true of the regeneration of roots. Accordingly a series of experiments was carried on to test this. Smith found for the cotyledons of Cucurbita, that darkness hastened the regeneration of roots. Before coming to a definite conclusion with regard to Lupinus, more data should be obtained, but what data I have, indicates that the difference is very slight.

Experiment VI.

Thirty cotyledons were placed in the usual manner in moist sand in a Koch's moist chamber and placed in the dark room where the temperature remained at 21°, during the time of the experiment. A similar dish was set up and placed in the greenhouse where the average temperature was about 21°. Since the greenhouse temperature could not be maintained fairly constant longer than thirty-three days, the experiment did not continue as long as usual, and other roots which formed after the temperature rose to 25° and came up every day for several days were not taken into account. During the thirty-three days, seven roots formed in each case. The results are tabulated below:

<table>
<thead>
<tr>
<th>No. of days to first appearance of roots</th>
<th>In Dark.</th>
<th>In Light.</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>14-</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>15-</td>
<td>2</td>
<td></td>
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<tr>
<td>16-</td>
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<tr>
<td>17-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>19-</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>25-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>33-</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>
This seems to indicate a slight hastening of root formation in the dark. A measurement of the roots shows that those which formed in the dark are longer than those which formed in the light and that the cotyledons do not increase in size as much in the dark as in the light. The average size of the cotyledons grown in the light was 20.8 X 18.1 mm, while the average size of those grown in the dark was 18.1 X 13 m.m. The average length of the roots grown in the light was 12.3 cm, while that of those grown in the dark was 16.1 cm.

Because of the objections which might be raised on account of the somewhat greater fluctuation in the temperature in the greenhouse than in the dark room, I carried on another experiment and obtained somewhat different results. However there are some objections which might be made to it and before the question can be considered settled more experiments will have to be carried on in which the temperature and moisture factors can be more perfectly controlled.

Experiment VII.

On April 19th forty-eight cotyledons were placed in Koch's moist chambers as before and placed in the greenhouse. Before placing the cover on the dish one half of the cotyledons was covered by a stiff paper cover which was pressed down into the sand so that the light could not enter. At the first appearance of roots (May 2) there were four roots in the light half, and four in the dark. May 22 there were five in dark and 6 in the light, that is, 20 per cent of those grown in the dark developed while 25 per cent of those grown in the light developed in the
same time. The temperature may have been slightly lower under the paper than in the other half of the dish. At the same time the half of the dish under the paper had a slight advantage in moisture content.

In Experiment II which was carried on in the light 80 per cent of the cotyledons in one dish formed roots. This is the best result that has been obtained. Next to this the best result was in the dark where 66 2/3 per cent of the cotyledons in one dish formed roots.

From the above data then, there is no conclusive evidence that darkness favors regeneration.

**Effect of Size and Character of Wound.**

Zellamy (1903) found that in the arms of the brittle star Ophicglypha lacertosa, the greater the number of arms removed (except in cases when all are removed) the greater is the rate of regeneration. He found for the scypho-medusa Cassiopea xamachana, that the rate of regeneration after cutting off one part increases with added injuries to other parts of the body up to an optimum which represents the amount of injury most favorable for regeneration. Beyond this point added injury causes a decline in the rate of regeneration.

My experiments for plants are scarcely comparable to Zellamy's for animals, since in my experiments the least amount removed was the whole plant axis and this extent of injury may have been greater than the optimum.

Table IV shows plainly that the method of procedure employed in these experiments the rate of regeneration and the percent of
cotyledons forming roots decreased as the size of the wound became greater.

Experiment VIII.

Eighteen cotyledons were cut off so that the long diameter of the cut was 8 mm., eighteen were cut off so that the long diameter of the cut was twelve mm. and a third eighteen, so that the long diameter was fourteen mm. These cotyledons were then placed in dishes in moist sand and under the conditions of moisture found to be most favorable to root formation in previous experiments, and under the best temperature conditions possible in the greenhouse. The results are given below in the form of a table.

<table>
<thead>
<tr>
<th>Date of Setting</th>
<th>Number of Roots</th>
<th>Per cent of Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>up Experiment</td>
<td>Forming</td>
<td>Forming</td>
</tr>
<tr>
<td>Mar. 23.</td>
<td>8 mm., 12 mm., 14 mm.</td>
<td>8 mm., 12 mm., 14 mm.</td>
</tr>
<tr>
<td>Dish no. 1</td>
<td>4, 3, 0</td>
<td>22.2, 16 2/3, 0</td>
</tr>
<tr>
<td>Dish no. 2</td>
<td>5, 2, 0</td>
<td>26.1, 11 1/9, 0</td>
</tr>
</tbody>
</table>

Experiment IX.

The object of Experiment IX was to determine the effect, if any, of cutting off the sides of the cotyledons parallel with the midrib.

A series of dishes were set up in the usual manner, except that in addition to having the plant axis removed the sides of eighteen of the cotyledons in a dish were cut off parallel to the midrib. Eighteen were treated as usual and served as a check.

Under the heading of "General Observations" reference was
made to the scanty callus which developed on the cut surface under these conditions. The calluses at the basal end of the cotyledons which had the sides cut off did not grow to be so large as did the calluses at the basal ends of the cotyledons no so treated; further the roots did not develop as rapidly, nor was the percent of cotyledons which formed roots so great. This is shown in Table V.

Experiment X.

The pieces cut off the sides in Experiment IX were placed with the cut surfaces down, in dishes arranged as nearly as possible like Experiment IX. They rotted in a week and did not form roots.

Experiment XI.

The only variation in conditions in Experiment XI was that triangular pieces were cut out at the apex of the midrib of twenty-eight cotyledons (See Fig. 3.) and twenty-one were not so treated and served as a check. Fifty-two per cent of the whole number of cotyledons formed roots in thirty-eight days while in the same length of time thirty two and one tenth percent of those with triangular pieces cut out formed roots.

Experiment XII.

It was thought that splitting the cotyledons might have some effect on regeneration. Half of the cotyledons in the dish were treated as usual and half in addition to the ordinary treatment were split for a short distance in the region of the midrib.
<table>
<thead>
<tr>
<th>Date of setting up experiment</th>
<th>Date of appearance of roots</th>
<th>Number and percent of roots forming without parallel cuts</th>
<th>Number and percent of roots forming with parallel cuts</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 23</td>
<td>April 10</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>April 21</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>April 23</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>May 4</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>May 10</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 total 16½, 6 total 33½</td>
<td>2 total 14½, 7 total 38½</td>
</tr>
</tbody>
</table>

Table X
Six out of eighteen split cotyledons formed roots and eight out of eighteen whole ones formed roots.

Experiments VIII, - XII show conclusively for Lupinus albus that the rate of regeneration and the percent of cotyledons that regenerate diminishes as the extent of injury increases. These experiments, together with general observations suggest a possible cause for this difference in rate and per cent which may be referred to here. McCallum's experiments show that development of the primordia takes place even though the plant is in a starving condition. He showed however that in those plants in which the food supply was removed, the development of the primordia took place more rapidly in the light where the plants formed chlorophyll, and photosynthesis was possible. While it is not probable that Geobel and Sachs are right in thinking that the cause of regeneration is the increase in the food supply due to the removal of a part, still it may be possible that regeneration will occur more quickly under conditions where the food supply is abundant. The experiments recorded in this paper seem to bear out such a supposition. The cotyledons with only the plant axes removed form large calluses and abundant roots readily. It seems possible that the food stuff which follows its natural channel through the vascular bundles to the base of the cotyledons, not being able to escape, collects there and forms the large calluses. Some of the material from the interior of the calluses was scraped off and tested for starch. The characteristic blue color was given with the iodine test. When more of the food supply was cut off, the calluses did not become so large and roots were less abundant and formed more slowly. This is
merely offered as a suggestion because the data of the present study is not sufficient to warrant a conclusion.

The effect of Cutting off Different Proportions of the Cotyledons.

An experiment was undertaken to determine how far above the base of the cotyledon the plant axis could be removed without preventing regeneration.

Experiment XIII.

Dish number one of this series contained thirty cotyledons with less than one third of each cotyledon cut off with the plant axis. Dish number two contained thirty-four cotyledons with one-third cut off; dish number three contained thirty cotyledons with between one-half and one-third cut off; dish number four contained thirty cotyledons with one-half cut off. Aside from this variation in the amount removed the conditions were uniform in the different dishes. Between February 3 and March 16 thirteen cotyledons (43 1/3 percent) from dish number one produced roots; from dish number two twelve cotyledons (35 1/3 percent) formed roots; in dish number three two roots (6 2/3 percent) were observed and in number four, no roots formed.

From this experiment it is plain that the proportion cut off has considerable influence on the number of the cotyledons which regenerate roots and that when as much as one-half of the cotyledon is removed, no roots will form.
Effect of the Age of the Cotyledons on Regeneration.

It is shown in the following experiment that when cotyledons are cut from the axis after soaking for twenty-four hours they regenerate roots much more readily than cotyledons which are cut from the plant axis after growing one, two, three or four days in addition to soaking twenty-four hours. When cut off after four days the cotyledons do not regenerate. Table VI gives these results.

Although several series of dishes containing cotyledons more than four days old were tried no roots developed from them. Each series of dishes was set up under similar conditions except for the age of the cotyledons, hence the difference in the results may be attributed to the difference in age.

The Formation of Shoots.

During this study no shoots were formed upon the cotyledons separated from the plant. This seems to be in accordance with the results obtained by investigators generally. Smith experimented with several species with negative results in all cases. He reports four investigators who claim to have obtained the formation of shoots on cotyledons which had been removed from the plant. Von Zabel had shoots to regenerate on the cotyledons of Borago officinalis and reports them upon Pisum and Phaseolus cotyledons. He speaks of "grüne Hockerchen" which form upon the wounded surface in the last two species. These he regards as buds. Von Küster however contends that they are merely calluses. Von Küster found that shoots formed more readily on the cotyledons
<table>
<thead>
<tr>
<th>Date of beginning of experiment</th>
<th>Percent of cotyledons which formed roots</th>
<th>Date of end of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soaked 24 hours</td>
<td>Grew one day</td>
</tr>
<tr>
<td>Jan. 9</td>
<td>50.6</td>
<td>36 2/13</td>
</tr>
<tr>
<td>April 7</td>
<td>66 2/13</td>
<td>46 2/13</td>
</tr>
</tbody>
</table>
of Cucurbita pepo than roots. He also demonstrated that shoots develop on cotyledons of Cucumis and Luffa. Van Tiegham claimed to have seen the formation of shoots on Helianthus cotyledons. However his data does not seem to be very certain.

No one, so far as I know, has observed the production of shoots on Lupinus cotyledons which have been entirely removed from the plant. When the cotyledons were removed together with the shoot primordia it was easy to get regeneration of shoots from these primordia and this occurred in a number of the present experiments.

It was thought that if the hypocotyl left on so that regeneration could not take place in the region where the root usually regenerated, and if the plumule together with the primordia present in the axils of the cotyledons were removed, that a shoot might be induced to develop from the cotyledons. The plumule and primordia were sometimes cut out and at other times they were made inactive by encasing them in plaster of Paris. The seedlings treated in this way were planted in moist sand in pots. Two seedlings which had been operated upon were placed in a pot with a normal plant. Though these seedlings remained green and healthy for two months, no shoots formed.

Summary.

1. Adventitious roots form readily upon cotyledons of Lupinus albus which have been separated from the plant axis.

2. Callus forms upon the cut surface of the cotyledons, most abundantly at the basal ends of the midribs.
3. Roots that develop early, develop from the callus, while roots that form later arise from the midrib not more than three millimeters above the base.

4. Cotyledons separated from the axis grow considerably and those cotyledons which develop roots grow considerably more than those which do not.

5. There is a great difference in the readiness with which different cotyledons produce roots. The two cotyledons from the same seed usually behave alike in this respect.

6. The moisture content influences both the rate of regeneration and the number of roots which form on cotyledons. The greater the moisture content, the greater the rate and per cent of regeneration until the optimum is reached; beyond this, regeneration is hindered by the increase of moisture.

7. An increase in temperature above 18°C. is favorable to regeneration up to an optimum, beyond which, however, regeneration is hindered by any further increase in temperature. In constant temperature as high as thirty degrees, regeneration is stopped altogether. In a constant temperature as low as eighteen degrees, no regeneration takes place.

8. The greater the extent of injury, the more regeneration is checked in cotyledons removed from the plant axis.

9. The greater the proportion of the cotyledon removed with the plant axis, the greater the check to regeneration. If as much as one-half be removed, no regeneration takes place.

10. The cotyledons lose their power of regeneration as they grow older. Cotyledons which have soaked twenty-four hours possess this power in the highest degree, while it is lost entirely
by cotyledons which have grown five days before separation from the plant.

12. No shoots formed upon the cotyledons separated from the plant. Repeated experiments with seedlings from which the plumule and axial primordia had been removed resulted negatively as far as the formation of shoots is concerned.

This paper is by no means an exhaustive treatise upon regeneration in the cotyledons of Lupinus albus. A good deal has been attempted, but in the time spent on the study everything could not be thoroughly carried out.

Whatever there may be of merit in this paper is due to the careful supervision and kind suggestions of Doctor Charles F. Hottes
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PLATES
Fig. 1. Cotyledon with root.
1. Callus.
3. Root

Fig. 2. Cotyledon with large callus.

Fig. 3. Cotyledon with triangular cut.
Fig. 4. Cotyledon which failed to form Root.
Fig. 5. Cotyledon soaked twenty-four hours.
Fig. 6. Cotyledon with root.
Fig. 7. Cotyledon with sides removed.
Figs. 8: Root developing early from callus.

Fig. 9: Root developing late from midrib.