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## *REGENERATION IN MUTILATED SEEDLINGS**

BY CARL D. LARUE

DEPARTMENT OF BOTANY, UNIVERSITY OF MICHIGAN

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Van Tieghem[9] in his studies on Helianthus and Mirabilis found that cotyledons and isolated hypocotyls could be induced to root and that new plants could be grown from them. Blociszewski[1] found that isolated cotyledons of Pisum and Lupinus developed roots. Zabel[10] made similar observations concerning Pisum and Borrago. Küster[6] carried out studies on regeneration in several species of the Cucurbitaceae, and Kanzler[4] observed rooting of cotyledons in a single species, *Galium aparine*. More recently Kowalewska[5] has experimented with Pisum and Phaseolus, and Fuja[3] has studied root and shoot regeneration from cotyledons of *Cucurbita pepo*, *Cucumis sativus* and *Lupinus albus*. The writer discovered regeneration of roots and buds in *Phaseolus vulgaris* by accident, and thereby became interested in determining whether the small number of species in which regeneration was known to occur could not be increased. The

present paper is a result of that interest. Some attention was given to both Monocotyledonea and Dicotyledoneae, but only the latter group is considered in this paper.

*Method.*—Seeds were sterilized with calcium hypochlorite according to the method described by Wilson.[8] They were then laid on moist filter paper in sterile petri dishes and allowed to germinate there. The length of time of exposure to the sterilizing solution was varied according to the resistance of the seeds to the solution, and the degree of difficulty of sterilization of each type of seed. In some cases numerous trials had to be made, but in practically all cases it was possible to secure sterile seedlings.

When the seedlings were well out of the seed coats they were mutilated in various ways with a sterile knife, and again placed on moist filter paper in sterile petri dishes to allow regeneration to take place.

In some cases agar made with Shive's nutrient solution[7] and 2% agar-agar was used in place of the wet filter paper. In other trials 1% of dextrose was added to the Shive's nutrient agar. Numerous cultures became contaminated with fungi but, except in cultures on the agar containing sugar, bacteria did not cause any difficulty. By exercising care cultures of nearly all species were kept sterile for a period long enough to test their regenerative powers.

All these tests were performed at room temperature in the laboratory. Trials were made in both light and darkness for many of the species, but some species were tested only in light.

TABLE 1

REGENERATION OF MUTILATED SEEDLINGS ON MOIST FILTER PAPER IN PETRI DISHES

| FAMILY | SPECIES | NUMBER OF DAYS REQUIRED TO DEVELOP ROOTS ON ISOLATED COTYLEDONS | NUMBER OF DAYS REQUIRED TO DEVELOP SHOOTS ON ISOLATED COTYLEDONS | NUMBER OF DAYS REQUIRED TO DEVELOP ROOTS ON BASES OF CUT HYPOCOTYLS |
|---|---|---|---|---|
| Urticaceae | *Cannabis sativa* | 23 | .. | 8 |
| Polygonaceae | *Fagopyrum esculentum* | 7 | .. | 4 |
| Caryophyllaceae | *Silene vulgaris* | 11 | .. | 16 |
| Calycanthaceae | *Calycanthus florida* | 20 | 40 | 21 |
| Cruciferae | *Rhaphanus sativus* (radish) | 9 | .. | 4 |
| Cruciferae | *Brassica oleracea* var. *capitata* (cabbage) | 7 | 25 | 4 |
| Cruciferae | *Brassica caulorapa* (kohlrabi) | 14 | 58 | 14 |
| Cruciferae | *Brassica pekinensis* (celery cabbage) | 23 | .. | 16 |
| Cruciferae | *Brassica napus* (rape) | 12 | .. | 19 |
| Cruciferae | *Brassica alba* (white mustard) | 16 | .. | 14 |
| Cruciferae | *Alyssum saxatile* var. *compactum* | 28 | .. | .. |
| Cruciferae | *Brassica rapa* (turnip) | 14 | .. | .. |
| Cruciferae | *Brassica oleracea* var. *gemmifera* (Brussels sprouts) | 12 | .. | 7 |

TABLE 1 (*Continued*)

| | | | | |
|---|---|---|---|---|
| Rosaceae | *Agrimonia eupatoria* | 10 | .. | None |
| Leguminoseae | *Pisum sativum* | 16 | 15 | 15 |
| Leguminoseae | *Lathyrus latifolius* | 37 | .. | None |
| Leguminoseae | *Lupinus albus* | 17 | 18 | 23 |
| Leguminoseae | *Vicia faba* | 9 | 7 | 7 |
| Leguminoseae | *Phaseolus vulgaris* | 10 | 16 | 8 |
| Leguminoseae | *Gleditsia triacanthos* | 30 | .. | None |
| Leguminoseae | *Thermopsis caroliniana* | 29 | .. | 29 |
| Leguminoseae | *Robinia pseudo-acacia* | 15 | 15 | None |
| Leguminoseae | *Medicago sativa* | None | 22 | 7 |
| Tropaeolaceae | *Tropaeolium majus* | 28 | .. | 5 |
| Linaceae | *Linum usitatissimum* | 4 | 22 | 4 |
| Linaceae | *Linum perenne* | 6 | 25 | 6 |
| Simarubaceae | *Ailanthus glandulosa* | 29 | 6 | .. |
| Euphorbiaceae | *Ricinus communis* | .. | .. | 15 |
| Aceraceae | *Acer negundo* | 35 | 41 | .. |
| Balsaminaceae | *Impatiens balsamina* | 9 | 10 | 4 |
| Malvaceae | *Hibiscus esculentus* | .. | 21 | 7 |
| Malvaceae | *Althea rosea* | 14 | .. | None |
| Umbelliferae | *Daucus carota* var. *sativa* | 20 | .. | 10 |
| Boraginaceae | *Cynoglossum chinense* | 7 | .. | 5 |
| Solanaceae | *Lycopersicon esculentum* | 7 | 12 | 4 |
| Solanaceae | *Capsicum frutescens* var. *grossum* | .. | .. | 43 |
| Cucurbitaceae | *Cucurbita pepo* | 9 | 17 | 9 |
| Cucurbitaceae | *Cucurbita maxima* | 17 | 20 | 12 |
| Cucurbitaceae | *Citrullus vulgaris* | 21 | 21 | 4 |
| Cucurbitaceae | *Cucumis sativus* | 10 | .. | None |
| Cucurbitaceae | *Cucumis melo* | 12 | 20 | 22 |
| Compositeae | *Helianthus annuus* | 8 | 17 | 9 |
| Compositeae | *Heliopsis pitcherianum* | 8 | .. | 8 |
| Compositeae | *Lactuca sativa* | 17 | .. | 10 |
| Compositeae | *Arctium lappa* | 29 | 18 | 13 |

*Regeneration of Cotyledons.*—The cotyledons were cut off with care to make sure that none of the hypocotyls were included, and an attempt was made to avoid including any axillary buds. Table 1 lists the species which formed roots and gives the rate of regeneration.

In plants with petioled cotyledons some of the cotyledons were tested with petioles intact, others with the petioles excised. The bases of the laminae rooted as readily and rapidly as the bases of the petioles.

The majority of the cotyledons with petioles produced roots at the bases of the petioles. Usually the roots emerged from the vascular bundles in the cut end of the petiole, and as a general rule, they appeared without the preliminary development of callus. In the tomato, and in the cabbage a small callus sometimes preceded the appearance of roots. In members of the Cucurbitaceae a small callus almost always developed before the roots.

Some anomalies in the position of roots on cotyledons have appeared. Pumpkin cotyledons have developed roots from the middle of the lamina, both along the midrib and away from the midrib. These roots arose from small masses of callus, and appeared to have been the result of wounds. The tomato has also produced roots from the midrib above the base of the cotyledon. The garden balsam retains its cotyledons for a long time, both on normal seedlings and on those from which the plumules have been removed. When these older cotyledons are allowed to regenerate they do not develop roots at the cut end of the petiole, but at varying distances above the base. The roots emerge from the upper, or the lower surface of the petiole depending on which is in contact with the wet paper. Frequently roots are produced from the midrib half-way up the blade of the cotyledon.

In the radish, and in buckwheat, roots have been formed on petioles or on midribs of cotyledons which had not been detached from their hypocotyls. Such cotyledons lay in contact with the moist surface of the filter paper, and it appeared that the roots had resulted from small wounds.

The time required for rooting of cotyledons ranged from four days in flax to 35 days in box-elder. Neither size of seed nor systematic position of the species appear to have any considerable influence on the rate of regeneration. Tomato, radish, buckwheat, sunflower, Brussels sprouts, garden balsam and pumpkin cotyledons all root quickly, though they vary greatly in size and family relation.

The age of the seedling when the cotyledons are detached appears to have some influence on the rapidity of regeneration as has been noted by Fuja.[3] But this influence is of less importance in some species than in others, depending on the rate at which the reserve food in the cotyledons is exhausted. In two species, *Helianthus annuus* and *Alyssum saxatile*, cotyledons from unripe seeds produced roots, though not more rapidly than those from ripe seeds.

Seedlings of tomato, garden balsam, kohlrabi and pumpkin were grown in soil for 58 days, then cut off and put in dishes on wet paper. The seedlings of another set of each of these species had the plumules cut out as soon as they began to grow. These seedlings were grown for 58 days also, when the cotyledons were considerably larger than those on the normal plants. These cotyledons were cut off also and placed on moist paper in petri dishes. Roots were formed by all the cotyledons, both from normal and from decapitated plants. The kohlrabi cotyledons rooted in 7 days, whereas cotyledons from freshly germinated seedlings of this species required 14 days to root. The old cotyledons of balsam rooted in 10 days, while those from young seedlings needed 9 days. Cotyledons from young tomato seedlings rooted in 7 days, while the 58-day cotyledons required 11 days for the process. Young pumpkin cotyledons rooted in 9 days, and the 58-day cotyledons did not form any roots until 14 days had elapsed.

None of these cotyledons had lost their regenerative capacity, and in two species it appeared to have been increased. The cotyledons on the decapitated seedlings were unable to use their food supplies in continued growth of the seedling, since all new buds had been cut off as rapidly as they appeared, and it was anticipated that they would be able to regenerate. However the cotyledons on the normal seedlings had an opportunity to pass their reserve food into the growing plants, and the fact that they still had sufficient food resources to form roots when detached, indicates that, in some species at least, the growing seedling does not draw on the cotyledons as heavily as it has been thought to do. In the common bean the food is withdrawn from the cotyledons very rapidly, and these organs soon wither and fall. In one case a cotyledon was noted which apparently had suffered an injury to its petiole which prevented the removal of food. It remained plump, and green, long after the normal cotyledons had fallen. The wide variability in the behavior of different plants makes it unsafe to generalize concerning the rate of diminution of regenerative capacity.

*Regeneration of Segments of Cotyledons.*—In addition to the preceding studies on whole cotyledons tests were made of the regeneration of portions of cotyledons. Cuts were made crosswise of cotyledons dividing them into apical and basal portions, and lengthwise of other cotyledons dividing them into right and left halves. Still other cotyledons were divided into smaller segments.

Basal halves of cotyledons root in quite the same way as whole cotyledons in most of the species tested. They differ only in having a smaller food supply, and ordinarily one would expect them to have food enough to enable them to root. The apical halves of cotyledons usually produce a pad of callus at the cut end of each of the main veins. These upper halves of cotyledons have produced roots in flax, Chinese cynoglossum, Brussels sprouts, tomato, sunflower, pumpkin, squash, celery cabbage, garden balsam and buckwheat. The period required for rooting these parts of cotyledons is from two to four times as long as for whole cotyledons. In some species roots are produced from the midrib only, but in others they are developed from the other principal veins as well, so that a row of roots extends across the cut surface.

Right and left halves of cotyledons cut lengthwise have produced roots in sixteen species. The roots developed at the basal tip of tissue. When cotyledons are halved in a diagonal direction roots are formed at the cut ends of the basal veins on each segment.

Small segments of cotyledons have formed roots in pumpkin, squash, buckwheat and tomato. In each of these a small mass of callus developed at the basal part of the fragment and from this a root appeared. The smallest part of cotyledon found capable of rooting was 3 mm. square. Pieces of tomato and squash cotyledons of this size produced roots.

*The Effect of Light.*—Neither light nor darkness appears to be capable of inhibiting root formation on cotyledons. Most species regenerated in either light or darkness, and appeared rather indifferent as regaded the influence of light. Tomato, watermelon, white mustard, flax, kohlrabi, Brussels sprouts, turnip, sunflower and pumpkin cotyledons rooted slightly sooner in light than in darkness, while radish cotyledons regenerated more rapidly in darkness. Once the roots had appeared, they developed more rapidly in the dark in almost all species. This was especially noticeable in the tomato.

*The Effect of Nutrient Solutions.*—Cotyledons of cabbage, white mustard, radish and garden balsam were tested on agar made by adding 2% of agar-agar to Shive's nutrient solution, and on agar made by adding 2% of agar-agar and 1% of dextrose to Shive's solution. On Shive's agar without sugar, radish and white mustard cotyledons rooted in 6 days. Balsam cotyledons rooted in 7 days and those of cabbage in 10 days. By comparison with table 1 it may be seen that, with the exception of the cabbage, the rate of regeneration has been increased greatly.

On Shive's agar with dextrose, radish cotyledons produced roots in 6 days; balsam in 17 days; cabbage in 19 days; and mustard in 20 days. The cultures of the last two species became contaminated with bacteria and fungi. It is possible that bacteria may have been present on the cotyledons of balsam though they were not apparent. Since no advantage appeared to result from the use of sugar in the nutrient solutions, and none was to be expected where the cotyledons were stored with food, no further steps were taken in this direction.

*Growth of Cotyledons on Soil.*—After they had become well rooted cotyledons of flax, pumpkin, watermelon, lettuce, cabbage, muskmelon, tomato, garden balsam, burdock, box-elder, kohlrabi, black locust, lupine, Chinese cynoglossum, horse bean and sunflower were planted in pots of earth. All of these grew but a number were destroyed by rats two or three weeks after they were planted. A number of others increased in size until they were twice the size of normal cotyledons on seedlings. In this enlarged form they were capable, apparently, of making their own food, for they were bright green. They formed plants without any means of extending their growth, but, otherwise able to lead an independent existence. Rooted cotyledons of several species lived for six months when they were abandoned. Others had lived nearly as long but had finally succumbed to attacks of thrips, sow bugs, etc., to which they were subject from time to time.

*Development of Shoots on Cotyledons.*—Some of the rooted cotyledons formed buds after a time as is indicated in table 1. Not all the cotyledons of a species formed buds, but some remained in the vegetative condition described in the preceding paragraph. Some other species produced shoots on the cotyledons while still in the petri dishes. In some

cases, okra for example, the cotyledons could not be planted in soil, for although shoots were formed, no roots were developed.

Whether the shoots were produced on cotyledons in the soil, or in culture dishes, they developed normally. In some cases they developed into plants, which ultimately became normal, though somewhat unusual in appearance at first. Species in which cotyledonary shoots were grown to considerable size are: *Acer negundo, Cucurbita pepo, Cucurbita maxima, Phaseolus vulgaris, Vicia faba, Lupinus albus, Brassica oleracea* var. *capitata, Lycopersicon esculentum, Linum usitatissimum* and *Pisum sativum.* Although not all the species noted in table 1 as having formed buds were carried through further stages in the development of those buds, there appears to be no reason why they could not have been grown to maturity from those shoots. An exception must be made of those forms which did not develop roots on the cotyledons, or on the shoots. The okra did not form any roots in the course of these experiments, and the pea usually failed to produce them on the cotyledons, but developed an adequate number at the base of the shoot just above the cotyledon.

The question of the origin of the buds on these cotyledons has not been answered as yet. If buds were present on the petioles they must have been somewhat removed from the axils for the petioles were cut off at a little distance from the hypocotyls. In one instance a bud arose on a petiole of a cabbage cotyledon about half-way between the base of the petiole and the blade, and a bud developed from a pad of callus almost midway between the base and the top of a cotyledon of box-elder. External examination has failed to show the presence of buds on the bases of any of the cotyledons observed in these studies. Fuja[3] has found that shoots on cotyledons of *Cucurbita pepo, Lupinus albus* and *Lupinus luteus* are developed from buds already formed when the cotyledons were cut off, and suggests that other cases of shoot regeneration are due also to the presence of pre-formed buds. She has observed a few cases in which buds were developed from callus on *Cucurbita pepo, Cucumis sativus* and *Lupinus albus,* and Kowalewska[5] has observed a similar origin of shoots in Phaseolus and Pisum. If all the species listed for shoot production in table 1 have pre-formed buds on their cotyledons, that condition must be much more common than has been suspected.

The following species have failed to develop roots on their cotyledons: *Beta vulgaris* (blood beet), *Beta vulgaris* var. *cicla,* (Swiss chard) *Aquilegia dichroa, Aquilegia hispanica, Aquilegia formosa, Geum urbanum, Geum tyroliensis, Colutea arborescens Albizzia julibrissin, Baptisia leucantha, Astrafalus cicer, Rhus glabra, Acer saccharum, Abutilon indicum, Solanum melongena* var. *esculentum, Pentstemon grandiflora* and *Prenanthes sp.* Possibly some of these might produce roots on further trial, for most of the cotyledons remained alive for long periods. However, two of the mem-

bers of the Chenopodiacea, the blood beet and Swiss chard, were tried repeatedly and it appears that members of that family lack the ability to regenerate either from cotyledons, or from cut hypocotyls. The egg-plant, *Solanum melongena* var. *esculenta*, was tested several times also, and contrasts strikingly with the behavior of the tomato. The pepper, *Capsicum frutescens* var. *grossum*, failed to develop roots on cotyledons though the hypocotyls rooted. Several other species are shown in table 1, in which the cotyledons did not root though the hypocotyls did. In the okra, *Hibiscus esculentus*, the cotyledons developed shoots but did not root.

*Regeneration of Hypocotyls.—Hypocotyls severed immediately above collet.*—After the seedlings had emerged from their seed coats the hypocotyls were severed at their bases just above the collet, or root collar. They are then placed on moist paper in the manner already described.

Table 1 shows the length of time required for regeneration of roots at the bases of the hypocotyls in the species in which it occurred. It should be noted that only those marked as failures in table 1 are known not to have rooted under the conditions of the experiment. The blanks indicate that no trial was made of the species concerned.

In almost all species the rooting is prompt and effective, so that the seedling resumes its growth with little interruption. In some species the rooted hypocotyls were planted in soil where they grew into normal plants. Most of the others appeared capable of growth to maturity, though a few were weak, and not well supplied with roots.

A comparison was made of hypocotyls cut just above the collet, and others severed near the cotyledons leaving only a short piece of hypocotyl intact. The short pieces of hypocotyl rooted as well as the longer ones, but the time required was usually twice as long as that needed by full length hypocotyls.

*Effect of Various Cultural Conditions.*—In general the hypocotyls will root in light, or in the dark, but certain preferences are shown, balsam and radish hypocotyls are alike in requiring four days' time for the appearance of the first root but while the radish shows more roots and larger ones in the dark than in light, the balsam acts in a directly opposite manner. The tomato is indifferent as to light affect, though the roots grow faster in the dark once they are initiated. Watermelon hypocotyls root in the dark in half the time which they require in light, while the cabbage roots in the dark in one-fifth the time which it needs for the process in light. Even after 20 days' time the roots are so few and so small that one can scarcely say that it really roots at all in light. White mustard roots rather feebly in light and not at all in the dark.

The use of agar made with Shive's solution, in place of filter paper wet with distilled water, increased the rate of root formation on hypocotyls to a remarkable extent. Cabbage, radish and garden balsam hypocotyls

rooted in three days on this substance, although they all required four days to root on filter paper wet with distilled water. Mustard hypocotyls were slow to root on wet paper, 14 days being needed for the process, but on Shive's agar they formed roots in 5 days.

Shive's agar with dextrose did not prove so good a medium for rooting as the same agar without sugar. Cabbage, garden balsam and radish all required 5 days to root on this medium, one day more than they needed on the filter paper. The sugar is certainly of no advantage, and makes the matter of keeping the cultures free of contamination a difficult one. It seems likely that contamination by unseen bacterial colonies was the explanation of the slowing effect of the sugar agar.

*Position and Polarity of Roots on Hypocotyls.*—The hypocotyls of all species appeared to produce roots all round, without regard to whether they appear on upper or lower sides of the stem. After the roots have emerged from the hypocotyls for a little distance they become normal in their geotropic response, and those which started upward from the stem, turn and grow downward. On some hypocotyls roots seemed to emerge from any point of their circumference, but on others the roots were formed at wide intervals, doubtless in connection with the arrangement of the vascular bundles in the steles.

*Hypocotyls Severed Immediately Above Collet and Immediately Below Cotyledons.*—Hypocotyls without cotyledons or roots are rigorously limited in their food stores, and it is not to be expected that many will be able to develop new roots, or buds, in the short time which is needed to bring them to the point of starvation. Perhaps the habit of storing much, or little, food temporarily in the hypocotyl may determine whether the structure produces new roots and shoots, or does not. By far the larger number of hypocotyls linger for a time and then perish without regenerating. Roots were developed on such hypocotyls in tomato, carrot, lettuce and lupine, while new buds appeared on tomato, carrot, pumpkin and watermelon.

*Hypocotyls Severed Immediately Below Cotyledons but with Roots Left Intact.*—Seedlings mutilated in this fashion are in somewhat less straitened circumstances than those in the preceding category, since the root system is not disturbed. However, the species which produced shoots are the same as in the preceding group. Flax was not tried, but Burns and Hedden[2] found that flax seedlings with roots in soil, and hypocotyls in a moist atmosphere were able to produce buds. Up to the present time relatively few species have been found to have the ability to form new buds under the conditions of the experiment of Burns and Hedden, and it is not surprising that so few were found to regenerate in the present trials.

*Experiments with Excised Roots.*—Excised roots of a number of species were kept on moist filter paper, and some of them remained alive for a month or more. At first they continued growth at the tip, but this growth

became less and less, until it finally ceased, after which the roots began to die at the upper ends and finally collapsed completely. Trials were made also with whole root systems of seedlings which had been cut away from the hypocotyls just above the collet. The short stub of hypocotyl proved unable to form buds, and usually perished before the roots. These root masses usually lived longer than excised individual roots, but were equally powerless to form buds.

*Seedlings with Excised Cotyledons.*—It would appear likely that seedlings from which the cotyledons had been removed leaving the plumules intact, would be able to continue growth from these plumules. However, such seems not to be the result when such seedlings are placed on wet filter paper in moist chambers. Several species were used, but in all of them the plumules failed to unfold, and death resulted without any change having taken place, except a limited amount of growth at the root tips, and a slight extension of the hypocotyls.

*Discussion.*—Blociszewski[1] found that detached cotyledons produced roots in peas and lupines, but reported that only the parts which had been attached to the embryonic plant was able to form roots. Fuja[3] failed to secure roots on the upper halves of lupine cotyledons sliced transversally, but did secure rooting of upper halves in *Cucurbita pepo*. In one case Kowalewska[5] rooted an upper half of a cotyledon of *Phaseolus vulgaris*. Beside *Cucurbita pepo*, the present investigation showed that nine other species were capable of such regeneration.

The rooting of severed hypocotyls seems to have been given little attention. Van Tieghem[9] reports that plumules were capable of developing into new plants in *Helianthus annuus*, and here the new roots must have been formed on a portion of the hypocotyl. In this paper it is shown that the hypocotyls of most species develop new roots in a short time, although a few species failed entirely in this respect.

So far as the rooting of cotyledons is concerned the results of the present study are in substantial agreement with Van Tieghem, Küster, Kowalewska and Fuja. However, they have greatly increased the number of species in which this type of regeneration is known to occur and have added to the knowledge of the conditions most favorable to it.

Now that it is known that cotyledonary regeneration is possible in many species, and a number of families, it becomes important to determine the internal conditions leading to such regeneration. This will be a difficult end to reach, but a step toward it will be the discovery, for comparison, of species, genera and perhaps families in which regeneration is impossible. The beet and Swiss chard have failed so completely to produce roots on either cotyledons or hypocotyls, that it appears that we have in them, two varieties of a species without any capacity for regeneration. If, indeed, they are surely incapable of developing any new growths, a

comparison of them, with regenerating species, may give some clue to the fundamental causes of all regeneration in plants.

*Summary.*—1. Regeneration of mutilated seedlings was tested on moist filter paper in petri dishes, and on agar made with Shive's solution. Regeneration took place more rapidly on Shive's agar than on the moist paper.

2. Rooting of excised cotyledons was observed in 41 plants belonging to 19 different families.

3. Basal halves of cotyledons rooted almost as rapidly as whole cotyledons, and apical halves were rooted in 10 species. Right and left halves of cotyledons formed roots in 16 species, and smaller fragments of cotyledons rooted in four species.

4. Shoots were developed on excised cotyledons in 22 species.

5. Seedlings, severed just above the root collar, formed new roots at the bases of the severed hypocotyls in 35 different plants belonging to 16 different families.

6. Hypocotyls severed below the cotyledons and above the root collar formed roots in four species, and shoots in four species.

7. In seedlings from which the cotyledons were removed, the plumules failed to develop.

8. Cotyledons and hypocotyls alike, form roots in either light or darkness, but some species develop best in the light, and others in the dark. The cotyledons and the hypocotyls of a given species may react differently to light.

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