REGENERATION IN MONOCOTYLEDONOUS SEEDLINGS [1]

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The experiments described in this paper were made for comparison with those conducted by the writer on dicotyledonous seedlings (La Rue, 1933). Sixty years ago Van Tieghem (1873) published an account of researches on germination in which he stated that maize seedlings from which the plumules had been removed were unable to form new shoots, though they lived for a month or more and formed adventitious roots. Andronescu (1920) found that maize embryos which had been removed from their endosperms were able to develop into mature, though small, plants. When he removed the scutella as well as the endosperms, he found the plumules unable to grow out from their coleoptiles. Brown (1922) studied the effect of mutilation of maize seeds on germination and yield. His experiments differ from other similar tests in that they were carried out under field conditions and were continued to maturity of the plants.

METHODS

Seeds were sterilized with calcium hypochlorite according to Wilson's (1915) method. Some molestation by fungi, such as *Aspergillus, Mucor, Fusarium,* and *Helminthosporium,* seems almost inevitable. Complete sterilization was often attended by complete failure of germination. Therefore the sterilization was so adjusted as to allow a high percentage of germination, and large numbers of seeds were prepared so that contaminated specimens might be discarded. After sterilization the seeds were placed on water-soaked filter paper in sterile Petri dishes. If a contaminated seed appeared in a dish, the whole dish was rejected. When germination had taken place, the seedlings were sectioned with a sterile knife and placed with sterile forceps on pads of sterile water-soaked filter paper in Petri dishes. In a few instances agar made with Shive's nutrient solution, or with Shive's solution plus 2 per cent of dextrose, was used as a substratum.

Seedlings of the following species were used in the experiments: Corn (*Zea Mays* L. var. "Pride of the North"), pop corn (*Zea everta* Sturt.), wheat (*Triticum sativum* Lam.), barley (*Hordeum sativum* Juss.), oats (*Avena sativa* L.), sorghum (*Holcus Sorghum* L.), Canary grass (*Phalaris arundinacea* L.), Sudan grass (*Holcus sudanensis* Bailey), iris (*Iris orientalis* Thunb.), yucca (*Yucca filamentosa* L.), allium (*Allium coerulium*

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Scheele and *A. delicatulum* Sievers), blackberry lily (*Belamcanda chinensis* [L.] DC.), canna (*Canna indica* L.), and asphodel (*Asphodelus fistulosus* L.).

The morphological and anatomical terminology used in this paper is that which Avery (1930) has employed for the seeds and seedlings of grasses.

SEEDS AND SEEDLINGS OF THE GRAMINEAE

Excised roots. Tests were made with excised roots of maize, wheat, oats, barley, Sudan grass, sorghum, and Canary grass. The results of Robbins (1922) indicated that regeneration from roots of any monocotyledonous plant was not likely to occur, and no surprise was felt when all the roots died without any sign of budding. In all species growth continued for a time, but in little more than a week development had almost completely ceased, though the roots remained alive for three weeks or longer.

Embryos deprived of roots. The seminal roots were cut off just below the seed, and the coleoptile was left intact. Wheat, barley, maize, pop corn, oats, sorghum, Canary grass, and Sudan grass were used in this experiment. In all these an abundance of adventitious roots was produced. Only in sorghum was any regeneration of seminal roots observed; in one instance the cut base of the main root threw out a secondary root. In all other plants the new roots arose from the scutellar node, the first internode, the coleoptilar node, or, less frequently, from the third node.

In sorghum some of the adventitious roots appeared in twenty-four hours. In Canary grass they were developed in two days; in barley and oats in three days; in wheat, maize, and pop corn in four days. Sudan grass, however, required a week. It is well known that these grasses regularly supplement the seminal roots by the production of adventitious roots above the scutellum, but in control plants no adventitious roots appeared until long after their appearance on plants from which the seminal roots had been excised, except in maize, in which adventitious roots arise normally very soon after germination.

Seedlings deprived of seminal roots proved capable of growth into normal plants. In fact, the loss of the seminal roots appeared to be of little consequence in the survival of all of the species used in the experiment.

Seeds split lengthwise. These seeds were halved by a cut which split the embryo and endosperm lengthwise. The results with corn, wheat, and barley were in agreement with those of Brown (1922) for corn. Most of the seeds developed roots and shoots. (Sometimes the cut had been made too far to one side, so that practically no part of the embryo, except scutellum, was left on that side. Such slices sometimes developed roots but not shoots.) Many of the shoots were rather torn and disheveled in appearance at first, but the later leaves developed normally.

Seeds with half of embryo cut away. The embryos of these seeds were halved lengthwise, and one half was removed, leaving the other intact on the endosperm. The same species were used as in the preceding trial, and the results were identical except that for some reason the regenerated shoots appeared weaker.

Seeds from which both roots and shoots were removed. Roots and shoots were cut off even with the surface of the seeds. A number of wheat seeds produced roots, and a few developed weak shoots in twelve days. Barley seeds developed roots in three days but no shoots. Corn grains produced no shoots, but adventitious roots, most of them from the region of the scutellar node. Oats, Canary grass, Sudan grass, and sorghum failed to show any growth.

Embryos deprived of endosperm. Removal of the endosperm from the embryo after germination had taken place diminished the rate and extent of growth, but did not stop it completely. Embryos of wheat and of Sudan grass were able to continue growth. In sorghum seedlings the scutellum enlarged considerably and adventitious roots were formed. The wheat and Canary-grass embryos appeared to be capable of continued growth, but were not actually planted in soil. Sorghum embryos were planted in soil successfully, but were not grown to maturity.

Embryos deprived of scutellum. Wheat embryos from which the scutellum had been removed continued growth and remained healthy for some time, but sorghum embryos so treated stopped growing, and the epicotyls failed to emerge from the coleoptiles, a result which Andronescu (1920) reported for maize embryos.

Seeds with shoots excised. The stems were cut off close to the seeds, which were otherwise uninjured. In twelve days wheat embryos were able to produce new shoots, which appeared almost normal. Embryos of barley and oats died within a week, but Canary grass, Sudan grass, and sorghum all showed a lively root growth and an extensive development of secondary roots, but no sign of shoot restitution. Maize seedlings developed numbers of adventitious roots, but no new shoots, a result reported by Van Tieghem (1873) long ago.

Excised shoots. The first internodes were severed close to the seeds, and the shoots were laid on moist filter paper in Petri dishes. The excised shoots of wheat showed little elongation and no tendency to form roots. The internodes of barley embryos elongated somewhat, but formed no roots. All first internodes of oat seedlings were well rooted in three days. Excised shoots of Canary grass rooted at the first node in two days; later they rooted at the second node as well and grew into normal plants when set out in soil.

First internodes of Indian corn and of pop corn produced from one to three roots each in four days. Later they were planted in soil where they were grown for three weeks and appeared capable of continued development.

Basal internodes of sorghum rooted in eight days but appeared to be rather weak. Fifteen days after the shoots had been excised they were set out in soil. Ten days later the plumules had not yet emerged from the coleoptiles, and at this time the shoots were discarded.

The excised shoots of Sudan grass showed a striking elongation of the internodes in three days, and most of them had produced roots. Nine days after they were cut off, the shoots were planted in soil, where they grew into normal plants.

Root and shoot removed completely from scutellum; scutellum left attached to endosperm. One half of the wheat seeds showed no sign of regeneration, but the other half produced adventitious roots and ragged green shoots in twelve days. In the same length of time barley grains gave practically identical results except that the shoots were more irregular. Canary grass and Sudan grass did not show any response. A few sorghum seeds produced weak shoots in eight days, and a few others developed roots.

Maize showed a more pronounced reaction than any other species tested. In a number of seeds the scutellum developed a bulging mass of tissue at the scutellar node, which sometimes gave rise to a mass of roots, quite normal in appearance. In addition to roots, some seeds produced curling leaf-like outgrowths, and a few developed actual shoots.

Isolated cotyledons. The production of roots and shoots from isolated cotyledons of dicotyledonous seeds reported in a previous paper (1933) led to trials with isolated cotyledons of monocotyledonous plants. The cotyledons were dissected from the endosperm, and the root and shoot removed as completely as possible. The cotyledons were put on moist filter paper, on agar made with Shive's solution, or on agar made with Shive's solution to which 2 per cent of dextrose had been added. The developments on these three types of substratum were not significantly different.

Wheat and barley cotyledons swelled to more than double the size they had reached when they were dissected from the endosperm without removal of shoots and roots, and exceeded by a considerable amount the largest size reached by cotyledons left attached to the endosperm. About one-fourth of the cotyledons produced roots and shoots from the region where they had been attached to the stems of the embryos. No other growth could be observed. The formation of masses of tissue, and later of roots and shoots at the ends of cut vascular bundles, is common in dicotyledonous embryos and seems to correspond with the behavior of these wheat and barley seedlings.

Cotyledons of Sudan grass and sorghum did not show much increase in size. Both developed new, though rather weak, roots. Sudan grass threw out weak shoot-like outgrowths in a few instances.

Maize cotyledons increased in size more than those which were in their normal position attached to the endosperm. Some of them developed on their surfaces small spots of tissue which resembled intumescences, but aside from these no new growth was to be found. In early trials a number of cotyledons produced fragmentary leaves, or stems. One such structure was obviously only a part of the outer cortex of the first internode. Later tests, in which the root and shoot were more nearly completely removed, showed no outgrowth resembling a leaf or stem, though roots were formed by nearly all the cotyledons. It appears that roots can be formed by the cotyledons at their point of attachment, but that shoots cannot be developed if the mesocotyl is completely extirpated. One cotyledon actually developed chlorophyll in its inner portion, but was unable to develop further. Van Tieghem (1873) reported that the scutellum never developed chlorophyll.

RESULTS WITH OTHER MONOCOTYLEDONOUS SEEDS

Seeds of *Allium delicatulum, Allium coeruleum, Asphodelus fistulosus, Yucca filamentosa, Iris orientalis,* and *Belamcanda chinensis* were germinated in the manner described for the seeds of the Gramineae. The cotyledon was then isolated from the remainder of the embryo by a cut across the base of the cotyledon. No isolated cotyledon showed any signs of regeneration.

Canna. Seeds of *Canna indica* seemed to offer especially good material for study on account of their large store of endosperm. Trials for regeneration were made on embryos deprived of endosperm; on embryos from which cotyledons and endosperms were excised; on isolated cotyledons; on cotyledons excised from embryos but left attached to endosperms; on embryos with excised plumules; on embryos with both plumules and endosperms removed; and on isolated root systems. Results were negative.

DISCUSSION

In general, if allowance is made for differences in morphology between the two groups, no great differences in capacity for regeneration are to be noted between monocotyledonous and dicotyledonous plants. Excision of shoots, for instance, means their separation not only from roots, but from cotyledon as well; yet in spite of this most of the isolated shoots of the Gramineae were able to produce roots, from the first internode, or from the coleoptilar node.

Isolated cotyledons contain less food than most of those of dicotyledonous plants, but are able to increase in size in about the same degree. Regeneration from them has been found to be localized in the region of attachment of the scutellum to the remainder of the embryo, the cotyledonary plate. There may be some question whether shoots are regenerated when all the tissue of the scutellar node has been removed. However, wheat and barley cotyledons throw out shoots after apparently complete excision of the scutellar node.

Root formation is possible in wheat, barley, Sudan grass, sorghum, and maize when all tissue of the scutellar node has been removed beyond question. This corresponds with the formation of roots on cotyledons of dicotyledonous plants at the cut ends of veins. When the scutellar node is all excised, the cut end of the vascular bundle leading into the scutellum is exposed, and apparently it is from this bundle that the mass of tissue develops which later gives rise to roots, possibly to shoots in some species. Maize cotyledons will not produce new shoots if the whole mass of tissue at the scutellar plate is removed, and in this respect maize resembles many cotyledons of dicotyledonous plants which can produce shoots from preformed buds, but cannot form new buds when the original buds have been removed.

The remarkably prompt development of adventitious roots after the excision of the seminal roots has not been noted heretofore. This characteristic of the seedlings of grasses may be of great importance in enabling them to survive if the seminal roots are destroyed by insects or by animals, or by fungi.

SUMMARY

1. Excised roots of monocotyledons proved to be incapable of regeneration.

2. Seeds of maize, wheat, and barley, split lengthwise, were able to produce roots and shoots.

3. Embryos of wheat, Sudan grass, and sorghum, deprived of endosperm, were capable of growing into normal, though weak, plants. Wheat embryos grew even when the scutellum also was excised, but growth of sorghum embryos ceased at once.

4. Wheat seedlings from which the shoots were removed were able to produce new shoots. Canary grass, Sudan grass, and sorghum seedlings deprived of shoots continued root growth and produced secondary roots, but no new shoots. Barley and oat seedlings died without response to this treatment.

5. Excised shoots of oats, Canary grass, Sudan grass, sorghum, maize, and pop corn developed adventitious roots. All these except oats and sorghum were able to continue growth when planted in soil. Excised shoots of wheat and barley died without rooting.

6. Isolated cotyledons, deprived of roots and shoots, usually grew to a greater size than control cotyledons with endosperm. Wheat and barley cotyledons regenerated roots and shoots. Maize cotyledons developed roots, but no shoots. Cotyledons of Sudan grass formed roots and weak, abortive, shoot-like outgrowths.

7. Mutilated seedlings of monocotyledonous plants, other than the grasses, showed no capacity for regeneration.

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