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An analysis of an unilateral reproductive isolation barrier in Secale L

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AN ANALYSIS OF
AN UNILATERAL REPRODUCTIVE ISOLATION BARRIER
IN SECALE L.

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INTRODUCTION AND REVIEW OF THE LITERATURE

In crosses between Secale cereale L. and S. africanum Stapf., seeds which are formed germinate only when S. cereale is the female parent (Schiemann and Nurnberg-Kruger, 1952). Also in crosses between S. cereale var. King II and S. montanum Guss., seeds which are formed have been reported to germinate only when S. cereale is the seed parent (Riley, 1955). Using the Copenhagen strain of S. montanum Price (1955) reported 78% germination of seeds when S. cereale was the female parent and 18% germination in the reciprocal cross in which S. montanum was the female parent. The hybrid seeds formed using an Iranian form of S. montanum showed 84% germination when S. cereale was the female parent and 4% germination when S. montanum was the seed parent.

The purpose of my study was to examine this unilateral germination barrier with emphasis on the S. africanum x S. cereale reciprocal crosses. Through this study a better understanding of reproductive barriers could be obtained.

Riley (1955) suggested that the reciprocal difference in germination of these seeds might result from seed incompatibility due to maternal tissue-endosperm relations. Studies of other crosses, e.g., Hordeum jubatum L. x S. cereale (Brink and Cooper, 1944, 1944a) and H. vulgare x S. cereale (Thompson and Johnston, 1945), show that it is also possible that seed failure may be due to hypofunction or disintegration of the endosperm. Another possibility might be that the hybrid embryo is incompatible with the endosperm developed from S. africanum polar nuclei. In

such cases, it should be possible to culture excised embryos following hybrid embryo initiation.

The hybrid, H. jubatum x S. cereale was obtained by Cooper, et. al. (1944) by culturing young hybrid embryos on a nutrient agar medium. Using similar techniques, hybrids of H. sativum and H. bulbosum were grown by Konzak, Randolph, and Jensen (1951). Hybrids of H. vulgare x H. bulbosum have been obtained by Davies (1960) using a medium modified from that used by Randolph and Cox (1943).

Much of the embryo culture work reported in the Graminae has been done using Hordeum. It has been observed that immature embryos grown in a nutrient medium fail to complete the later stages of normal embryonic development, and instead, germinate into miniature weak seedlings (Kent and Brink, 1947; Zieber, et. al., 1950).

Improved growth of Hordeum embryos in an agar medium was obtained when Hordeum endosperm was placed around the excised embryos (Zieber and Brink, 1951). Norstog (1961) reported that coconut milk added to a nutrient agar solution produced a noticeable increase in growth of Hordeum embryos, but his results were highly variable. Some coconut milk added to the culture medium produced nearly normal embryonic growth, while milk from other coconuts was ineffective. Using coconut milk in the medium, Norstog (1961) was able to grow pro-embryos as small as 60 u. Norstog and Smith (1963) observed that a complex medium containing vitamins and amino acids suitable for the germination of barley embryos proved inadequate for reliable germination of rye embryos. The rye embryos which they used were reported to have grown erratically on this medium.

The cause of seed sterility is often due to abnormal development of the endosperm. In the cross between H. jubatum (n = 14) and S. cereale

(n = 7), Brink and Cooper (1944, 1944a) reported that the initial division of the primary endosperm nucleus was delayed, and that mitotic divisions became disorderly resulting in nuclei which varied in size and shape, and which never became cellular. Since the antipodal cells had cytoplasmic and nuclear irregularities, it was suggested that seed-collapse in this hybrid resulted from failure of the S. cereale sperm to stimulate H. jubatum antipodals to full functional activity, which in turn failed to secrete a sufficient amount of some essential material needed for growth and division of the endosperm nuclei. The endosperm then disintegrated resulting in the starvation and death of the embryo. Thompson and Johnston (1945) also noticed abnormal development of the hybrid endosperm in the cross H. vulgare (n = 7) x S. cereale (n = 7). In this hybrid no endosperm cells were formed, and there were irregularities in the endosperm nuclei. These irregularities included disorderly mitotic divisions, large nuclei of a variety of shapes, slower growth, and early disintegration of the nuclei. The embryo appeared normal, and seed failure appeared to be due to the starvation of the embryo. No irregularities in the antipodals were reported.

A reciprocal germination barrier was reported to exist in crosses between species of wheat differing in chromosome number (Thompson and Camaron, 1928; Thompson, 1950). When members of the vulgare series (n = 21) were crossed with members of the emmer series (n = 14), the seeds were wrinkled and usually failed to germinate when vulgare series plants were male parents. In the reciprocal crosses the seeds were plump and healthy. Thompson (1930) explained these differences in terms of endosperm conditions. From the results of investigations between chromosome conditions and shrivelled endosperm in many types of backcross and F_2 seeds, Thompson (1930) concluded that the endosperm was well developed

whenever it was diploid or triploid for the whole set of extra vulgare chromosomes or wherever it contained none of the vulgare chromosomes as in pure emmer wheat. The endosperm was wrinkled when it was haploid for the extra seven vulgare chromosomes, and even more so when incomplete sets were doubled or tripled.

Boyes and Thompson (1937) also reported frequent nuclear and cytoplasmic abnormalities in the endosperm of wheat crosses in which the female parent had a lower chromosome number than the male parent. These abnormalities included nuclei of irregular shapes and sizes, irregularly staining cytoplasm, persisting non-cellular regions, and in extreme conditions, abortion.

Kihara and Nishiyama (Reviewed by Brink and Cooper, 1947) found that Avena strigosa (n = 7) after pollination by A. fatua (n = 21), or A. sterilis (n = 21) set seeds, but the kernels were very shrivelled and failed to germinate. The reciprocal crosses yielded a few poorly developed, occasionally viable seeds. The embryos appeared to the investigators to be normal in the developing seeds, and the irregularities occurred in the endosperm.

In the examples cited above, with the exception of H. vulgare x S. cereale, the reciprocal differences in seed sterility resulted only from crosses between plants with a difference in chromosome number. An unusual characteristic of the unilateral barrier found in Secale is that no polyploidy exists; all species of Secale have seven pairs of chromosomes.

METHODS AND PROCEDURES

Embryo Transplant Experiments

In order to determine whether or not an endosperm-embryo incompatibility exists between S. africanum and S. cereale, embryos of S. cereale were transplanted to the seeds of S. africanum. Secale cereale embryos were also transplanted to a paste prepared from S. africanum seeds.

At first, ten mature embryos were transplanted from S. cereale to the seeds of S. africanum. No controls were run with this experiment. Later twenty-five mature embryos were excised from S. cereale and transplanted onto the seeds of S. africanum. For controls, twenty-five mature embryos of S. cereale were transplanted onto seeds of S. cereale. This experiment was run twice.

In order to reduce fungal contamination, all seeds were handled with sterilized forceps, and were passed through the flame of an alcohol lamp. The embryos were cut from the seeds under a dissecting scope with a sterilized razor blade. The scutellum was left with the seed, and all recognizable endosperm was carefully trimmed from the embryo.

The site on the seeds used as acceptors for transplanted embryos was prepared by removing the embryos and carefully exposing an area of endosperm. The transplanted embryo was set at this site. These seed-embryo combinations were then placed on moist paper in a petri dish and put in a growth chamber set for an eighteen hour 75° F. day and a six hour 70° F. night.

Twenty-five S. cereale embryos were then grown on a paste of S.

africanum flour and on a paste of S. cereale flour. This flour was prepared by grinding flamed seeds, from which the embryos had been removed, at high speed in a two-speed Waring blender for about fifteen to twenty-five minutes. The fine powder which stuck to the sides of the blender was collected and mixed with water in a petri dish. Excised embryos were then positioned on the resulting paste and set in the growth chamber. This experiment was repeated once.

Hybridization Techniques

In order to detect reciprocal differences in seed viability, hybrid seeds were obtained from reciprocal crosses between S. cereale and S. africanum, S. cereale and S. montanum, and S. cereale and S. vavilovii Grossh. Seeds of S. cereale, of the Merced variety, were donated by the Agronomy Department of the University of California at Davis. Secale africanum seed was originally acquired from the Botanical Garden of Sweden. The S. montanum was an Iranian form originally growing around Mt. Demavand, and S. vavilovii seed came from plants growing near Mr. Arart, Turkey.

Starting in early November, seeds of S. cereale var. Merced were planted at weekly intervals in a greenhouse with lighting conditions controlled to produce seventeen hours of light per day. Plants of S. africanum which had wintered in the garden were periodically brought into the greenhouse starting in early January. The S. vavilovii plants were grown completely under greenhouse conditions from seeds sown the preceding spring. The S. montanum plants were placed in a 5^{o} C., seventeen-hour lighted chamber for six weeks, and then moved to the seventeen-hour-day greenhouse.

All crosses were made in the spring of 1967 using one of three

methods. The heads of the female parents of crosses were emasculated by use of forceps. In one method, the pollen-donating culms were clipped from a plant and placed in a vial of water tied to a bamboo stake. The female heads, along with the pollen heads, were then bagged by rolling a paper towel around them and tying the ends with twine. A variation of this method was to bag the emasculated heads with the heads of the pollen parent which were still attached to the plant. The third method consisted of simply dusting bagged emasculated receptive heads with pollen which was collected minutes earlier. The spikes were then rebagged. This method was of great value when the exact time of pollination was required.

To check for germination, the seeds were first rinsed in the disinfectant Ves Phene (½ oz. per gal.), then in distilled water, and planted on moist filter paper in petri dishes. One-hundred seeds of the S. africanum ♀ x S. cereale ♂ cross and one-hundred seeds of the reciprocal cross, S. cereale ♀ x S. africanum ♂ were planted in this way. Seventy seeds of the cross S. montanum ♀ x S. cereale ♂ and sixteen seeds of the reciprocal cross were planted. Twenty-four seeds from the cross S. vavilovii ♀ x S. cereale ♂ and twenty-five seeds from the reciprocal cross were planted. One-hundred S. cereale x S. cereale seeds harvested in the spring of 1967 were planted as a control and seventy-five seeds derived from self-pollinated S. africanum, also harvested this spring, were used as another control. No seeds harvested in the spring of 1967 from S. montanum or S. vavilovii were available for planting.

Seed set was determined by comparing the number of seeds formed to the total number of florets.

Embryo Culture Techniques

The media used for embryo culture were basically like that used by Davies (1960). The medium which will be referred to as "standard" was prepared as follows: seven grams of agar were dissolved in one liter of boiling distilled water. Twenty grams of sucrose, 5 ml of solution A, 5 ml of solution B, and 3 ml of solution C were added (see Table 1). The pH was adjusted to 5.7 with NaOH. The medium was autoclaved and poured into sterilized petri dishes.

Five-hundred ml of a solution which will be referred to as "rye supplemented medium" was also prepared. It differed from the above "standard medium" only in that distilled water was replaced by an extract from boiled rye seeds. This extract was prepared by boiling twenty grams of S. cereale flour in 500 ml of distilled water. The mixture was filtered through cheese cloth and the supernatent was diluted to 500 ml with distilled water.

To reduce contamination in embryo cultures a crude isolation chamber was constructed of polyethylene. It consisted simply of a wooden frame six feet long, three feet wide, and five feet high enveloped with polyethylene and equipped with two flap doors. In this chamber were placed a small table, chair, and dissecting scope. Prior to use, the chamber, table, and chair were soaked with a solution of the disinfectant Ves Phene.

All seeds were soaked in a solution of Ves Phene for a few minutes before embryo excision. They were then rinsed in distilled water and placed on a piece of tissue paper. All excision and transfer instruments were dipped in 95% alcohol and flamed before use.

TABLE 1

SOLUTIONS USED TO PREPARE "STANDARD MEDIUM" FOR EMBRYO CULTURES

(After Davies, 1960, and Randolph and Cox, 1943)

Solution A (500 ml)

$Ca(NO_3)_2 \cdot H_2O$	23.6 g
KNO_3	8.5 g
KCl	6.5 g
Distilled H_2O	500 ml

Solution B (500 ml)

$(NaPO_3)_n$ Calgon	1.0 g in 250 ml distilled H_2O
$MgSO_4 \cdot 7H_2O$	3.6 g in 250 ml distilled H_2O

Solution C (100 ml)

$FeC_6H_5O_7 \cdot 3H_2O$

100 ml distilled H_2O

Standard Medium

5 ml Solution A

5 ml Solution B

3 ml Solution C

7 g Agar

20 g Sucrose

1 liter Distilled H_2O

Two twenty-six day old embryos were excised from hybrid seeds of S. africanum ♀ x S. cereale ♂ and placed on a rye supplemented medium. A transfer to a new medium was later required because contamination ensued. The transfer was accomplished by cutting out cubes of agar containing the embryos and placing these on the standard medium of a new petri dish.

In addition to the above, a total of twenty-one other embryos were excised from hybrid seeds ranging in age from twenty to twenty-six days. These were placed on standard medium. Because of contamination it was necessary to transfer each of the embryos to fresh sterile media. In some cases, in the transfer, the embryos which were removed from contaminated agar were rinsed thoroughly in distilled water before being placed in new sterile medium. In others, where contamination was not widespread, a cube of agar containing the embryo could be removed and transferred directly.

To determine whether or not germinated embryos were capable of further growth, three were transferred to sterilized soil in a deep petri dish; one at nine days, one at fourteen days, and the other at sixteen days after germinating.

Seed Development

In order to compare the anatomical development of hybrid seeds of S. africanum ♀ x S. cereale ♂ to that of S. africanum, seeds were fixed in Formalin-Aceto-Alcohol at 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, and 25 days following pollination. Secale africanum seeds were fixed at the same intervals following anthesis, except no material was fixed at 9 or 19 days. Five different plants of S. africanum were used for this part of the study.

The seeds were dehydrated with tertiary-butyl alcohol and imbedded in paraffin according to methods described by Johansen (1940). The material was sectioned at 12 u with a rotary microtome and stained with Conant's Quadruple Stain.

RESULTS

Embryo Transplants

The criteria for germination used in these experiments were the elongation of the coleoptile and development of chlorophyll.

All ten of the initial S. cereale embryos transplanted to the seeds of S. africanum germinated (figs. 1, 2).

Five days after transplanting twenty-five S. cereale embryos to the seeds of S. africanum, twenty-one had germinated (fig. 3; see Table 2). At this time eighteen controls, S. cereale embryos transplanted to S. cereale seeds, had also germinated (fig. 4; see Table 2).

When this experiment was repeated, similar results were obtained. At five days, twenty-three embryos of S. cereale transplanted onto the seeds of S. africanum had germinated (fig. 5; see Table 2). Twenty of the control transplants had germinated (fig. 6; see Table 2).

Of the twenty-five S. cereale embryos that were placed on a paste of S. africanum flour, fourteen germinated at three days (see Table 2). Seventeen embryos of S. cereale transplanted to a S. cereale paste germinated at the same time (see Table 2).

At three days in the repeated experiment, fourteen S. cereale embryos placed on S. africanum paste germinated (see Table 2). Sixteen of the control transplants germinated (see Table 2).

Despite all attempts to avoid contamination, nearly all the above transplant cultures were overtaken early by contamination.

Fig. 1. *Secale cereale* to *S. africanum* embryo transplants.

Fig. 2. *Secale cereale* to *S. africanum* embryo transplants.

Fig. 3. *Secale cereale* to *S. africanum* embryo transplants.

Fig. 4. *Secale cereale* to *S. cereale* embryo transplants.

Fig. 5. *Secale cereale* to *S. africanum* embryo transplants.

Fig. 6. *Secale cereale* to *S. cereale* embryo transplants.

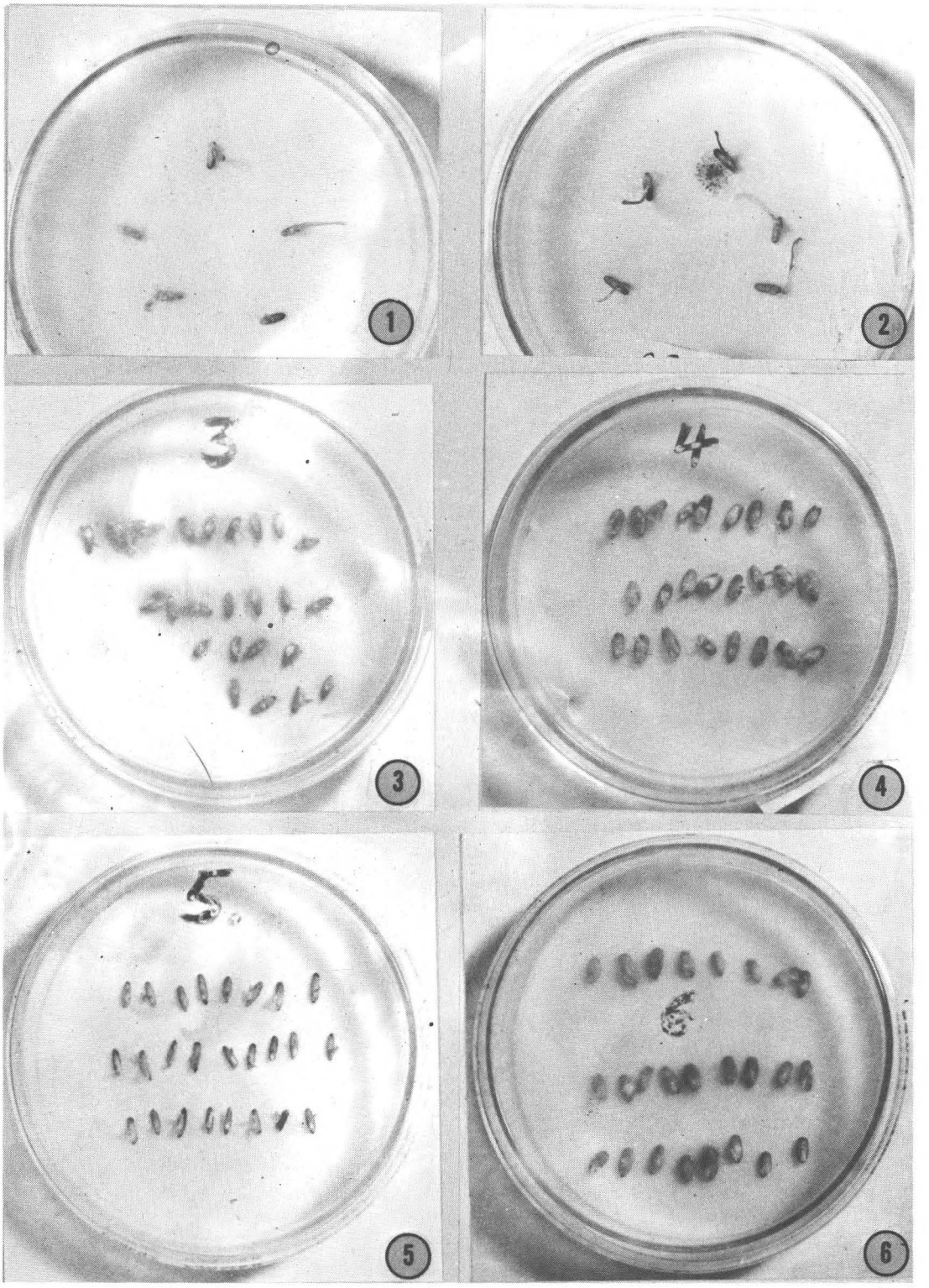
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TABLE 2

EMBRYO TRANSPLANTS

Embryo to seed transplants

	Embryos transplanted	Embryos germinated	Coleoptile length*
cer. on afr.	25	21	$\angle$8 mm
cer. on cer.	25	18	$\overline{\angle}$5 mm
cer. on afr.	25	23	3-7 mm
cer. on cer.	25	20	$\angle$5 mm

Embryo to flour-paste transplants

	Embryos transplanted	Embryos germinated	Coleoptile length*
cer. on afr.	25	14	$\overline{\angle}$4 mm
cer. on cer.	25	17	3-10 mm
cer. on afr.	25	14	7 mm
cer. on cer.	25	16	$\overline{\angle}$7 mm

*The coleoptile lengths are only approximations. In order to have obtained precise measurements, the dishes would have had to been opened. This would have exposed the cultures to contaminates.

Description of Hybrid Seed, Seed Set, and Germination

The hybrid seed obtained from the crosses between S. africanum ♀ x S. cereale ♂ were all extremely shrivelled and brittle at maturity (fig. 9). The dry brittle endosperm was brown in color and was much less abundant than in normal S. africanum seeds. The embryos were also dried out and could not be readily distinguished from the endosperm. Seeds derived from reciprocal crosses, S. cereale ♀ x S. africanum ♂, were usually slightly wrinkled and smaller than S. cereale seeds but otherwise appeared normal (fig. 8). The endosperm was normal in appearance or slightly discolored with a normal appearing embryo.

Of the seventy-six seeds obtained from crosses of S. montanum ♀ x S. cereale ♂, fifty-six were slightly wrinkled or slightly shrivelled, fourteen were extremely shrivelled, and six were not well developed (fig. 13). In the reciprocal cross, sixteen seeds were obtained. These seeds all had a wrinkled seed coat, more so than seeds of S. cereale ♀ x S. africanum ♂ crosses. The seeds were also smaller than S. cereale seeds (fig. 12).

Of twenty-four seeds examined from the cross S. vavilovii ♀ x S. cereale ♂, ten were plump, six were slightly wrinkled, and eight were extremely shrivelled (fig. 17). In the reciprocal cross, large plump seeds were obtained (fig. 16). With few exceptions, these seeds were actually larger than the average S. cereale seeds.

Seeds from crosses between S. cereale and S. africanum germinated only when S. cereale was the female parent. No strong reciprocal differences were observed in crosses between S. cereale and S. montanum or S. cereale and S. vavilovii. For a complete tabulation of seed set and germination of seeds from the above crosses, see Table 3 and 4.

TABLE 3

SEED SET IN SECALE CROSSES

Cross	Florets Emasculated	Seed Set	% Seed Set
afr. x cer.	534	213	40
cer. x afr.	789	145	18
cer. x cer.	212	72	34
afr. selfed	410	151	37
mon. x cer.	229	76	33
cer. x mon.	46	16	35
vav. x cer.	188	34	18
cer. x vav.	108	35	32

TABLE 4

SEED GERMINATION

Cross	Seeds Planted	Seeds Germinated	% Germination After 4 weeks	Plump Seeds Remaining
afr. x cer.	100	0	0	0
cer. x afr.	100	40	40	56
cer. x cer.	100	43	43	56
afr. selfed	75	63	84	12
mon. x cer.	70	54	77	1
cer. x mon.	16	13	81	2
vav. x cer.	24	12	50	1
cer. x vav.	25	24	96	1

Since over 50% of the S. cereale ♀ x S. africanum ♂ seeds and over 50% of the control seeds, S. cereale x S. cereale, had not germinated at the end of four weeks but appeared plump and viable, the low seed germination of these seeds was attributed mainly to dormancy rather than seed incompatibility.

Embryo Cultures

The two twenty-six day old S. africanum ♀ x S. cereale ♂ embryos which were placed on the rye-supplemented medium grew; the most vigorous of these two germinated at 37 days. This seedling was transferred to the soil fourteen days later. At this time it consisted of two leaves and one root (fig. 19). Although it appeared healthy at the time of transplanting, it died in the soil.

The other embryo germinated at fourteen days. The coleoptile elongated to only about 3 mm and later died back. However, the root system continued to grow and many branches formed (fig. 20).

Of the twenty-one hybrid embryos placed on standard media; two germinated; two elongated slightly and developed a trace of chlorophyll before being lost to contamination; eight showed no signs of elongation; and nine were lost to contamination.

The two embryos which germinated did so at nine days and one was transferred to the soil sixteen days later. At that time it consisted of two leaves, the longest of which was 22 mm long, and two roots about 3 mm long. The seedling died in the soil. The other seedling was transferred to the soil nine days later. This seedling continued to grow and differentiated into a vigorous plant. Fig. 21 shows this seedling prior to planting and fig. 22 shows the plant into which it grew.

Fig. 7. *Secale cereale* seeds.

Fig. 8. *Secale cereale* ♀ x *S. africanum* ♂seeds.

Fig. 9. *Secale africanum* ♀ x *S. cereale* ♂seeds.

Fig. 10. Self pollinated *S. africanum* seeds.

Fig. 11. *Secale cereale* seeds.

Fig. 12. *Secale cereale* ♀ x *S. montanum* ♂seeds.

Fig. 13. *Secale montanum* ♀ x *S. cereale* ♂seeds.

Fig. 14. *Secale montanum* seeds.

Fig. 15. *Secale cereale* seeds.

Fig. 16. *Secale cereale* ♀ x *S. vavilovii* ♂seeds.

Fig. 17. *Secale vavilovii* ♀ x *S. cereale* ♂seeds.

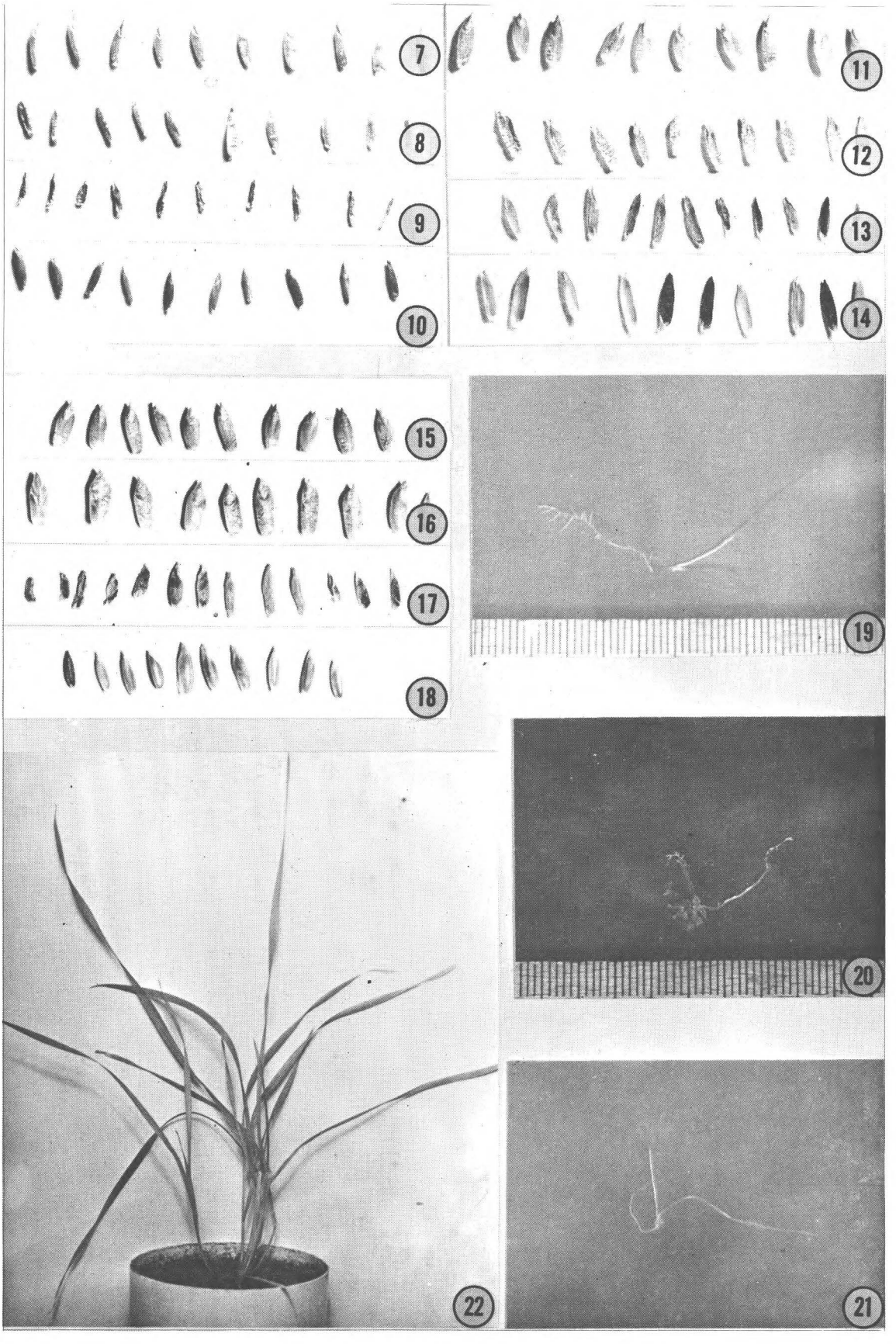
Fig. 18. *Secale vavilovii* seeds.

Fig. 19. A *S. africanum* ♀ x *S. cereale* ♂seedling obtained by embryo culture.

Fig. 20. Differentiated root system of a germinated embryo of *S. africanum* ♀ x *S. cereale* ♂obtained by embryo culture.

Fig. 21. A *S. africanum* ♀ x *S. cereale* ♂seedling obtained by embryo culture.

Fig. 22. A *S. africanum* ♀ x *S. cereale* ♂plant grown from the seedling shown in fig. 21.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Seed Development

Secale africanum

Seed development in Secale africanum Stapf. usually requires about 25 days. As shown in fig. 23, a small proembryo is apparent at three days following anthesis. At seven days a many-celled undifferentiated embryo is evident (figs. 25, 26). Differentiation appears to begin at about eleven to thirteen days (figs. 28, 30) and the embryo is highly differentiated by the fifteenth to seventeenth day (figs. 31, 32).

Endosperm is entirely free-nuclear at three and five days following anthesis (figs. 23, 24) and becomes cellular rather suddenly at about seven days (figs. 25, 26). The endosperm at seven days fills the lumen of the embryo-sac and contains starch grains. From seven days onward, the endosperm becomes progressively more abundant, and contains more and larger starch grains. As these starch grains increase in size and number, the nuclei in the cells disappear.

Secale africanum ♀ x Secale cereale ♂

In contrast to seed development in S. africanum, S. africanum ♀ x S. cereale ♂ seeds develop very abnormally. A proembryo consisting of several cells is present at three days following pollination (fig. 33). In the material studied, the proembryo consisted of several cells at seven days (fig. 38) but it was much smaller than the embryo of S. africanum at the comparable stage. The embryo observed at eleven days was also smaller than those of S. africanum and did not appear to have many more cells than it had at seven days. The thirteen and fifteen day old embryos were large and undifferentiated (figs. 40, 42). Embryo differentiation in the hybrid seed was first observed at the seventeenth day

Fig. 23. A section of a S. africanum seed at three days following anthesis showing a young proembryo and free nuclear endosperm. X 33.

Fig. 24. A section of a S. africanum seed at five days following anthesis showing free nuclear endosperm. X 33.

Fig. 25. A section of a S. africanum seed at seven days following anthesis. X 13.

Fig. 26. A section of a S. africanum seed at seven days following anthesis showing cellular endosperm and a many-celled undifferentiated embryo. X 33.

Fig. 27. A section of a S. africanum seed at eleven days following anthesis. X 13.

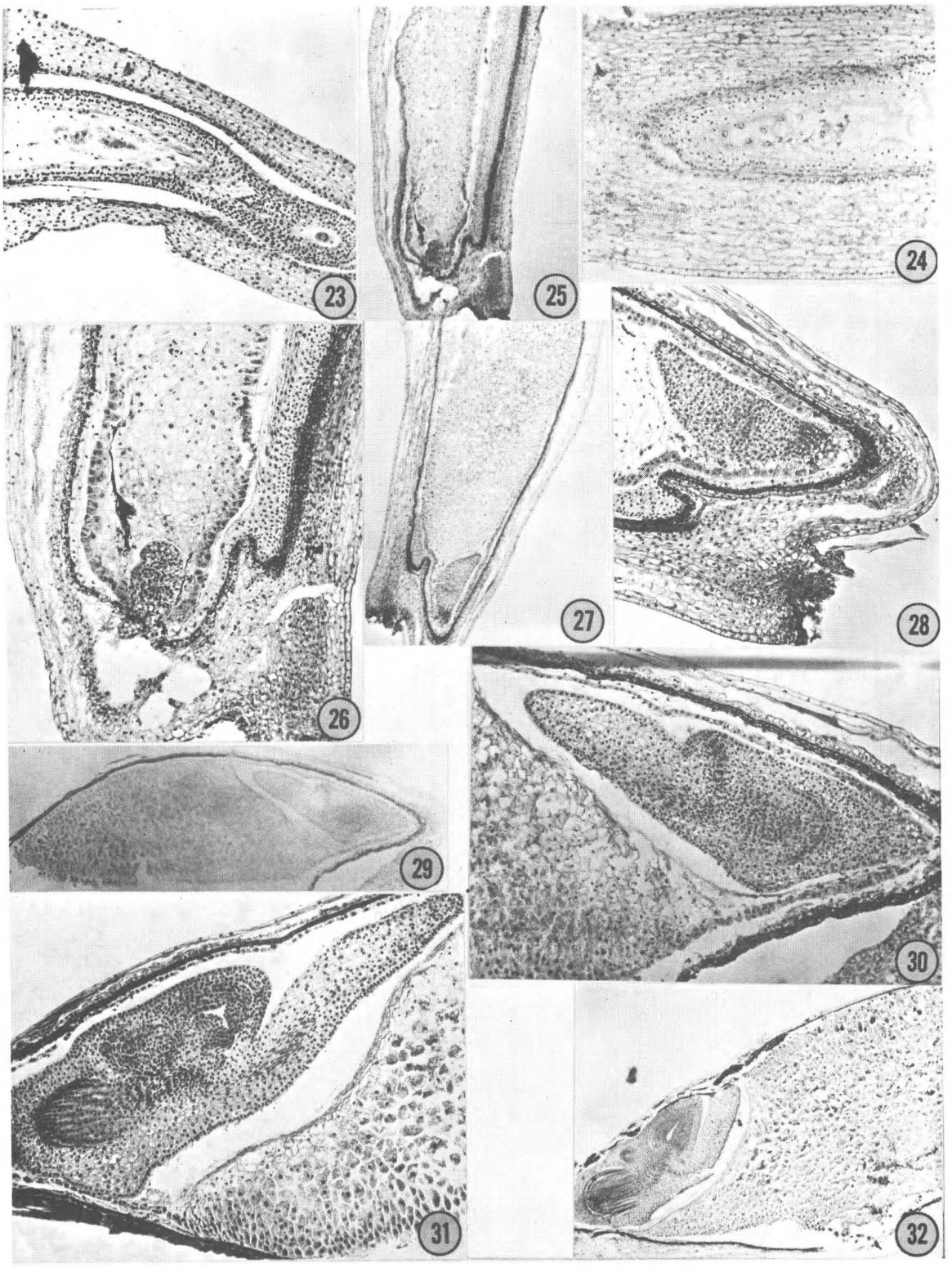
Fig. 28. A section of a S. africanum seed at eleven days following anthesis showing the embryo. X 33.

Fig. 29. A section of a S. africanum seed at thirteen days following anthesis. X 13.

Fig. 30. A section of a S. africanum seed at thirteen days following anthesis showing differentiation of the embryo. X 33.

Fig. 31. A section of a S. africanum seed at fifteen days following anthesis showing a differentiated embryo. X 33.

Fig. 32. A section of a S. africanum seed at seventeen days following anthesis showing a well differentiated embryo. X 13.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of development (fig. 44), as compared to thirteen days in the control, but it is apparent in fig. 46 that some embryos are not yet differentiated even at nineteen days. The cells of the 19 day hybrid embryo appear densely stained and by twenty-three days the hybrid embryo appears to be unhealthy or dead (fig. 48).

Endosperm formation in the hybrid also varies markedly from that of S. africanum. As in S. africanum, the S. africanum ♀ x S. cereale ♂ endosperm is free nuclear at three and five days (figs. 33, 34, 35, 36). However, after this time it develops much more slowly than that of S. africanum. At seven days the endosperm is cellular, or nearly so, but does not fill the lumen of the embryo sac (fig. 37). No starch grains are present in the hybrid endosperm at seven days as there are in the endosperm of S. africanum. Starch grains apparently do not form until about seventeen days (fig. 45). They were never abundant, nor were they observed past nineteen days. The endosperm in seeds examined at eleven days did not completely fill the lumen of the embryo sac (fig. 39). The thirteen day old endosperm was cellular and fairly abundant; however, abnormalities were present. These abnormalities took the form of curdly patches in the endosperm, which looked like areas of collapsed cells; and granular cytoplasm in the endosperm cells near the embryo (figs. 40, 41). Many endosperm cells appeared to be collapsed at fifteen days, and a few irregularly shaped endosperm nuclei were observed. An apparent increase in collapsed endosperm cells was observed after fifteen days of development and by twenty-three to twenty-five days the endosperm had completely broken down (figs. 47, 49).

No significant difference in maternal tissue development or in mitosis in endosperm nuclei was observed in the hybrid seeds in comparison to S. africanum.

Fig. 33. A section of a S. africanum ♀ x S. cereale ♂ seed at three days after pollination showing a proembryo and free nuclear endosperm. X 33.

Fig. 34. A section of a S. africanum ♀ x S. cereale ♂ seed at three days after pollination. X 13.

Fig. 35. A section of a S. africanum ♀ x S. cereale ♂ seed at five days after pollination. X 13.

Fig. 36. A section of a S. africanum ♀ x S. cereale ♂ seed at five days after pollination showing free nuclear endosperm. X 33.

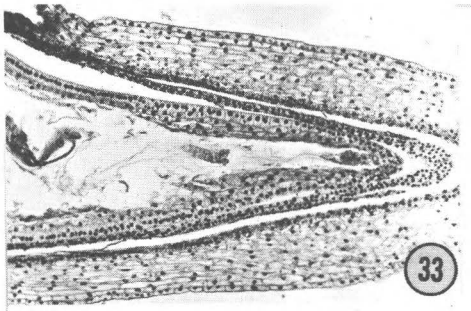
Fig. 37. A section of a S. africanum ♀ x S. cereale ♂ seed at seven days after pollination showing the endosperm. X 33.

Fig. 38. A section of a S. africanum ♀ x S. cereale ♂ seed at seven days after pollination showing the embryo. X 33.

Fig. 39. A section of a S. africanum ♀ x S. cereale ♂ seed at eleven days following pollination showing the endosperm. X 13.

Fig. 40. A section of a S. africanum ♀ x S. cereale ♂ seed at thirteen days following pollination showing the embryo and adjacent endosperm. X 33.

Fig. 41. A section of a S. africanum ♀ x S. cereale ♂ seed at thirteen days following pollination showing a portion of the endosperm. X 33.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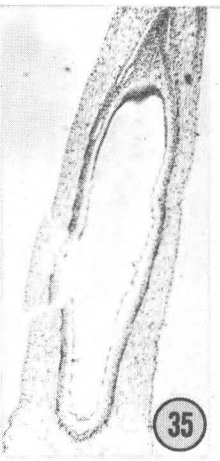
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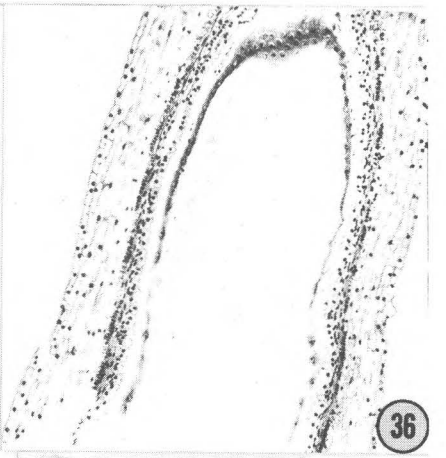
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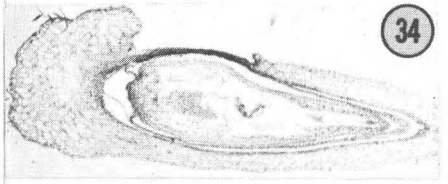
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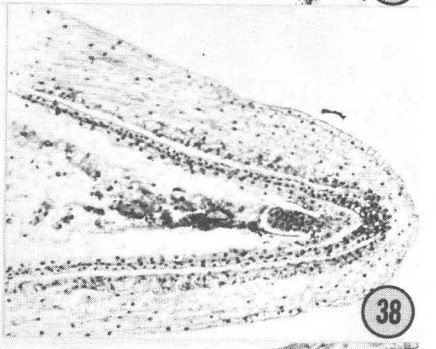
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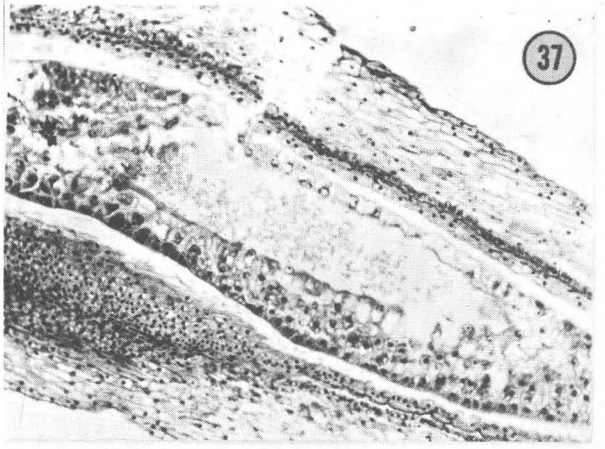
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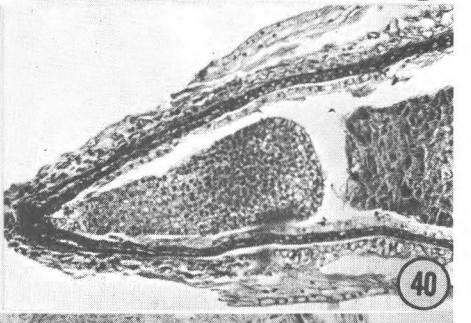
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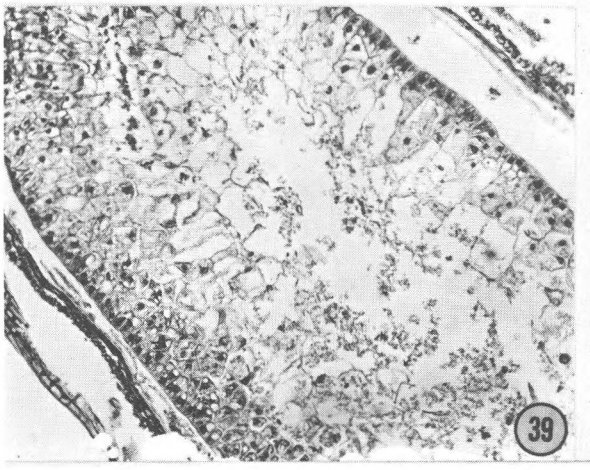
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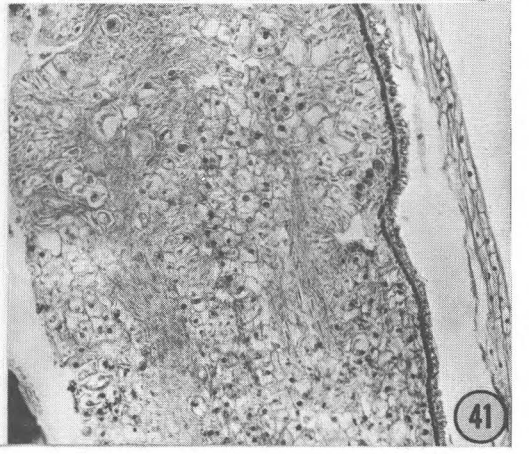
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Fig. 42. A section of a S. africanum ♀ x S. cereale ♂ seed at fifteen days following pollination showing the embryo. X 33.

Fig. 43. A section of a S. africanum ♀ x S. cereale ♂ seed at fifteen days following pollination. X 13.

Fig. 44. A section of a S. africanum ♀ x S. cereale ♂ seed at seventeen days following pollination showing a differentiated embryo. X 33.

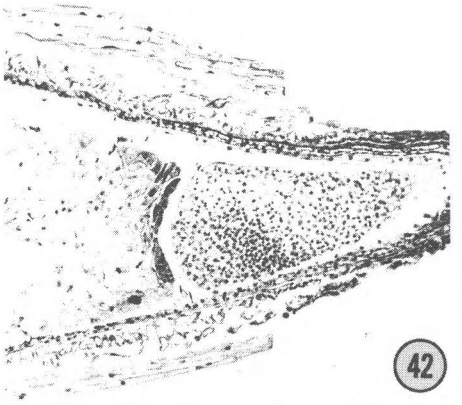
Fig. 45. A section of a S. africanum ♀ x S. cereale ♂ seed at seventeen days following pollination showing a portion of the endosperm. X 33.

Fig. 46. A section of a S. africanum ♀ x S. cereale ♂ seed at nineteen days following pollination showing the embryo and adjacent endosperm. X 33.

Fig. 47. A section of a S. africanum ♀ x S. cereale ♂ seed at twenty-three days following pollination. X 13.

Fig. 48. A section of a S. africanum ♀ x S. cereale ♂ seed at twenty-three days following pollination showing the embryo. X 33.

Fig. 49. A section of a S. africanum ♀ x S. cereale ♂ seed at twenty-five days following pollination showing a portion of the endosperm. X 33.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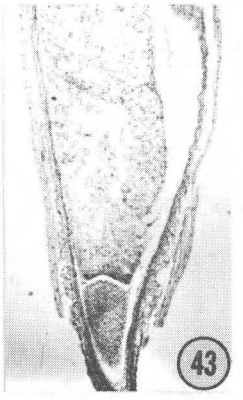
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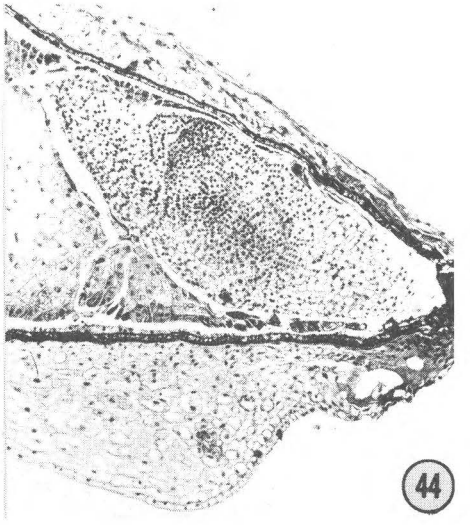
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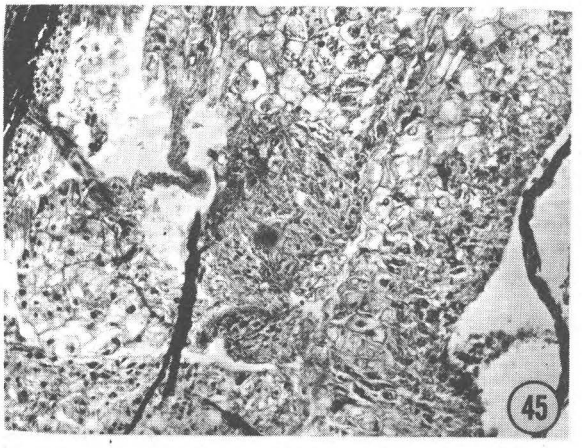
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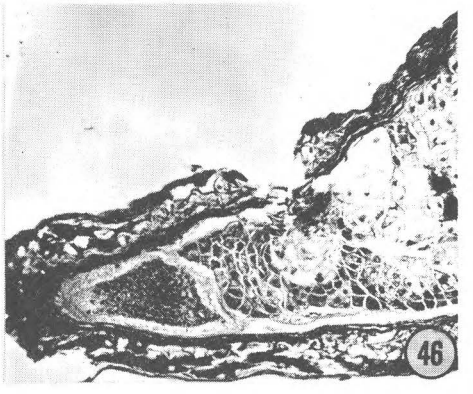
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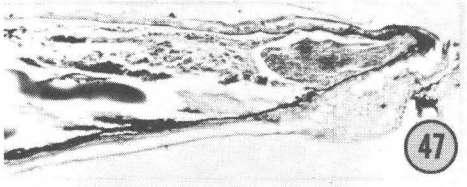
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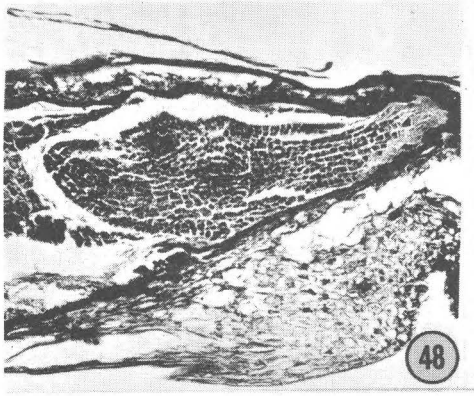
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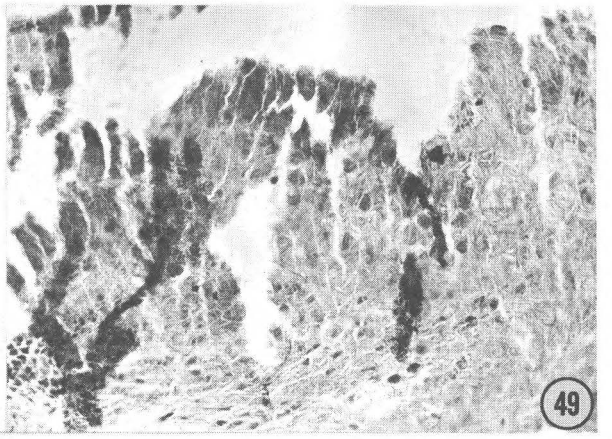
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DISCUSSION

Since S. cereale embryos germinated just as well on S. africanum endosperm as on the endosperm of S. cereale, S. africanum endosperm apparently produces no inhibiting effect on the germination of S. cereale embryos. This was also indicated from the transplants of S. cereale embryos to the pastes formed from S. africanum and S. cereale flour, in which no significant difference in germination resulted. Therefore, it may be inferred that endosperm-embryo incompatibility cannot account for the reciprocal difference in germination of S. cereale ♀ x S. africanum ♂ and S. africanum ♀ x S. cereale ♂ hybrid seeds.

Also, because some embryos of S. africanum ♀ x S. cereale ♂ were cultured on nutrient agar media, and one was even transplanted to soil and grown to a vigorous plant, the cause of seed failure in S. africanum ♀ x S. cereale ♂ crosses cannot be attributed to genic failure of the embryo.

Furthermore, since there appears to be no significant difference in maternal tissue development in the hybrid, S. africanum ♀ x S. cereale ♂ as compared to that of S. africanum as shown in figures 23 to 48, Riley's hypothesis of seed failure in Secale hybrids due to maternal tissue-endosperm relations does not seem to be correct.

Therefore, since (1) an endosperm-embryo incompatibility apparently does not exist, (2) the embryo is potentially viable, and (3) there is apparently no abnormal maternal tissue development, the primary cause of seed failure appears to lie in the endosperm development itself. This

is graphically corroborated in figures 37 to 49 where it can be seen that the hybrid seeds in the cross S. africanum ♀ x S. cereale ♂ fail to germinate because of abnormal endosperm development followed later by collapse of endosperm cells, resulting in starvation and death of the embryo.

A unilateral isolation barrier as strong as the one which exists between S. cereale and S. africanum suggests that it is the product of intensive differential selection. Indeed the barrier exists in the direction which would be expected if it were the product of natural selection. Since S. africanum is a stabilized uniform species, occupying a rather restricted ecological niche, it would be expected to offer less advantage to products of introgression from a foreign species than would S. cereale which, because of its wide distribution as a roadside weed, an invader of barley and wheat fields, and as a cultigen itself, would be expected to be most successful with a heterogenous gene pool. Therefore, it might be expected that natural selection would continually intensify such a differential barrier. It would also be expected that where avenues were available, such unilateral barriers might become established between S. cereale and all other Secale species which have restricted habitat requirements.

Because of the differential germination of seeds in S. cereale x S. montanum reciprocal crosses, Stutz (1957) suggested that such a barrier might have been established by natural selection as a way of keeping out variability resulting from introgression of S. cereale genes into S. montanum.

Since the intensification of barriers by natural selection requires two species to be in contact so that hybridization is possible, a stronger barrier might be expected in crosses of S. montanum x S.

cereale than in *S. africanum* x *S. cereale* crosses. This might be expected because of the long history of isolation of *S. africanum* from all other *Secale* species. However, as reported above, the unilateral isolation barrier is weaker in *S. montanum* than in *S. africanum*. In fact, in these studies there is no good evidence for reciprocal differences in seed germination in hybrids of *S. cereale* x *S. montanum* at all.

It is possible that the *S. montanum* used in this study was from an area where introgression from *S. cereale* may have eroded a previously established barrier and that the *S. montanum* showing the strong unilateral barrier in crosses with *S. cereale* studied by Price (1955) and Riley (1955) may be from areas where these barriers are still effective.

Secale africanum has apparently been quite free from hybridization and introgression from *S. cereale*. This may be the reason why the barrier is more intense in *S. africanum* than in *S. montanum*; but in any case it is difficult to attribute it to the direct effect of natural selection. More likely, there must be something inherent in the *S. cereale* and *S. africanum* genomes which results in the breakdown of *S. africanum* ♀ x *S. cereale* ♂ seeds. This probably reflects differences in each species which are quite independent of the isolation barrier itself.

Since in reciprocal crosses, the genotypes of the embryos are identical, the only genotypic difference in fertilization products must lie in the endosperm. In *S. cereale* ♀ x *S. africanum* ♂ crosses, the endosperm has two genomes from *S. cereale* and one from *S. africanum*. However, in the reciprocal crosses, *S. africanum* ♀ x *S. cereale* ♂ the endosperm has two genomes from *S. africanum* and only one from *S. cereale*. Therefore, the basic cause of hybrid seed failure in *S. africanum* ♀ x *S. cereale* ♂ crosses could very well be related to disturbed genetic

conditions in the endosperm.

Since S. cereale is a highly variable, cross-pollinated species with wide ecological tolerances, it might be supposed that plants possessing mutations which have occurred in this species would have a fair chance of finding a niche in which they might survive. In this way, mutations could have accumulated in S. cereale providing for abundant genetic variation. Similarly, mutations affecting the steps in endosperm development might have also accumulated and thereby provided a variety of alternate routes for normal endosperm development.

On the other hand, since S. africanum is so uniform in its ecological requirements, being restricted to a very narrow ecological habitat in South Africa, and since it is normally self-fertilized, very little genetic variation would be expected to have accumulated in this species. Consequently, endosperm formation in S. africanum might be expected to be monotonously the same in all plants with a very low tolerance of alternate pathways having been attained.

Therefore, in the cross S. africanum ♀ x S. cereale ♂, the genes introduced to S. africanum endosperm from S. cereale may be very different from those of S. africanum, and since S. africanum is apparently not adapted to utilize variation, endosperm development could be severely affected. However, since endosperm development in S. cereale may be accomplished in a variety of ways, the introduction of a single genome of S. africanum may not seriously affect endosperm development when S. cereale is the female parent.

Reciprocal differences in hybrid seed development have often been reported in crosses between species with different chromosome numbers (see Brink and Cooper, 1947), and it is generally true that hybridization is more successful when the female parent has the higher chromosome

number. For example, in wheat, Thompson (1930) observed that when members of the emmer series (n = 14) are crossed with members of the vulgare series (n = 21), seeds which form are wrinkled and usually fail to germinate when plants from the emmer series are used as the female parents; but when vulgare plants are the female parents, hybrid seeds are plump and healthy.

Also, Boyes and Thompson (1937) observed frequent endosperm abnormalities, and in extreme cases endosperm abortion, in crosses of wheat in which the female parent had a lower chromosome number than the male parent. When the female parent was a polyploid, and the male parent had a lower chromosome number, the endosperm was usually normal except for slower growth and a smaller ultimate size.

The basis for this differential endosperm development is not known but it is very likely related to the inability of a single genome from a diploid plant to seriously affect the genetic balance when associated with the multiple genomes of the female polyploid species. Reciprocally, since a diploid female contributes two genomes to developing endosperm, a single genome from a polyploid male parent could seriously affect the genetic balance in the hybrid endosperm.

It has been suggested by Woodrell (1960) that some reciprocal seed germination barriers in strictly diploid crosses may actually involve the same principles as polyploid-diploid crosses. For example, Valentine (1956) explained reciprocal germination of seeds in British Primula crosses in terms of "genetic value", believed to be controlled by several unlinked genes having an additive effect. Woodrell (1960) suggested that this may be due to duplications which would bring the genetic complement of the cells toward a polyploid state without an increase in chromosome number.

Woodrell (1960), also suggested that if evolution in primulas involved an accumulation of these "duplications", then an artificial autotetraploid crossed with its diploid ancestor should yield seeds resembling those of extreme interspecific crosses. This was indeed observed when such crosses were made (Woodrell and Valentine, 1961).

Although all species of Secale are diploid, it is possible that S. cereale may have accumulated numerous duplication during its evolution which would have resulted in some genes behaving as though they were polyploid. Evidence that duplications are common in S. cereale was demonstrated by Muntzing and Prakken (1941), and Stutz (1957). If duplications have accumulated in S. cereale which effect endosperm development, then it might be expected that S. cereale may perform like a polyploid when crossed with S. africanum. In this situation a sperm of S. africanum may not seriously affect the two genomes of S. cereale in the cross S. cereale ♀ x S. africanum ♂, but in the cross S. africanum ♀ x S. cereale ♂ a single S. cereale genome containing additional genetic material due to the duplications could seriously affect the genetic balance in the hybrid endosperm. If this were true, then crosses of autotetraploid rye with its diploid ancestor should yield seeds resembling those of S. africanum x S. cereale crosses. As reported by Hakansson and Ellerstrom (1950), mitotic irregularities were common and cell wall formation was delayed or failed in the endosperm of 2n ♀ x 4n ♂ crosses. Seed development was more successful in 4n ♀ x 2n ♂ crosses; cell wall formation occurred early and no mitotic irregularities were observed.

If the barrier to seed germination in the cross, S. africanum ♀ x S. cereale ♂ is caused by extra genetic material in S. cereale, then it can be postulated that in crosses between an autotetraploid S.

africanum plant used as the female parent and S. cereale as the male parent, the barrier to normal endosperm development should be weakened.

SUMMARY

1. A strong unilateral reproductive isolation barrier is inherent in crosses between S. africanum and S. cereale. Seeds germinate only when S. cereale is the female parent.

2. Since embryo transplants from S. cereale to intact endosperm of seeds of S. africanum germinate just as well as they do on S. cereale endosperm, and S. cereale embryo transplants show no significant difference in germination on pastes prepared from S. africanum and S. cereale flour, an endosperm-embryo incompatibility therefore apparently does not exist.

3. Since hybrid seedlings of S. africanum ♀ x S. cereale ♂ were obtained by embryo culture, the cause of seed failure in the cross S. africanum ♀ x S. cereale ♂ is not due to genic failure of the embryo.

4. Seed failure does not appear to be due to abnormal maternal tissue-endosperm relations.

5. Very marked disturbances observed in endosperm and embryo development in the S. africanum ♀ x S. cereale ♂ seeds suggest that seed failure is due to abnormal development of the endosperm leading to endosperm disintegration, resulting in starvation and death of the embryo.

6. The basis for abnormal endosperm development in the cross S. africanum ♀ x S. cereale ♂ appears to be due to disturbed genetic conditions in the endosperm brought about by either S. africanum endosperm not being able to tolerate variation introduced from S. cereale, or to upset genetic balance resulting from the S. cereale genome which possibly contains additional genetic material.

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AN ANALYSIS OF AN UNILATERAL REPRODUCTIVE ISOLATION BARRIER IN *SECALE* L.

An Abstract of a Thesis

Presented to the

Department of Botany

Brigham Young University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Harold James Price

August 1967

ABSTRACT

In the cross, S. cereale ♀ x S. africanum ♂, near normal seeds were formed. They showed 40% germination. In the reciprocal cross, S. africanum ♀ x S. cereale ♂, the seeds were dry, brittle, extremely shrivelled at maturity and none germinated. Reciprocal differences as sharp as those in crosses between S. cereale and S. africanum were not noted in crosses of S. cereale with S. montanum or with S. vavilovii. Seeds from the cross S. cereale ♀ x S. montanum ♂ showed 81% germination, whereas seeds from the reciprocal cross S. montanum ♀ x S. cereale ♂ showed 77% germination. Seeds from the cross S. cereale ♀ x S. vavilovii ♂ showed 96% germination, and seeds from the reciprocal cross S. vavilovii ♀ x S. cereale ♂ showed 50% germination.

The possible causes of seed inviability which were investigated in S. africanum ♀ x S. cereale ♂ crosses were: (1) endosperm-embryo incompatibility, (2) embryo inviability, (3) maternal tissue-endosperm interaction, (4) abnormal endosperm development.

To determine whether an endosperm-embryo incompatibility existed, embryos were transplanted from S. cereale to the seeds of S. africanum as well as to seeds of S. cereale, and also to a paste prepared from both S. africanum seeds and S. cereale seeds. Since S. cereale embryos germinated just as well on S. africanum endosperm as on the endosperm of S. cereale, S. africanum endosperm apparently produced no inhibiting effect on the germination of S. cereale embryos.

To determine whether the embryos were viable or inviable in the

S. africanum ♀ x S. cereale ♂ seeds, hybrid embryos were excised prior to seed maturity and placed on nutrient agar media. Since four out of twenty-three germinated, and one of these even grew to a vigorous plant when transferred to the soil, the cause of seed failure cannot be attributed to genic failure of the hybrid embryo.

To determine whether or not there was abnormal maternal tissue-endosperm development or abnormal endosperm development, seeds were fixed and sectioned at various ages following pollination. Since no abnormalities were observed in the maternal tissue of the hybrid seed, seed failure probably cannot be attributed to abnormal maternal tissue-endosperm relations. However, very marked disturbances in endosperm and embryo development were observed with the eventual disintegration of the endosperm.

The cause of seed failure in the cross S. africanum ♀ x S. cereale ♂ is apparently due, therefore, to abnormal development of the endosperm resulting in starvation and death of the embryo.

This abstract by Harold James Price is accepted in its present form by the Department of Botany of Brigham Young University as satisfying the abstract requirement for the degree of Master of Science.

28 July 1967
Date

Typed by Susan C. Daines