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DEVELOPMENT OF THE EMBRYO SAC, ENDOSPERM AND EMBRYO IN *IRIS MUNZII* AND THE HYBRID *I. MUNZII* X *I. SIBIRICA* 'CAESAR'S BROTHER'

LEE W. LENZ

INTRODUCTION

Ever since the pioneering work of Foster and Dykes in iris breeding it has been known that it is sometimes possible to effect very "wide" interspecific crosses within the genus. In a number of instances the resulting hybrids not only have proven to be desirable horticultural subjects themselves but in some instances they have led to the production of whole new races of hybrids. Probably the most remarkable example of this is 'Wm. Mohr', a cross between the oncocyclus species *I. gatesii* and the diploid tall-bearded iris 'Parisiana'. This hybrid, while a difficult parent, has led to the development of a whole series of easily grown hybrids which combine much of the exotic beauty of *I. gatesii* with better known and easily grown tall-bearded iris. Another example is the production of 'Royal Californian' which resulted from a cross between one of the native California irises, *I. douglasiana*, and *I. sibirica*. In the production of these "wide" crosses it is often necessary to pollinate a very large number of flowers in order to obtain a single viable seed. More recently (Lenz, 1954, 1956) it has been shown that in some instances it is possible to culture embryos from partially mature seeds and thus obtain seedlings from what would otherwise be considered incompatible crosses. The purposes of the present investigation were two-fold: (1) to determine, if possible, the cause of seed failure in a typical "wide" cross and (2) to study the development of the embryo as a background for further work on the application of the excised embryo technique to the handling of immature embryos.

MATERIALS AND METHODS

Plants of the native California iris, *I. munzii* R. C. Foster (RSABG 5788-1) and the horticultural variety of *I. sibirica* hort. known as 'Caesar's Brother', hereafter referred to only as *I. sibirica*, used in this investigation were grown in the experimental gardens of the Rancho Santa Ana Botanic Garden at Claremont, California. During the blooming season individual plants were enclosed in insect-proof cages and the flowers were pollinated the morning of their opening. The three anthers were carefully removed and the appropriate pollen dusted on the triangular stigmatic surfaces by means of a small camel's-hair brush and the flowers then tagged showing date and hour of pollination. In some instances it was necessary to use flowers on plants not protected by cages. In this case the anthers were removed from buds which were just ready to open, the stigmas dusted with pollen and the buds then covered with a cellophane bag which was allowed to remain until the flower had withered. In order to make certain that self-incompatibility factors might not interfere with results in the cross *I. munzii* X *I. munzii*, the flowers used were always dusted with pollen obtained from other clones of the same species.

Developing capsules were harvested at intervals of from 24 hours to 7 weeks after pollination. At the time of fixation the upper and lower one-third of the capsule were discarded and the remaining portion cut into sections $\frac{1}{4}$ - $\frac{1}{3}$ inch long. As the

capsules matured it was found advisable to trim away a portion of the ovary leaving only the placenta and attached ovules. The sections were fixed in either Formalin-Propionic-Alcohol or CRAF solution. Because of the air trapped in the sections it was found necessary to place them in a vacuum for a few minutes in order to obtain adequate fixation. This was accomplished by means of a water-operated aspirator attached to a dessicator jar into which vials containing the sections and fixing solution were placed. No damage to the material could be detected from this treatment. Material fixed in FPA was allowed to remain in that solution until needed. The sections fixed in CRAF solution were transferred, after 24 hours, to 70% alcohol for storage. Dehydration was carried out by means of the tertiary-butyl alcohol method and the sections were embedded in Parlux. Sectioning was done by means of a rotary microtome at thicknesses of from 12-20 microns. Safranin-fast green was found to be a satisfactory staining combination and it was used throughout the course of this investigation. Photographs were made by means of a Leitz Aristophot attachment on a microscope and the film used was Eastman Kodak Contrast Process Panchromatic developed in Dektol diluted 1 part Dektol to 3 parts of water.

EMBRYO SAC

In *Iris* the female gametophyte is of the monosporic, 8-nucleate type and at the time that the micropylar megaspores have degenerated the functional chalazal megaspore is embedded in a many-celled nucellus. Surrounding the nucellus is an inner integument which is quite uniformly two cells in thickness and this in turn is surrounded by a much thicker outer integument 8-10 cells thick. Figure 1 of *I. munzii* shows the functional megaspore and portions of the three degenerated nuclei. At the two-nucleate stage (Figure 2) the young embryo sac has elongated considerably and the nuclei are situated near the two ends. At the four-nucleate stage (Figure 3) two nuclei lie near one another in the chalazal end while the other two remain in the micropylar end. At this stage the embryo sac has become more or less ovoid in outline with the chalazal end being considerably narrowed. The eight-nucleate stage was not observed in this material.

The mature embryo sac (Figure 4) is of the "Normal type" with a three-celled egg apparatus, three antipodal cells and two polar nuclei located near the center of the embryo sac. By this time the embryo sac has enlarged considerably and consists of a thin layer of cytoplasm surrounding a large vacuole with the central pair of polar nuclei connected to the peripheral layer by means of thin strands of cytoplasm. In many instances at the time that the flower opens, the polar nuclei have not as yet approached one another and fusion may be delayed as much as 72 hours after pollination. However, in no instance was fusion seen to be delayed as late as the arrival of the male nuclei. This is in agreement with the findings of Sawyer (1925) working on *Iris pseudacorus* and differs from those of Guignard (1899) who, in an undisclosed species, figured the three nuclei in contact. Sawyer further reports that each polar nucleus contains a conspicuous nucleolus and that upon fusion two nucleoli are present. Later a single large nucleolus is usual and is always the condition when the second male nucleus arrives. In material of *I. munzii* more than one nucleolus is sometimes found in one of the polar nuclei and at times three nucleoli (or nucleoli-like bodies) are clearly evident (Figure 6). Figure 3 which shows the four-nucleate stage in embryo sac formation also shows three nucleoli in the nuclei at the chalazal end of the embryo sac. The matter of multiple nucleoli will be discussed later in connection with the endosperm.

FERTILIZATION

In *Iris munzii* pollen tubes were not found in the micropylar canal earlier than

approximately 96 hours after pollination. Sawyer (1925) reports that in *I. pseudacorus* she was able to detect pollen tubes in from 77 to 78 hours. The exact time required for pollen tubes to reach the nucellus probably is dependent at least to a certain extent upon environmental conditions prevailing at and immediately following pollination. In the spring of 1955 the weather was unusually cold and rainy during a portion of the pollinating season and pollen tubes were absent from the micropylar canal as late as 120 hours after pollination. Thus, in some of the 1955

Fig. 1-4. Development of embryo sac in *Iris munzii*. Fig. 1, functional megaspore, x 560; fig. 2, two-nucleate stage, x 560; fig. 3, four-nucleate stage, x 560; fig. 4, mature embryo sac, x 350; fig. 5, pollen tube of *I. sibirica* entering *I. munzii* embryo sac, x 560; fig. 6, polar nuclei showing one with three nucleoli, x 560; fig. 7, metaphase of first endosperm division in *I. munzii*, x 560. (All figures slightly reduced).

material identical stages in ovular development were not reached until several days after they were in material grown in 1954. Figure 5 (*I. munzii* $\times$ *I. sibirica*) shows the pollen tube inside the embryo sac and what is believed to be the fertilized egg cell showing two nucleoli, the smaller one presumably that contributed by the male nucleus. Apparently there is little or no difference in rate of pollen tube growth between *I. munzii* pollen on flowers of that species and pollen of *I. sibirica* on *I. munzii*. In both instances pollen tubes were found in the micropylar canal at about the same time. Sawyer (1925) reported a similar situation in that she found pollen tubes of *I. versicolor* grew as rapidly in the styles of *I. pseudacorus* as pollen tubes of *I. pseudacorus* in the styles of that species.

HYPOSTASE

One of the most conspicuous structures in the developing iris ovule is the highly developed hypostase. This structure is located at the level of the origin of the integuments and below the embryo sac. As pointed out by Maheshwari (1950) this body is often a well-defined but irregularly outlined structure of nucellar cells which are usually poor in cytoplasmic contents but have partially lignified or suberized walls composed of a highly refractive material. In *I. munzii* there appear to be three types of cells involved in the structure of the hypostase. The first sign of differentiation which occurs approximately ten days after pollination, appears to be the formation of a band of 3-5 cells in thickness which have quite irregular and somewhat thickened cell walls that stain with safranin. Immediately above this relatively thin band of cells lies a group of cells which, in the more advanced stages of ovular development, is quite massive (Figure 8). The cells composing this tissue appear not to have thickened walls but inside the cells there are large masses of an amorphous material which stains deep brown or reddish with safranin. This material appears to be built up of an accumulation of small often spherical bodies which fuse, or partially fuse, to form the large bodies (Figure 9). Lying above this mass of deeply stained material is another group of cells which are thin-walled and stain only with fast-green. In at least some instances they have a definite palisade-like arrangement with their long axis perpendicular to the cells below them. Figure 10 shows a portion of a longitudinal section through a partially mature ovule of one of the Hexapogon irises. Here the firm cellular endosperm has been removed. It can be seen that the upper thin-walled cells form a biscuit-like body which protrudes into the lower end of the endosperm. In fresh material this biscuit-like body is filled with chlorophyll.

As reported by earlier workers (Maheshwari, 1950; Dahlgren, 1939) the exact function of the hypostase is in doubt. However, it is a very characteristic feature of the seeds of certain families and genera. Lying as it does at the termination of the funicular vascular strand, it is certainly associated with the nutrition of the embryo and endosperm. The role played by the chlorophyll in some of the cells is not clear. The development of the hypostase in the hybrid *I. munzii* $\times$ *I. sibirica* is identical to that described for *I. munzii*. In no case was there ever any sign of an overgrowth or degeneration of this structure in ovules containing embryos and either normal or degenerating endosperm.

Mature iris seeds very often have a distinct dimple or wrinkled depression at the end of the seed opposite the micropyle and it would seem possible that this slight depression may be caused by a collapse of the cells of the hypostase at the time when the seed reaches maturity and there is no longer a need for water and nutrients to be carried into the endosperm.

Fig. 8, portion of young ovule showing funicular vascular strand and hypostase lying below cellular endosperm, x 80; fig. 9, portion of hypostase showing cell inclusions, x 560; fig. 10, hypostase in one of the *Hexapogon* irises with endosperm removed, x 80.

ENDOSPERM

The great significance of the role played by the endosperm in the life of the embryo has been admirably discussed by Brink and Cooper (1947). They point out that the major role of the endosperm is that of the development and maintenance of a medium suitable for the growth and development of the young embryos. Whereas, the endosperm in some groups does not persist in the mature seed, the iris endosperm, as in the case of the Gramineae, becomes a massive storage organ.

As Brink and Cooper and others have shown, the problems of the endosperm arise from the peculiarities of its origin and development. Physically it occupies a position between two sporophytic generations while genetically it differs from either the surrounding maternal tissue or the enclosed embryo. In diploid iris the endosperm is the normal $3n$ type with two complements being contributed by the maternal parent and one complement by the pollen parent. In *I. munzii* $3n=60$.

The first division of the primary endosperm nucleus apparently follows very shortly after the fusion of the $2n$ polar nucleus with the second male nucleus. Ninety-six hour material which first showed pollen tubes in the micropylar canal would usually also exhibit other ovules with the metaphase of the first endosperm division (Figure 7). After the first division the two nuclei appear to migrate to opposite sides of the embryo sac and the second division follows very shortly, so that in the ovules of a single capsule there would be those in which fertilization had not yet taken place to those having completed the second endosperm division. In iris the endosperm is at first coenocytic with cell formation not occurring, in the case of *I. munzii*, until approximately 25 days after pollination. In *I. pseudacorus* $\times$ *I. versicolor* Sawyer (1925) reports that wall formation begins to take place at 13 days.

The early endosperm nuclei divisions appear to be synchronous, but later as the nuclei become more numerous and are arranged in a thin layer of cytoplasm around the periphery of the embryo sac, the divisions occur in a wave-like series starting at the micropylar end of the cell. At times the nuclei near the young embryo will be in anaphase, those toward the center of the cell in metaphase, while those at the chalazal end are still in prophase. In *I. munzii* the divisions occur rapidly and the endosperm expands at the expense of the nucellus. In the hybrid, *I. munzii* $\times$ *I. sibirica* the rate of the nuclear divisions appear to approach that of *I. munzii* up until approximately ten days after pollination. In a few instances development in the hybrid appeared to be greatly retarded. Figure 14 shows one case of a relatively small number of endosperm nuclei still occupying the center of the embryo sac 21 days after pollination. Cases such as this were, however, exceptional.

As mentioned earlier, the number of nucleoli or nucleoli-like bodies was observed to vary in the nuclei during the development of the female gametophyte. Figure 6 shows two polar nuclei, one of which has a single nucleolus, while the other one apparently has three. On occasion four nucleoli were clearly evident after fusion of the two polars. One of the most conspicuous characteristics of the endosperm nuclei of both the *I. munzii* $\times$ *I. munzii* and the *I. munzii* $\times$ *I. sibirica* material was variation in the size and number of nucleoli present. In the *I. munzii* material the number varied from three to as many as fifteen per nucleus. Scott (1944) reported very much the same thing in the endosperm nuclei of *Echinocystis macrocarpa*. There she found that the nucleoli varied in size, in consistency and in number and she graded them as small, intermediate or large. The small ones usually had the conventional spherical or elliptical outline but the larger ones, according to Scott, often displayed fantastic forms. The same variation in size, and form was observed in the iris material studied here. In the developing ovules of *I. pseudacorus* $\times$ *I. versicolor*

Fig. 11, subnormal cellular endosperm in the hybrid *I. munzii* $\times$ *I. sibirica*, x 560; fig. 12, endosperm in *I. munzii* shortly after cytokinesis has begun, x 560; fig. 13, later stage in developing endosperm in *I. munzii* showing thickening of cell walls, x 560; fig. 14, embryo sac of *I. munzii* 21 days after pollination with *I. sibirica*, x 265. (All figures slightly reduced).

Sawyer reported that at 20 days after pollination some of the ovules showed endosperm nuclei that appeared normal except for the number of nucleoli present. According to her figure the abnormal nuclei were very similar in appearance to those found here in the normal *I. munzii* $\times$ *I. munzii* and the *I. munzii* $\times$ *I. sibirica* endosperm.

Figure 12 shows the endosperm of *I. munzii* shortly after cell formation has begun. The cells toward the periphery tend to be more or less hexagonal and usually contain a single rather large nucleus although occasional cells are found which are binucleate. Once cellular development has begun it takes place rather rapidly. At the time when the first cell walls are laid down the process occurs around the entire periphery of the cell. However, after the first few cell layers are established, divisions appear to be more rapid at the micropylar end of the ovule and the young embryo is surrounded by cellular endosperm some time before that near the chalazal end has become cellular. After the first few layers of cells have been established, the later ones tend to become oriented with their long axes directed inward and toward the chalaza (Figure 8). The later formed cells tend also to be larger than those first formed and the final part of the endosperm to become cellular is a cone-shaped area lying just above the hypostase. In this area the cells are irregular in size and shape and appear more loosely packed than in the rest of the endosperm. After the endosperm becomes cellular it very quickly becomes firm and is difficult to section or study. In the mature seed the endosperm is an extremely hard ivory-like white body which according to Mitchell (1930) in the case of *I. pallida* consists of hemicelluloses. She also reports that Colin and Auger found hemicelluloses to be present in *I. germanica* and *I. pseudacorus*. Figure 13 shows a section of partially mature endosperm. Thickening of the cell walls is clearly evident.

ENDOSPERM DEVELOPMENT IN HYBRID

Iris sibirica has 28 somatic chromosomes, thus in the hybrid *I. munzii* ($\varnothing$) $\times$ *I. sibirica* (σ) the embryo has 34 chromosomes while the endosperm contains 54. As reported in an earlier section, there is apparently little difference in the rate of pollen tube growth in the styles of *I. munzii* between *I. munzii* pollen and pollen from *I. sibirica* since pollen tubes were found in the micropylar canal at about the same time. However, there was never as high a percentage of fertilized ovules in the *I. munzii* $\times$ *I. sibirica* capsules as there was in *I. munzii* $\times$ *I. munzii* capsules even though an abundance of *I. sibirica* pollen was placed on the styles of *I. munzii*.

Fusion of the male nuclei of *I. sibirica* with the egg cell and the polar nucleus of *I. munzii* was not observed, but material fixed at 5 and 7 days after pollination showed divisions of the endosperm nuclei as well as pro-embryos. The early endosperm divisions appeared to be normal although the total number of nuclei present in the hybrid endosperm appeared, in some instances, to be fewer than in the corresponding *I. munzii* material.

At approximately ten days after pollination certain irregularities in the endosperm nuclear divisions were clearly evident in the hybrid material. In the 20 and 26 day old material these nuclear irregularities were fairly common in some ovules while in others they were lacking or infrequent. The photomicrographs in Figures 19-26 show the appearance of some of the nuclei. A series of *I. munzii* divisions (Figures 15-18) are included for comparison. All material was fixed and handled in exactly the same manner and all photographs were made at the same magnification. In some

anaphase separations there appeared to be connections which might be interpreted as chromatin bridges. One of these connections is shown in Figure 23. In this same dividing nucleus there were three other similar connections on different planes and therefore invisible in the photograph. Whether these connections are true bridges such as Brock (1954) has shown to be the case in interspecific hybrids in *Lilium*, or whether they are caused by chromosome stickiness it is not possible to say. Lagging chromosomes or fragments are also seen in some divisions. One of the most conspicuous features found in the endosperm of many of the hybrid ovules is the presence of "giant" nuclei. Two of these are shown in Figures 27 and 28. The great size that some of these nuclei attain can be seen in Figure 28 which shows a single giant nucleus beside a portion of an embryo. In still other instances peculiar dumb-bell shaped nuclei are present often associated with others that are variously lobed. In no instance were these irregularities found in the *I. munzii* $\times$ *I. munzii* material.

Another difference noted between the endosperm nuclei of the hybrid and *I. munzii* was that of size. Figure 29 shows *I. munzii* endosperm 25 days after pollination and just at the time that it was beginning to become cellular. Figure 30 shows the hybrid endosperm in 26-day old material. A measurement of the diameter of 150 nuclei from both the hybrid and *I. munzii* endosperm gave the following results: *I. munzii* $\times$ *I. munzii*, average 25.4 μ ; *I. munzii* $\times$ *I. sibirica*, average 16.4 μ . It seems entirely possible that at least a part of this size difference can be associated with differences in chromosome numbers of the two, i.e. *I. munzii* with 3 $n=60$ and *I. munzii* $\times$ *I. sibirica* with 3 $n=54$.

In approximately twenty-five days after pollination the *I. munzii* endosperm begins cytokinesis and, as mentioned earlier, in the next few weeks it becomes a firm white body entirely filling the central portion of the ovule. In no instance did the hybrid ovules reach such a stage of development. Very occasionally one would be found which contained a small amount of what appeared to be a rather soft endosperm usually located in the micropylar end of the seed and surrounding the embryo. Figure 11 shows a section of the endosperm found in an ovule of the hybrid seven weeks after pollination. Figures 11 and 12 are both shown at the same magnification. It was not possible to show a section of *I. munzii* endosperm of the same age because it becomes too hard to section before that length of time. In the endosperm of the hybrid it can be seen that the cells are irregular in both size and shape compared to the twenty-five day old *I. munzii* endosperm. It is impossible to say what differences there may be in the nuclei, since usually as endosperm matures, the nuclei tend to deteriorate. Nevertheless the size differences of the nuclei within the hybrid endosperm are conspicuous. Of all the ovules of this cross that were examined, not a single one was found which contained a firm normal appearing endosperm.

EMBRYO DEVELOPMENT

As mentioned earlier, one of the purposes of the present investigation was to determine the relative growth rate of the iris embryo compared to the endosperm. This information would be useful in determining at approximately what age the embryos would be large enough so that they might be successfully cultured by means of the excised embryo technique. It would appear that in iris, as in the case of a great many other plants, the endosperm develops rapidly following the fusion of the male nucleus with the two already fused polar nuclei and that the embryo does not develop to any great extent until after the endosperm has become fairly abundant. Ten days after pollination embryos of *I. munzii* are usually in about the 8-cell stage. Figures 31, 33, 35, and 37 show embryos at 10, 25, 30, and 36 days after

Fig. 27-28, endosperm nuclei in the hybrid showing giant nuclei, x 350; fig. 29, endosperm nuclei in *I. munzii* before cytokinesis, x 350; fig. 30, endosperm nuclei in the hybrid, x 350.

pollination. Even at 30 days after pollination the embryo is still a relatively small body but shortly thereafter there appears to be a rapid acceleration in its growth. Once this acceleration begins it continues, and by the end of eight weeks the embryo is 1-1.5 mm. in length and capable of independent growth when removed from the ovule and placed on nutrient agar.

Embryos of the hybrid appear to grow and develop in very much the same manner as those of *I. munzii* with the exception that at least some of them appear to be slower in growth. Figure 35 shows an *I. munzii* embryo 25 days after pollination and Figure 34 shows a hybrid embryo 26 days after pollination. Figure 37 shows an *I. munzii* embryo at 36 days, while Figure 36 shows a hybrid embryo at 49 days. The growth of the hybrid embryos continue in ovules in which the endosperm has not degenerated entirely and the final stage of growth which the embryo reaches appears, in this cross, to be determined by how far the endosperm has developed. In Figure 36 the embryo is surrounded by endosperm that is still non-cellular but the embryo itself, except for being somewhat small, appears to be entirely normal. From the number of seedlings which have been grown by use of the excised embryo technique it is abundantly evident that at least some of the embryos resulting from this cross reach the stage of development where they can continue growth *in vitro* under favorable conditions.

No mitotic irregularities were ever seen in nuclear divisions in the hybrid embryos.

DISCUSSION

In the cross *I. munzii* $\times$ *I. sibirica* the cause of failure of the seeds to reach normal maturity appears to be a failure of the endosperm with the result that many embryos perish due to lack of nourishment. As pointed out by Brink and Cooper (1947) this type of failure is common in interspecific hybrids in many genera. In studying the endosperm of the hybrid *Avena strigosa* $\times$ *A. fatua* Kihara and Nishiyama (1932) reported giant nuclei, irregular mitosis, nuclei fusing and degenerating endosperm. Cooper and Brink (1944) report chromosome bridges, dumb-bell shaped nuclei and highly polyploid nuclei in the endosperm of the hybrid *Hordeum jubatum* $\times$ *Secale cereale*. Brock (1954) in studying abnormal endosperm mitosis in *Lilium regale* and the hybrid 'Phyllis Cox' was able to show that the abnormalities appear to commence with single chromatid bridges and in 'Phyllis Cox' these abnormalities accumulated, resulting in the formation of multiple bridges, ring chromosomes, fragments, dicentric chromosomes and micronuclei. Failure of anaphase separation followed by restitution resulted in the formation of highly polyploid nuclei in which mass spontaneous breakage occurred. Examination of dividing endosperm nuclei of *I. munzii* $\times$ *I. sibirica* showed what appeared to be chromatin bridges along with fragments or lagging chromosomes, lobed and dumb-bell shaped nuclei and giant restitution nuclei. Usually the entire endosperm would later degenerate but in a few instances the endosperm continues to function so that a relatively small amount of subnormal cellular endosperm would be found around the embryo. As has been shown in another cross (*I. douglasiana* $\times$ *I. sibirica*), very occasionally an ovule may continue development to the point where it is capable of germination under ordinary conditions. In the above-mentioned cross several hundred flowers were pollinated over a two-year period and all shrivelled seeds and

Fig. 31, *I. munzii* embryo at 10 days, x 350; fig. 32, hybrid embryo at 26 days, x 350; fig. 33, *I. munzii* embryo at 25 days, x 265; fig. 34, hybrid embryo at 26 days, x 265; fig. 35, *I. munzii* embryo at 25 days, x 265; fig. 36, hybrid embryo at 49 days, x 265; fig. 37 *I. munzii* embryo at 36 days, x 265. (All figures slightly reduced).

chaff were planted. Out of the entire lot one single seed germinated and produced a seedling that has since been grown to maturity (Lenz, in press). In the *I. munzii* $\times$ *I. sibirica* cross embryos were excised from immature ovules and grown on by use of the embryo culture technique. Thus, in crosses between species of the *Californicae* and *Sibiricae* series of the genus, it would appear that at least some of the hybrid embryos have a potential capacity for continued growth if they are removed from the defective ovules and placed in a suitable medium. This has also been shown to be true in a number of other plants and immature embryos have been grown to maturity as reported by Brink (1944) for *Hordeum jubatum* $\times$ *Secale cereale*, by Keim (1953) for the cross *Trifolium ambiguum* $\times$ *T. hybridum*, and by Konzak, Randolph and Jensen (1951) in the case of *Hordeum sativum* $\times$ *H. bulbosum*.

According to Brock (1954) lily endosperms with uniformly distributed chromosome breakage indicative of abnormality first occurring at an early stage of development were small, while those with localized areas of chromosome breakage, indicating that the initial abnormality occurred later, were larger, while those with no abnormalities were larger still. It seems reasonable to assume that somewhat the same thing may occur in the case of the hybrid iris endosperm reported here and that the final stage which is reached in the ovule is dependent upon the rate of accumulation of the mitotic errors in the endosperm nuclei and its ultimate total collapse. This could then account for the occasional ovule which reaches the threshold of normal germinability.

SUMMARY

1. In *Iris munzii* the embryo sac is of the monosporic, 8-nucleate type and the polar nuclei are fused before the arrival of the second male nucleus.

2. Pollen tubes of *I. sibirica* ($2n=28$) grow as rapidly in the styles of *I. munzii* ($2n=40$) as pollen tubes of that species. In both instances fertilization takes place not earlier than 96 hours after pollination and it may be delayed as late as 120 hours.

3. In *I. munzii* the endosperm ($3n=60$) is at first coenocytic and becomes cellular about 25 days after pollination. Endosperm nuclear divisions are at first synchronous but later they occur in a wave-like series beginning at the micropylar end of the ovule. The number of nucleoli may vary from 3 to 15 per nucleus. Once cytokinesis commences it proceeds rapidly and in the mature ovule the endosperm is a massive storage organ surrounding the embryo.

4. In the hybrid, *I. munzii* $\times$ *I. sibirica*, the endosperm ($3n=56$) divisions appear at first to be normal but by 10 days after pollination mitotic irregularities are sometimes found. By 26 days after pollination some ovules display numerous irregularities such as sticky chromosomes or chromatin bridges, lagging chromosomes and/or fragments, lobed, dumb-bell shaped nuclei and giant restitution nuclei. In the majority of cases the endosperm later breaks down entirely. However, in a few instances development continues and there is a small amount of subnormal cellular endosperm formed at the micropylar end of the ovule. In no instance is a firm white endosperm found such as is present in mature seeds of *I. munzii*.

5. In *I. munzii* embryo development is at first slow and 10 days after pollination they are in about the 8-cell stage. About 30-35 days after pollination the embryos start a rapid development and by the end of 8 weeks are about 1-1.5 mm. long. Early development of the hybrid embryos is the same as those of the species except that in some instances they may be slower growing. No mitotic irregularities were ever observed in the hybrid embryos which appear to continue development as long as there has not been a complete breakdown of the endosperm. No evidence was found of any excessive or abnormal growth of maternal tissue.

6. Immature embryos were removed from some of the developing ovules of the hybrid and grown on by use of the embryo culture technique. The hybrid seedlings are vigorous and healthy. No viable seeds were obtained when the capsules were allowed to remain on the plants until maturity.

LITERATURE CITED

1. Brink, R. A. and Cooper, D. C. 1947. The endosperm in seed development. *Bot. Rev.* 8: 423-541.
2. Brock, R. D. 1954. Spontaneous chromosome breakage in *Lilium* endosperm. *Ann. Bot.* n.s. 18: 7-14.
3. Cooper, D. C. and Brink, R. 1944. Collapse of seed following the mating of *Hordeum jubatum* $\times$ *Secale cereale*. *Genetics* 29: 370-390.
4. Dahlgren, K. V. O. 1939. Endosperm und Embryobildung bei *Zostera marina*. *Bot. Notis.* 1939 pp. 607-615.
5. Guignard, I. 1899. Sur les antherozoides et la double copulation sexual chez les vegetaux angiosperms. *Compt. Rend.* 128: 864-871.
6. Keim, W. F. 1953. Interspecific hybridization in *Trifolium* utilizing embryo culture techniques. *Agron. Jour.* 45: 601-606.
7. Kihara, H. and Nishiyama, I. 1932. Different compatibility in reciprocal crosses of *Avena*, with special reference to tetraploid hybrids between hexaploid and diploid species. *Jap. Jour. Bot.* 6: 245-305.
8. Konzak, C. F., Randolph, L. F., and Jensen, N. F. 1951. Embryo culture of barley species hybrids. *Jour. Hered.* 42: 124-134.
9. Lenz, L. W. 1954. The endosperm as a barrier to intersectional hybridization in *Iris*. *El Aliso* 3: 57-58.
10. ———. 1956. Two new and unusual iris hybrids. *El Aliso* (in press).
11. Maheshwari, P. 1950. An introduction to the embryology of angiosperms. 453 pp. McGraw-Hill Book Co.
12. Mitchell, E. M. 1930. A microchemical study of hemicelluloses of endosperm and cotyledons. *Am. Jour. Bot.* 17: 117-138.
13. Sawyer, M. L. 1925. Crossing *Iris pseudacorus* and *I. versicolor*. *Bot. Gaz.* 79: 60-72.
14. Scott, F. M. 1944. Cytology and microchemistry of nuclei in developing seed of *Echinocystis macrocarpa*. *Bot. Gaz.* 105: 329-338.