EMBRYO DEVELOPMENT AND CROSSABILITY BARRIERS IN WHEAT-RYE HYBRIDS

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Sharon Lynne Keyworth

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ABSTRACT

The causes for poor seedset, inviable seed and large variations in success in crossing particular wheat genotypes with rye were investigated using 2 hexaploid and 3 tetraploid wheats.

A crossability barrier prior to egg cell fertilization was indicated by poor kernel set in the hexaploid Sonora wheat X rye line. This was likely due to one or more Kr genes in the dominant form. Chinese Spring which produced a high seedset has both Kr genes in the recessive form when pollinated with rye.

Tetraploid wheat, in contrast to hexaploid wheat, when crossed with rye expressed a barrier at the time of embryo and endosperm differentiation rather than at the pre-fertilization period. The barrier manifested itself in a lack of embryo differentiation, aberrant endosperm nuclei, endosperm failure and a cessation of embryo volume growth. The tetraploid wheat-rye fertilized ovules generally appeared to cease developing by 10 days, whereas, those of the hexaploid wheat-rye hybrids were still slowly growing at 18 days.

INTRODUCTION

One of the limiting factors in synthesizing new amphiploids of triticale (X Triticosecale Wittmack) is the low survival rate of hybrid embryos. Moss (1970) and Kaltsikes (1974) have both stated that higher seed viability in vivo can be obtained from crosses involving hexaploid wheat (Triticum aestivum L. em Thell.) and rye (Secale cereale L.) than from crosses involving tetraploid wheat (Triticum turgidium L.). In tetraploid wheat-rye crosses the embryo frequently aborts at 12 to 15 days post-fertilization unless it is cultured on artificial media, and even then the percentage of normal seedlings produced is frequently small (Taira and Larter, 1977a; 1977b). This presents problems in introducing a wide and diverse population of tetraploid wheats into a triticale breeding program. As well, there is often difficulty in obtaining sufficient seedset in wheat-rye crosses, particularly when using hexaploid bread wheat (Krolow, 1970; Pienaar and Marais, 1976; Prabhahara Rao, 1968).

The crossability of wheat and rye is controlled by two genes; Kr1 located on the long arm of chromosome 5B, and Kr2 probably situated on chromosome 5A (Riley and Chapman, 1967; Lange and Riley, 1973). Lange and Wojciechowska (1976) found

the crossability barrier as controlled by the Kr gene complex to retard and eventually inhibit pollen tube growth in the stylar tissue of hexaploid wheat. Moss (1970) stated that a different barrier exists in the tetraploid wheat-rye crosses which is expressed via endosperm failure and a lack of embryo development.

Although Bennett et al (1973, 1975), Kaltsikes (1973) and Wojciechowska and Lange (1977) have studied early embryo development in wheat, rye and wheat-rye hybrids, few workers have examined embryo development in these species past the 5 day post-fertilization period.

The present study was undertaken to examine and describe the development and growth of hybrid embryos derived from crosses between wheat and rye over an 18-day period. Two criteria were used in the comparative study: (1) embryos from hexaploid wheat-rye crosses were compared to those produced from tetraploid wheat-rye crosses to investigate the reasons for higher embryo viability in the former, and (2) to locate the stage of development at which the cross-ability barriers occur in these two types of crosses.

LITERATURE REVIEW

Wheat-Rye Crossability

The success of crossing wheat with rye varies greatly between genera, species, subspecies and varieties. Tetraploid wheats were found to cross more readily with rye than were hexaploid wheats. Krolow (1970) obtained a mean seedset of 13.36% for tetraploid wheat-rye hybrids compared to a mean seedset of 4.96% for hexaploid wheat-rye hybrids. Pienaar and Marais (1976) reported a similar trend with a mean seedset of 45.38% for tetraploid wheat-rye hybrids and 24.34% for hexaploid wheat-rye hybrids.

Riley and Chapman (1967) stated that within the hexaploid wheats the central Chinese varieties crossed more readily with rye than the European varieties. Pienaar (1973) found a mean seedset of 34.4% for 4 Triticum aestivum sphaerococcum varieties as compared to a mean seedset of 3.9% for 10 Triticum aestivum vulgare varieties. The same pattern was observed by Pienaar and Marais (1976). Prabhahara Rao (1968) noted a generally low seedset for all of the bread wheats of various origins tested except for Chinese Spring, and possibly two Indian varieties.

The ability of tetraploid wheats to cross with rye also

reflects differences between species, subspecies and varieties. Papers by Pienaar (1973), Pienaar and Marais (1976), Moss (1970) and a summary by Kaltsikes (1974) show diversity in mean seedset between subspecies. Krolow (1970) reports variation between varieties within a subspecies.

The ability of wheat to cross with rye depends on at least two genes designated as Krl and Kr2. Chinese Spring, a good combiner with rye, is believed to have Krl and Kr2 in the recessive form while Hope, a poor combiner, is thought to have Krl and Kr2 in the dominant form. Using Chinese Spring - Hope substitution lines, Riley and Chapman (1967) located Krl on chromosome 5B and Kr2 probably on chromosome 5A. Lange and Riley (1973) further established Krl to be on the long arm of chromosome 5B. Of the two genes, Krl is the more effective inhibitor.

With the use of Chinese Spring/Hope 5B substitution lines, Lange and Wojciechowska (1976) discovered the Kr genes crossability barrier in hexaploid wheat to be due to "retardation and eventually inhibition of the pollen tube growth at the style base and in the transmitting tissue of the ovary wall".

It is believed that the Kr genes are more significant in hexaploid wheat-rye hybrids than in tetraploid wheat-rye hybrids (Moss, 1970; Riley and Chapman, 1967). According to Krolow's (1970) classification the number of dominant Kr genes in a variety is expressed as the percentage seedset; i.e., Krl Krl Kr2 Kr2, Krl Krl kr2 kr2, krl krl Kr2 Kr2 and krl krl kr2 kr2

yield 0 to 10%, 10 to 30%, 30 to 50% and 50% or greater seedset respectively. Using that classification, Kaltsikes (1974) in a summary of the literature discovered that more than 75% of the hexaploid wheats reported had the Kr1Kr1Kr2Kr2 condition as compared to 41% for the tetraploid wheats. Riley and Chapman (1967) suggested that this gene barrier may have developed because it conferred agricultural and evolutionary advantage to the hexaploid wheats by preventing the production of sterile wheat-rye hybrids which would act as weeds within the crop.

Hexaploid wheat-rye crosses produce viable seeds more frequently than do tetraploid wheat-rye crosses. Krolow (1970) stated that the mean germination capacity of the tetraploid wheat-rye hybrids was 1.16% and the mean germination capacity of hexaploid wheat-rye hybrids was 61.38%. Moss (1970), Pienaar and Marais (1976), Pienaar (1973) and May (1977) found confirming trends.

Moss (1970) described a crossability barrier which manifests itself particularly in tetraploid wheat-rye hybrids. It is expressed in aberrant endosperm nuclei, endosperm failure and consequent inviable seed. The cause of this barrier is not known. Lange and Wojciechowska (1976) suggest that it may be an expression of the Kr genes at another site. Experiments by Krolow (1970, 1973) and Pienaar and Marais (1976) showed that when tetraploid wheat with an added A or D genome is crossed with rye, viability

and germination of the seed was raised to a level comparable to that of a hexaploid wheat-rye hybrid. Hsam and Larter (1974) found the incorporation of hexaploid cytoplasm into tetraploid wheat which was later crossed with rye, resulted in embryo development and hybrid plant vigor similar to the hexaploid wheat-rye hybrid.

The Embryo Sac Prior to Fertilization

The embryo sac prior to fertilization has been described for wheat, rye and triticale (Bennett et al, 1973, 1975; Bennett, 1973; Kaltsikes, 1973; Wojciechowska and Lange, 1977; Bhatnagar and Chandra, 1977; Morrison, 1955; and Hakansson, 1946).

Before fertilization the embryo sac consists of an egg apparatus in the micropylar end, a central cell in the upper middle section and a varying number of antipodal cells in the chalazal end. The egg apparatus is comprised of three pyriform cells, the larger egg cell flanked by two smaller synergid cells. The central cell is larger than the egg cell. It contains two bilaterally symetrical polar nuclei and a band of cytoplasm stretching from the egg cell to the nearest antipodal cell. The antipodal cells take up more than 50% of the embryo sac volume and their numbers are genotypically determined.

Fertilization and First Mitosis

Fertilization of the egg cell has been studied in wheat by Bennett et al (1973), Wakakuwa (1934), Batygina (1974), Morrison (1955), Wojciechowska and Lange (1977) and Hoshikawa (1959); in rye by Hakansson and Ellerstrom (1950); and in wheat-rye hybrids by Wojciechowska and Lange (1977). After the two sperm nuclei entered the embryo sac, one migrated to the egg cell, penetrated the egg cell membrane and moved through the cytoplasm to the egg nucleus. After the sperm nucleus entered the egg nuclear membrane it enlarged and became diffuse (Bennett et al 1973; Wojciechowska and Lange 1977; Batygina 1974). There was then a resting stage preceding mitosis. Morrison (1955) and Hakansson and Ellerstrom (1950) noted that at prophase the chromosomes of the sperm and egg were indistinguishable. Bennett et al (1973) reported the sperm nucleus to be diffuse but still discernible from the egg cell at 11 hours after pollination. At metaphase all the chromosomes oriented themselves on one spindle and division proceeded normally. The times recorded for fertilization and first mitosis varied with the temperature and genotype. Table 1 summarizes the data available.

Bennett el al (1973) found that the time required for the pollen tube of wheat to enter the embryo sac and for first mitosis to be completed was temperature dependent. In plants grown at 15^{o}C, pollen tube growth and first mitosis

TABLE 1. Events of fertilization and first mitosis in wheat, rye and triticale, the time of their occurrence at specific temperatures as reported by various authors

Event	Species	Temp. (°C)	Time after pollination	References
Pollen tube in embryo sac	Wheat	20	41 min	Bennett et al (1973)
		25	30	" " " "
		15	70	" " " "
		-	15-20	Batygina (1974)
		-	6 hrs	Morrison (1955)
		20	15 min	Hoshikawa (1959)
	Rye	20	40 min	Bennett et al (1975)
	Triticale	21	4 hrs	Kaltsikes (1973)
		20	40 min	Bennett et al (1975)
Fusion of egg & sperm	Wheat	field	15 hrs	Wakakuwa (1934)
		field	10-16	Bhatnagar & Chandra (1977)
		18-25	1-2	Wojciechowska & Lange (1977)
		23	18-20	Bennett et al (1973)
		20	3-5	Hoshikawa (1959)
	Rye	field	6-10 hrs	Hakansson & Ellerstrom (1950)
	Triticale	21	10-15 hrs	Kaltsikes (1973)
		field	15	Wakakuwa (1934)
		18-25	>2	Wojciechowska & Lange (1977)
First mitosis	Wheat	23	24 hrs	Bennett et al (1975)
		-	24	Batygina (1974)
		-	1-2 days	Morrison (1955)
		18-25	>24 hrs	Wojciechowska & Lange (1977)
	Rye	20	26 hrs	Bennett et al (1975)
		field	48	Hakansson & Ellerstrom (1950)
	Triticale	21	25-35 hrs	Kaltsikes (1973)
		20	<24	Bennett et al (1975)
		18-25	>24	Wojciechowska & Lange (1977)

was retarded compared to the same events at 20°C. Pope (1943) also discovered that the time of fertilization in barley was temperature dependent.

Embryo Growth Measurements

Early embryo development can be measured by an increase over time of cell numbers, cell volume, and embryo volume. Most papers report only cell numbers versus time which seldom go beyond 5 days due to the difficulty in obtaining an accurate count. Bennett et al (1975) used embryo cell number, cell volume and embryo volume to study embryo growth in the Triticeae.

A relationship between cell volume, cell numbers and embryo volume was noted by Bennett et al (1975). Cell number and embryo volume both increased with time, however, embryo volume made a noticeably slower start. This difference is explained by a sharp decrease in cell volume that occurred for the first 3 days and then a subsequent levelling off. Thus, even though the embryo was growing rapidly in cell numbers for the first three days, the embryo volume did not expand appreciably due to the concomitant decrease in cell volumes. When the cell volume reached equilibrium, the embryo volume expansion became proportional to cell number growth.

Embryo growth rates differ between and within a species. Both Bennett et al (1975) and Boyes and Thompson (1937) reported rye embryo development to be slower than that of

wheat. Wide variation in the rate of cell number growth can be seen in the embryos of the triticale lines as reported by both Kaltsikes (1973) and Bennett et al (1975). The four wheat genotypes used by Bennett et al (1975) showed that embryo growth rate varied with ploidy level.

Wojciechowska and Lange (1977), Boyes and Thompson (1937) and Cooper and Brink (1944) studied intergeneric hybrids and reported embryo cell number growth rates which were in contradiction. Wojciechowska and Lange (1977) found the wheat-rye hybrid embryos to develop faster than those of the maternal parent. However, Boyes and Thompson's (1937) study on wheat-rye hybrids and Cooper and Brink's (1944) experiment with barley-rye hybrids both indicated that the intergeneric hybrid embryos started slower and usually lagged behind the development of the embryos of the maternal parent. Bennett et al (1975) using disomic addition lines of rye chromosomes in wheat discovered that all rye chromosomes except number III (2R) reduced the rate of embryo cell development in the hybrid.

Discrepancies in growth rates found in the data of various studies can probably be explained by differing environmental conditions. Bennett et al (1973) noted that the duration of the division cycle of the embryo cells of wheat cv. Chinese Spring was longer when grown at 15°C than at 20°C. Using barley, Pope (1943) discovered embryo growth rate to be temperature dependent. Taira and Larter

(1977b) reported that normal embryo development and viability of wheat-rye hybrids were influenced by temperature and other environmental conditions. They found that an optimum temperature of 17°C resulted in maximum embryo development.

For wheat, Morrison (1955), Wakakuwa (1934) and Wojciechowska and Lange (1977) obtained comparable results in terms of embryo cell number but Boyes and Thompson (1937) and Bennett et al (1975) reported a faster growth rate than that reported by other workers. Morrison (1955) and Boyes and Thompson (1937) gave no indication of the environmental conditions under which their experiments were conducted, while Wakakuwa's (1934) experiment was conducted in the field. A growth room at 20°C with continuous light was employed in Bennett et al's (1975) experiment. Wojciechowska and Lange (1977) using a temperature range of 18-25°C in a glasshouse with natural light, pointed out that their discrepancies with Bennett et al's (1973, 1975) results were probably due to environmental differences.

The embryo cell numbers versus time for triticale lines reported by Kaltsikes (1973) and Bennett et al (1975) are in reasonable agreement. The growing conditions of Kaltsikes' (1973) experiment--21°C in a greenhouse with 16 hours of light--were similar to those used by Bennett et al (1975).

Embryo Differentiation

Brown (1960) described the stages of differentiation in

the grass embryo. The spherical to club-shaped proembryo develops a notch on one side. The area above the notch expands to form the coleoptile and the area below produces the shoot apex. The base of the coleoptile extends until it completely surrounds the shoot apex. The scutellum elongates rapidly from the tissue adjacent to the coleoptile. At the same time, the primary root develops endogenously, with the external tissue becoming the coleorhiza. An outgrowth of the coleorhiza called the epiblast appears in Triticum, Hordeum and Avena species but not in Secale (Reeder 1957).

Boyes and Thompson (1937) noted the time that these anatomical developments take place in wheat, rye and wheat-rye hybrid embryos. In 7-day-old Triticum aestivum embryos the coleoptile is beginning to grow around the stem apex and the root is starting to extend. At 9 days the epiblast is forming and the scutellum is elongating. The second foliage leaf and the lateral rootlets are present at 14 days. By 21 days the embryo is mature. The development of young embryos of durum wheat is slow relative to those of hexaploid wheat. After 14 days, however, embryos of the two species are comparable in size. Secale cereale embryo differentiation and growth is slower and more varied when compared to T. vulgare. Embryo differentiation in wheat-rye hybrids is slower than in the maternal wheat parent. At 21 days the hybrid embryos are usually normally differentiated but smaller

than those of the parental wheat.

Descriptions of embryo differentiation versus time are generally scarce in the literature. Cooper and Brink (1944) in two figures illustrate embryo differentiation over time in barley and a barley-rye hybrid. The hybrid embryo appeared to differentiate normally but slower and was more elongated in shape than that of the maternal Hordeum parent. Hakansson and Ellerstrom (1950) make reference to the pattern of embryo development in rye. At 14 days the scutellum is extending beyond the embryo and at 21 days the embryo is fully differentiated. Taira and Larter (1977a) depicted by photograph the level of differentiation in wheat, rye and wheat-rye hybrid embryos at 15 days post-fertilization. The wheat and rye embryos were almost fully differentiated at 15 days with the scutellum, coleoptile, coleorhiza and (in the wheat) epiblast well developed. The wheat-rye hybrid embryos at that age were generally considerably smaller in size and poorly differentiated, or undifferentiated.

Taira and Larter (1977a) noted that the undifferentiated hybrid embryos will not form normal seedlings in vitro. May (1977) reported comparable findings in that tetraploid wheat-rye hybrid embryos which were abnormally developed produced only a small percentage of seedlings when cultured.

Aberrant Endosperm Nuclei and Endosperm Failure

Aberrant endosperm nuclei and consequent endosperm

degeneration are commonly found in intergeneric hybrids, developed triticale lines, and occasionally in the parental lines. While studying a barley-rye hybrid, Cooper and Brink (1944) observed many aberrant endosperm nuclei of differing size and shape. Endosperm growth was delayed and failed to reach cellularization in the hybrid as compared to the parental barley line. Moss (1970) always found aberrant endosperm nuclei present in tetraploid wheat-rye hybrids but existing in only approximately 10% of the hexaploid wheat-rye hybrids. The endosperm tissue of the tetraploid wheat-rye hybrids was composed of large groups of mostly hyperpolyploid nuclei. No normal endosperm was produced in these hybrids and the seed at maturity was shrivelled. Kaltsikes et al (1975), Kaltsikes and Roupakias (1975), Kaltsikes (1973), and Bennett (1973) all noted some level of aberrant endosperm nuclei in triticale lines. As well, aberrant nuclei were occasionally observed in the parental species (Kaltsikes et al, 1975; Bennett, 1973; Moss, 1970). These aberrant endosperm nuclei have been reported to first appear at the 7 - 8^{th} endosperm division (Kaltsikes et al, 1975; Kaltsikes and Roupakias, 1975), 48 hours post-fertilization (Bennett, 1973), and 12 hours post-fertilization (Cooper and Brink, 1944). They were located in groups throughout the endosperm, however, most were found near the embryo (Moss, 1970; Kaltsikes et al, 1977; Bennett, 1973).

The number of normal endosperm cells produced is

profoundly affected by the occurrence of aberrant nuclei. Kaltsikes et al (1975) showed that during the geometric cell increase period until 4 days post-fertilization, aberrant nuclei which undergo an increase in size and DNA content but which fail to divide, reduce the overall number of endosperm nuclei. This is because after 4 days, cellularization begins and the size of the cambium which gives rise to further endosperm development is limited to the number of normal endosperm cells present. After cellularization, aberrant nuclei may also poison or impede neighbouring cell development leading to pockets of necrosis in the final endosperm tissue (Kaltsikes et al, 1975; Bennett, 1973).

The occurrence of aberrant endosperm nuclei has been associated with kernel shrivelling attributable to specific rye chromosomes. Kaltsikes et al (1975) reported that "the Kendall coefficient of rank correlation between the shrivelling score of triticale, wheat and rye lines and the number of aberrants per normal nucleus was significant at the 5% level". Rye chromosomes 5R, 4R, 6R, 3R, $6R^L$ and 1R were found to have an effect on the production of aberrant endosperm nuclei (Kaltsikes and Roupakias, 1975). Darvey (1973) found that chromosomes 4R/7R, 5R and 6R of rye have a major consequence on seed shrivelling.

Kaltsikes and Roupakias (1975) suggested that it may not be rye chromosomes alone which result in aberrant endosperm nuclei but rather an interaction between both the

wheat and rye chromosome complements. Thus, in tetraploid wheat-rye crosses Taira et al (1977) demonstrated that it is the interaction of the rye and wheat genotypes which dictates the rate and extent of both embryo and endosperm development in the hybrid.

Bennett (1977) found a relationship between aberrant endosperm nuclei, kernel shrivelling and the heterochromatin bands on the rye chromosomes in triticale. The heterochromatin bands on the rye chromosomes are slow replicating and result in bridges between the products of mitosis. These bridged nuclei become the aberrant nuclei. Bennett (1977) also reported a positive correlation between aberrant nuclei and grain shrivelling.

The relationship of aberrant nuclei and consequent endosperm failure to hybrid embryo viability is not completely understood. However, as Moss (1970) noted, it seems to function as a crossability barrier between wheat and rye frequently resulting in hybrid seed inviability.

MATERIALS AND METHODS

Genotypes

The two hexaploid wheats (*Triticum aestivum* L. em Thell) used in this study were cultivars Chinese Spring, a good combiner with rye, and Sonora, a poor combiner. The tetraploid wheats (*Triticum turgidum* L.) used were cultivars Jori, U. of M. accession No. 4B925, both of which were believed to be good combiners with rye, and cultivar Cocorit, a poor combiner. The paternal rye parent used in all crosses was a self-fertile, inbred line, U. of M. accession No. 2D82-S10 (Prolific). Control studies were conducted using embryos of selfed Jori wheat and selfed 2D82 rye. The crosses between the rye line 2D82 and the wheat parents Jori, Cocorit, 4B925, Sonora, and Chinese Spring will hereafter be referred to as Jori X rye, Coco X rye, 4B X rye, Son X rye and C.S. X rye, respectively.

Growth Conditions

The maternal wheat parents were grown in pots in a controlled growth room. Growing conditions were maintained at a day/night temperature regime of 20° C/16° C for an 18-h photoperiod. Relative humidity was maintained at 61%

during the day and 65% during the night.

Preparation of Material

The maternal parents were emasculated and bagged approximately 2 days before pollen shedding. Upper and lower florets of the spike were removed, leaving only the central florets which have a more uniform development. They were pollinated at approximately 2 days after emasculation. At intervals of 1, 2, 3, 4, 6, 8, 10, 12, 14, 16 and 18 days after pollination the heads were cut from the plants, fixed in Farmer's fixative and then stored in refrigeration until required. The florets were removed from the spike, hydrolyzed for 10 minutes in 1N HCL and subsequently stained in Feulgen solution for at least 24 hours before dissection.

The dissecting was done under a Wild Heerbrugg M5 dissecting microscope. The embryo sac was teased from the ovary walls with small dissecting needles in drops of Feulgen stain. The small 1 to 6-day-old embryos were transferred to a clean slide and observed under a compound light microscope, whereas the larger 8 to 18-day-old embryos were examined under the dissecting microscope. Size measurements were recorded using an eyepiece micrometer mounted on the microscope. Photomicrographs of the smaller embryos were taken with the Zeiss Photomicroscope II while for the larger embryos, a 35 mm camera was mounted on the dissecting microscope.

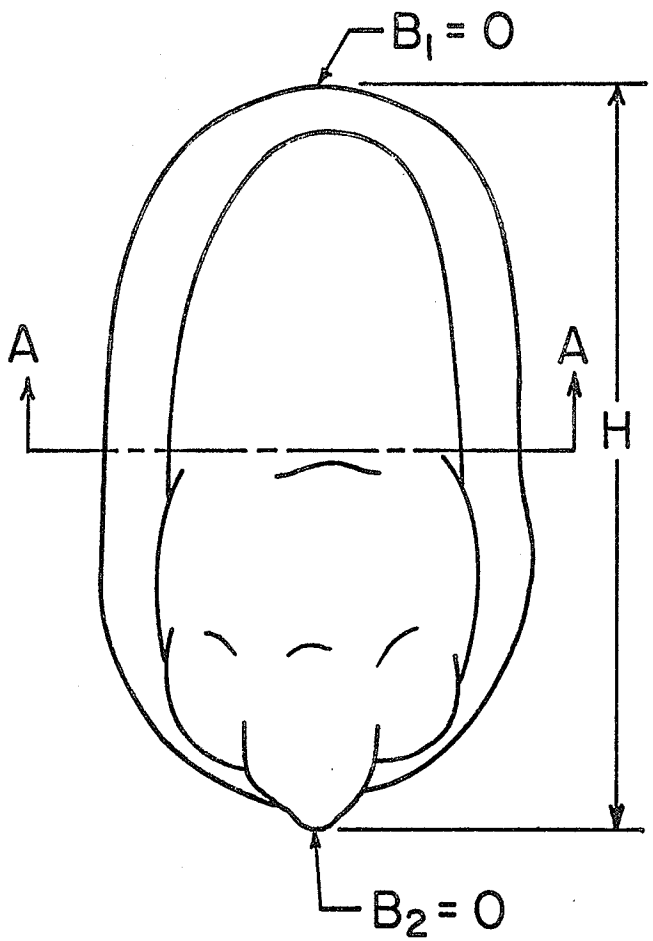
Measurements Recorded

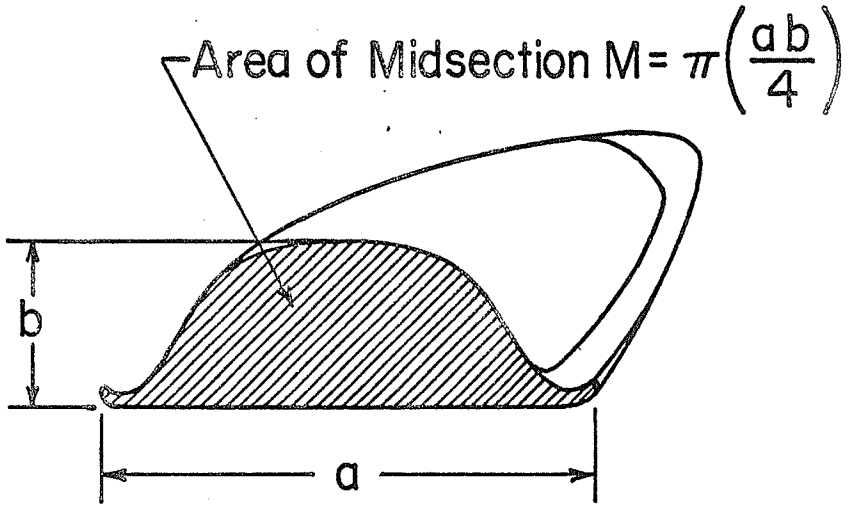
The seedset as visually observed on the spikes was recorded and calculated as a percentage of florets pollinated.

The number of cells in the embryo were counted up to the 4-day stage of development in all the embryos except the rye control embryos which were counted up to 6 days. Beyond this time, the number of cells became too numerous for an accurate count.

The embryo volume was measured up to and including 18 days for both hexaploid wheat-rye embryos and control embryos, and up to 14 days for the tetraploid wheat-rye embryos. The volume of the embryo was calculated on the basis of a sub-spherical object according to the formula $4/3\pi \left(\frac{D+d}{4}\right)^3$, where "D" is the larger diameter measurement and "d" is the diameter measurement taken at right angles to "D". This formula was fairly accurate for the determination of embryo volume, up to approximately the 8-day-old stage of growth because the embryos were approximately spheroidal. After 8 days, differentiation began and the formula applied was that for the volume of a general prismatoid where Volume = $\frac{1}{6} H (B_1 + 4M + B_2)$: "$B_1$" and "$B_2$" being the end areas, "M" the midsection area and "H" the length of the longitudinal axis. Since the embryo is pointed at both ends, "B_1" and "B_2" were taken to be zero. The midsection area was calculated by the formula; Area = $\pi \left(\frac{ab}{4}\right)$ where "a" and "b" represent, respectively, the

FIGURE 1. Measurements taken to calculate volume for differentiated embryos using the prismatoid formula (Based on the shapes of the differentiated embryo in Figure 2)





Section A - A

$$V = 1/6\, H\,(B_1 + 4M + B_2)$$

Fig. 1

cross-section diameter and height of the embryo measured at its midsection (Fig. 1). This formula was a more correct measure of the volume of the differentiated embryo because it includes the three major axes.

The volume of individual embryo cells was found by dividing each embryo volume by the number of cells in that embryo. Volume was calculated for embryos up to the 4-day-old stage in all genotypes except the rye control where it was calculated up to and including the sixth day.

Endosperm development was categorized as; 1) well developed (Fig. 3), 2) partially developed, including fragments and thin membranes (Fig. 4) and 3) no endosperm (Fig.5). The number of observations in each category was reported as a percentage.

The level of embryo differentiation was similarily recorded. The categories established were; 1) well differentiated (Fig. 6), 2) differentiated (Fig. 7), 3) slightly differentiated (Fig. 8) and 4) not differentiated (Fig. 9). The criteria used for classifying an embryo into one of these specific categories are illustrated in Fig. 6 to 9.

Photomicrographs

Photographs were used to record the first mitotic division and abnormalities, also to augment data on normal and abnormal embryo and endosperm development.

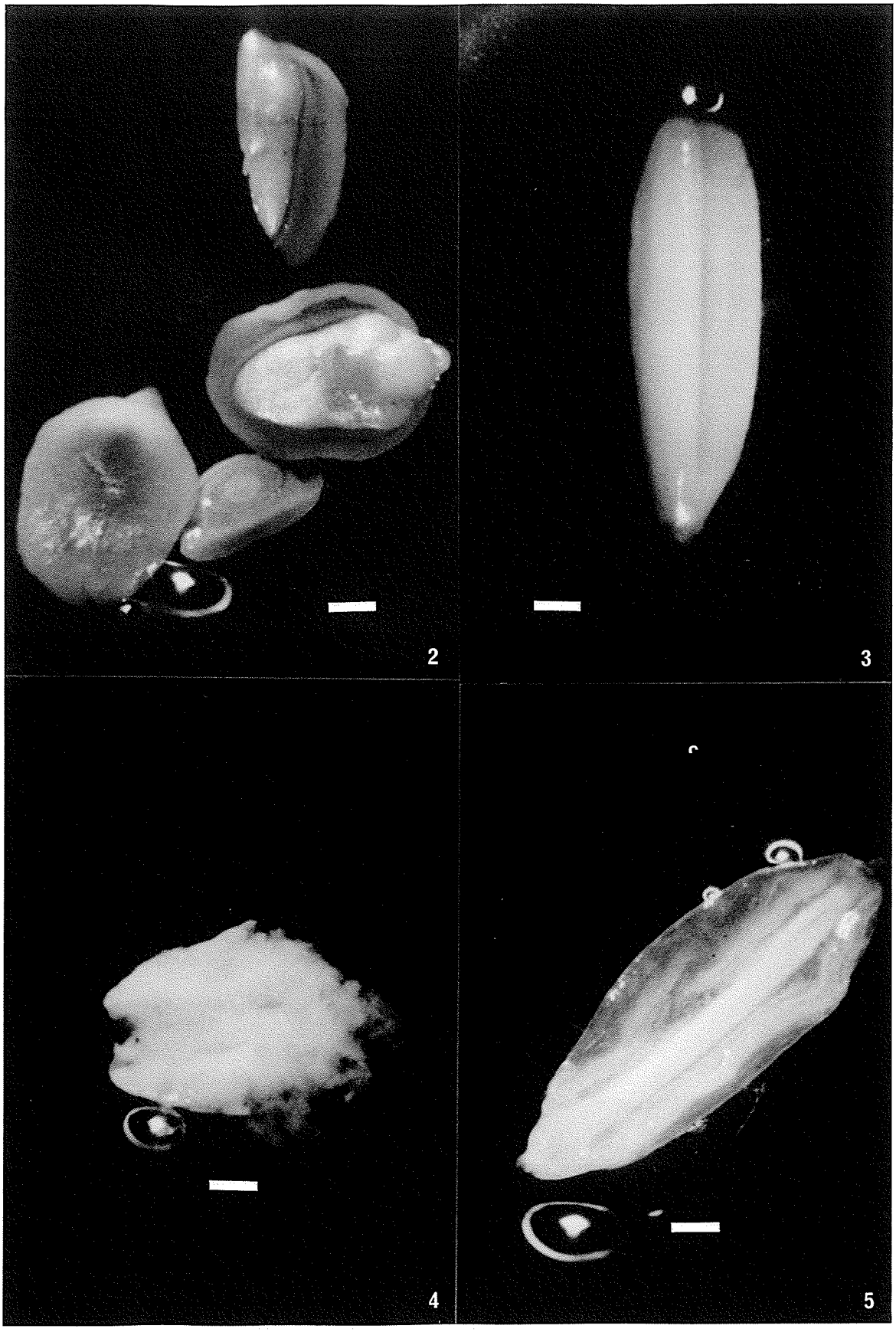
FIGURES 2-5. Fig. 2. Embryos fully differentiated (top, bottom, longitudinal and cross-sectional views) cultivar Jori 18 days

Fig. 3. Well developed endosperm 2D82 rye, 8 days

Fig. 4. Partially developed endosperm (fragmented) 4B925 X 2D82, 10 days

Fig. 5. No endosperm (exterior view of embryo sac) Cocorit X 2D82, 10 days

Bar in Fig.2-5 represents approximately 1600 μm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5

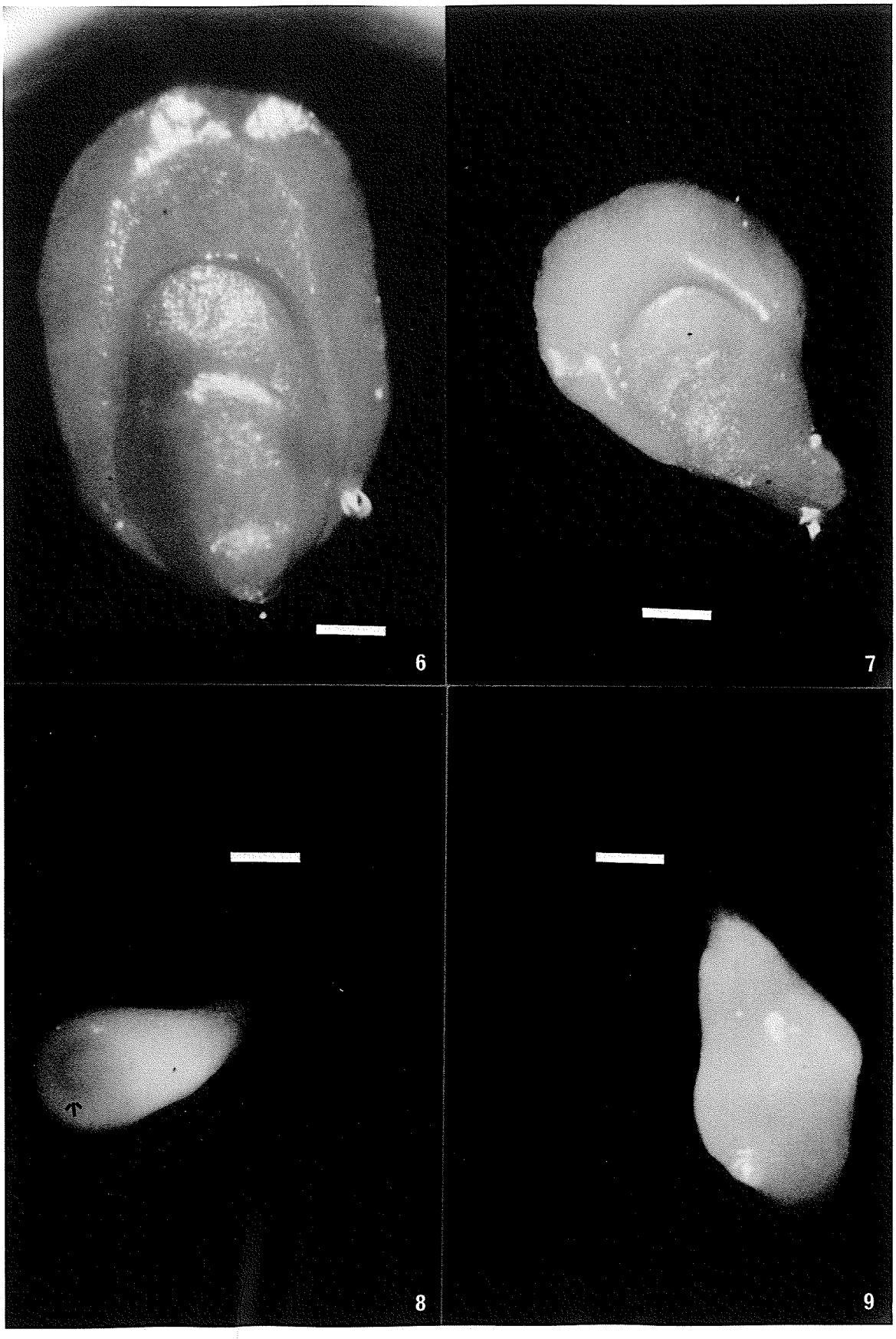
FIGURES 6-9. Fig. 6. Well differentiated embryo (scutellum, coleoptile, primary root, coleoriza, epiblast are all well developed) Jori wheat, 12 days

Fig. 7. Differentiated embryo (same parts are present as in "well developed" but they are not fully developed) Sonora X 2D82, 10 days

Fig. 8. Slightly differentiated embryo (the arrow shows the notch which separates the coleoptile and shoot apex) 4B925 X 2D82, 8 days

Fig. 9. Non-differentiated embryo (no signs of differentiation present) Jori X 2D82, 12 days

Bar in Fig. 6-9 represents approximately 200 μm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RESULTS AND DISCUSSION

Fertilization and First Mitosis

Twenty-four hours after fertilization the egg cells in the majority of the experimental material were fertilized and were undergoing first mitosis. The synergids were still present at this stage and the endosperm was multinuclear (Fig. 14).

The embryos of the three tetraploid wheat-rye crosses were at a similar stage of development at 24 hours. Their mean cell numbers were 1.54 for Jori X rye, 1.63 for Coco X rye and 1.69 for 4B X rye. A few ovules were unfertilized and some were fertilized but not yet dividing.

The hexaploid wheat-rye ovules varied in the number that were fertilized and undergoing first mitosis. The Son X rye and C.S. X rye embryos had mean cell numbers of 1.33 and 1.91 respectively. Thus, many of the fertilized Son X rye embryos were not yet dividing after 24 h. (Fig. 15). While the number of unfertilized ovules resulting from the C.S. X rye cross was low, the number from the Son X rye combination was very high. This relationship is reflected in the small number of embryos observed in the Son X rye

FIGURES 14-17. Fig. 14. First mitosis in 4B925 X 2D82, 1 day; synergids (s) and endosperm cells (ec) present.

Bar represents approximately 50 μm

Fig. 15. Fertilized egg cell in Sonora X 2D82, 1 day; the sperm nucleus (sn) is visable but the cell is not dividing.

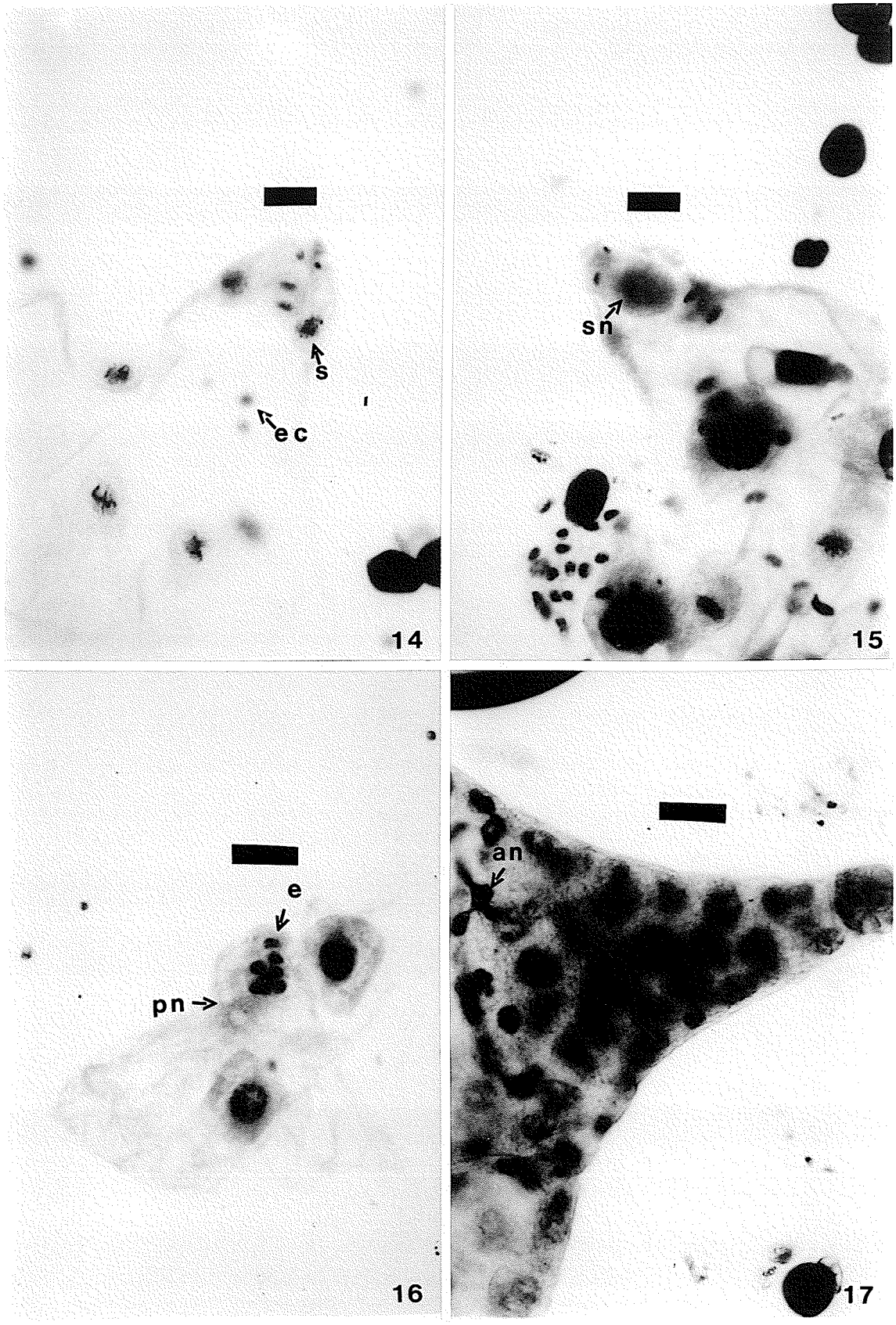
Bar represents approximately 50 μm

Fig. 16. Normally developed embryo (e) in Cocorit X 2D82, 3 days; note polar nuclei (pn) are not fertilized

Bar represents approximately 60 μm

Fig. 17. Aberrant endosperm nuclei (an) proximal to the embryo in Jori X 2D82, 4 days

Bar represents approximately 60 μm


s
ec
14
sn
15
e
pn
16
an
17

cross (Appendix Table 1).

The self-fertilized controls, Jori wheat and 2D82 rye had mean cell numbers of 1.67 and 1.00 respectively. The rye showed no signs of dividing at the 24 h. post-fertilization stage.

Of the seven genotypes, all but rye were in the process of first mitosis 24 h. after pollination. This agrees with Bennett et al's (1975) finding that rye at 24 hours has a mean cell number of 1. That neither of the hexaploid wheat-rye hybrid embryos had completed first mitosis by 24 h. agrees with the results of Wojciechowska and Lange (1977).

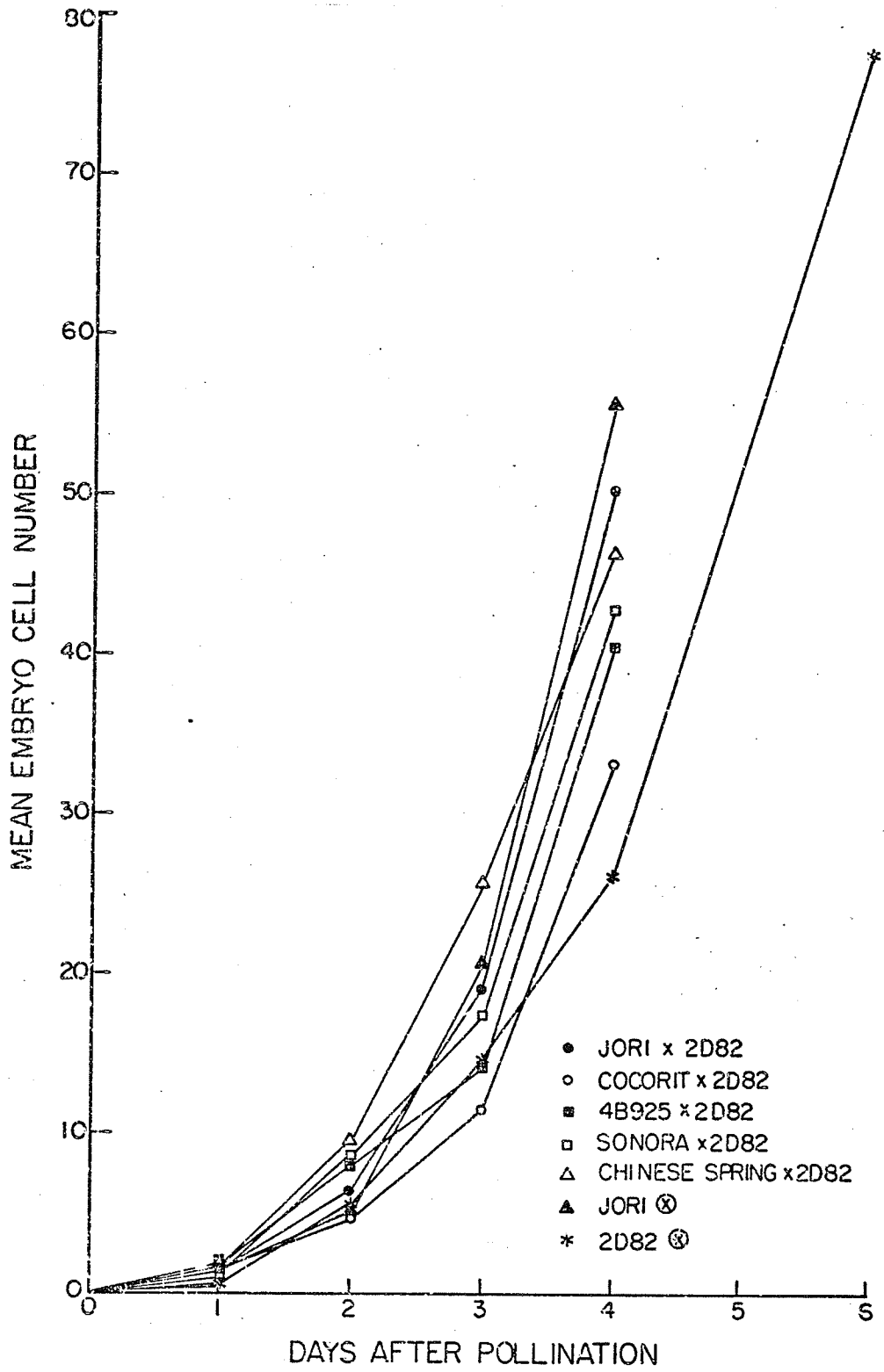
Embryo Cell Number versus Time

Embryo cell numbers were recorded for 4 days in embryos of all genotypes except the rye control which was measured up to 6 days. The cell numbers of all embryos showed an approximate geometric increase over time (Fig. 18).

Of the tetraploid wheat-rye crosses, embryo growth of Coco X rye lagged behind that of Jori X rye and 4B X rye. At 4 days Jori X rye embryos were well advanced with respect to cell numbers over other tetraploid wheat-rye embryos (Fig. 18). An abnormality was observed in a 3-day-old Coco X rye hybrid. The embryo was a normal looking 7-celled proembryo but the polar nuclei were still present and unfertilized (Fig. 16).

In the hexaploid wheat-rye crosses the C.S. X rye embryos maintained a slightly higher rate of cell division over

FIGURE 18. Mean embryo cell number versus days after pollination


MEAN EMBRYO CELL NUMBER
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
S
DAYS AFTER POLLINATION
JORI x 2D82
COCORIT x 2D82
4B925 x 2D82
SONORA x 2D82
CHINESE SPRING x 2D82
JORI ⊗
2D82 ⊗

those of Son X rye throughout the period of study (Fig. 18).

Of the controls, Jori embryos were generally faster growing than the 2D82 embryos (Fig. 18).

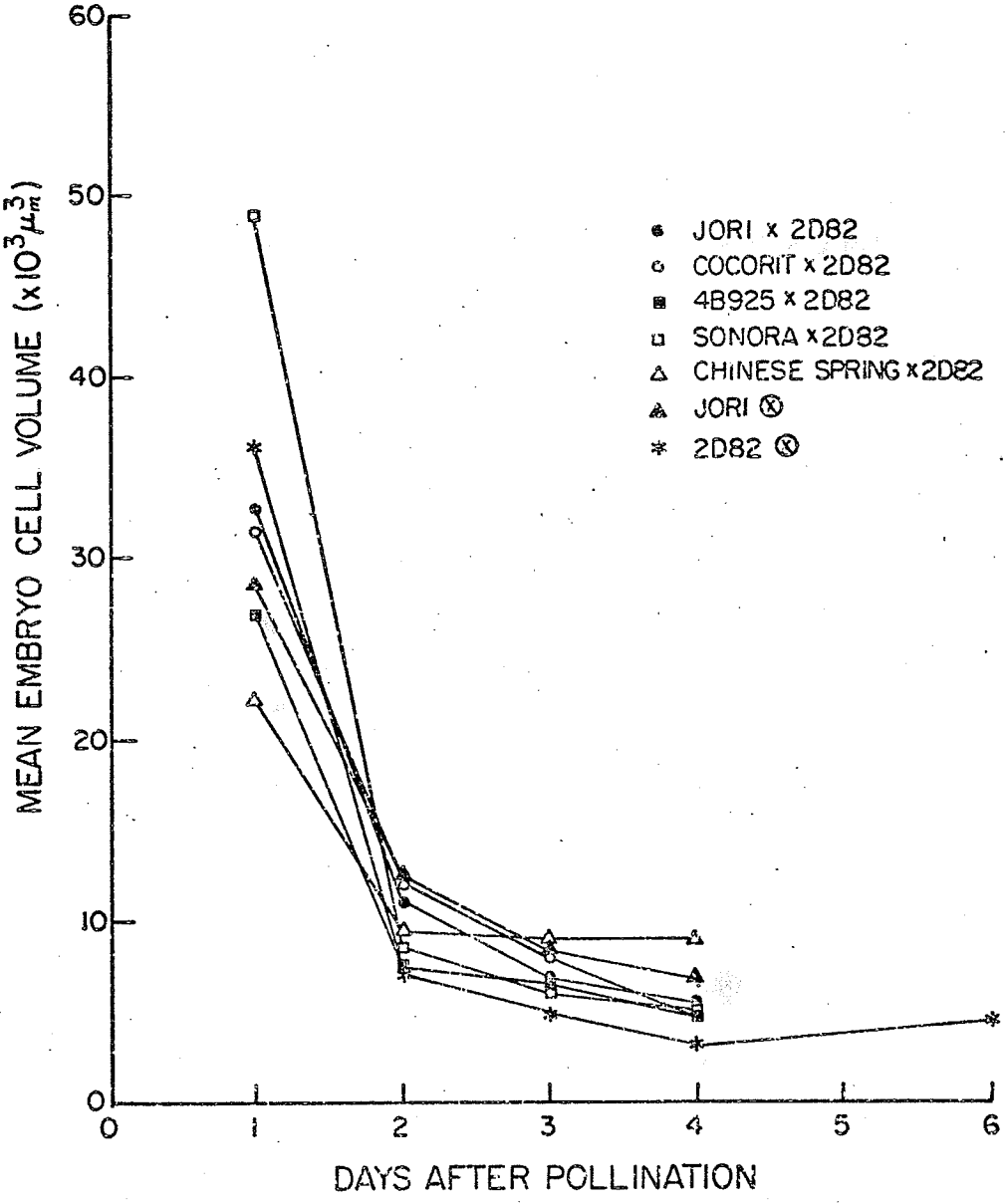
The embryos of all genotypes initially grew at a uniform rate but by 4 days some differential in rate of cell number increase existed. The control Jori (selfed) exhibited the highest cell number at 4 days. The remaining genotypes in descending order of growth rate were Jori X rye, C.S. X rye, Son X rye, 4B X rye, Coco X rye and 2D82 (selfed). On the basis of these results it did not appear that the inter-generic hybrid embryos were faster starting (Wojciechowska and Lange, 1977) or slower starting (Cooper and Brink, 1944; Boyes and Thompson, 1937) than those of the selfed maternal parent. The embryos from selfed Jori and those of the Jori X rye hybrid were very similar in cell number throughout the duration of the experiment. The smaller cell number for rye compared to wheat was in agreement with the findings of Bennett et al (1975).

Embryo Cell Volume versus Time

Cell volume of embryos of all lines decreased sharply during the first 2 days after fertilization, levelling off on approximately the third day (Fig. 19). Bennett et al (1975) found the same pattern in the Triticeae.

The tetraploid wheat-rye embryo cells had similar initial volumes and decreased uniformily until the fourth

FIGURE 19. Mean embryo cell volume ($X10^3 \mu m^3$) versus days after pollination


MEAN EMBRYO CELL VOLUME (x10³μm³)
60
50
40
30
20
10
0
0
1
2
3
4
5
6
DAYS AFTER POLLINATION
JORI x 2D82
COCORIT x 2D82
4B925 x 2D82
SONORA x 2D82
CHINESE SPRING x 2D82
JORI ⊗
2D82 ⊗

day (Fig. 19). The embryo cells of the 4B X rye hybrid had the smallest mean volumes until the 4th day.

There was wide variation in embryo cell volumes between the two hexaploid wheat-rye hybrids. At day 1, the Son X rye embryos had a very large mean cell volume ($49.07 \times 10^3 \mu m^3$) while those of C.S. X rye had a relatively small mean cell volume ($22.19 \times 10^3 \mu m^3$). The Son X rye embryo cell volume decreased very sharply on the second day to $8.68 \times 10^3 \mu m^3$ and more slowly until the fourth day. The C.S. X rye embryo cell volume decreased in the initial 1 to 2 day period but after 2 days, the volume remained relatively constant. (Fig. 19).

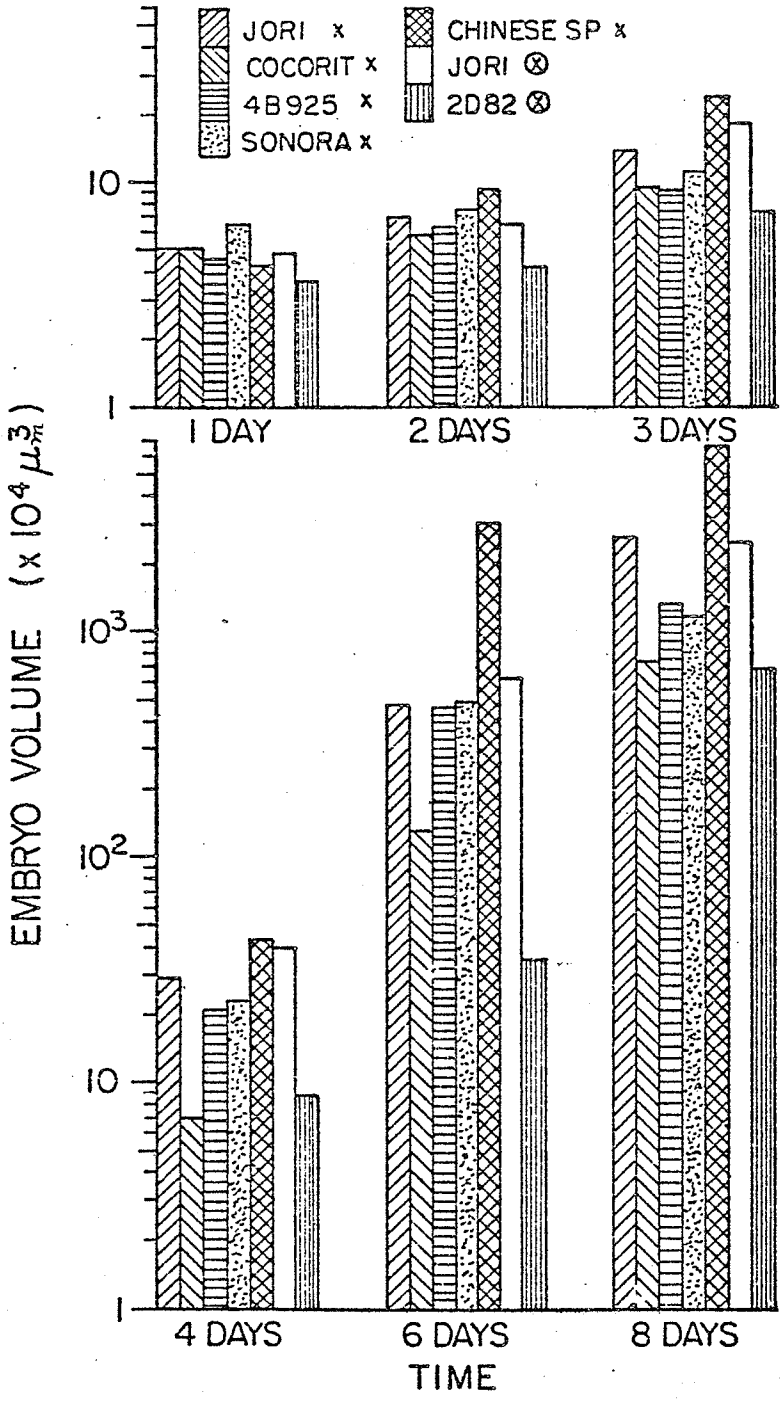
In the controls the 2D82 embryo cells had a larger initial volume but a faster volume decline than the wheat embryo cells.

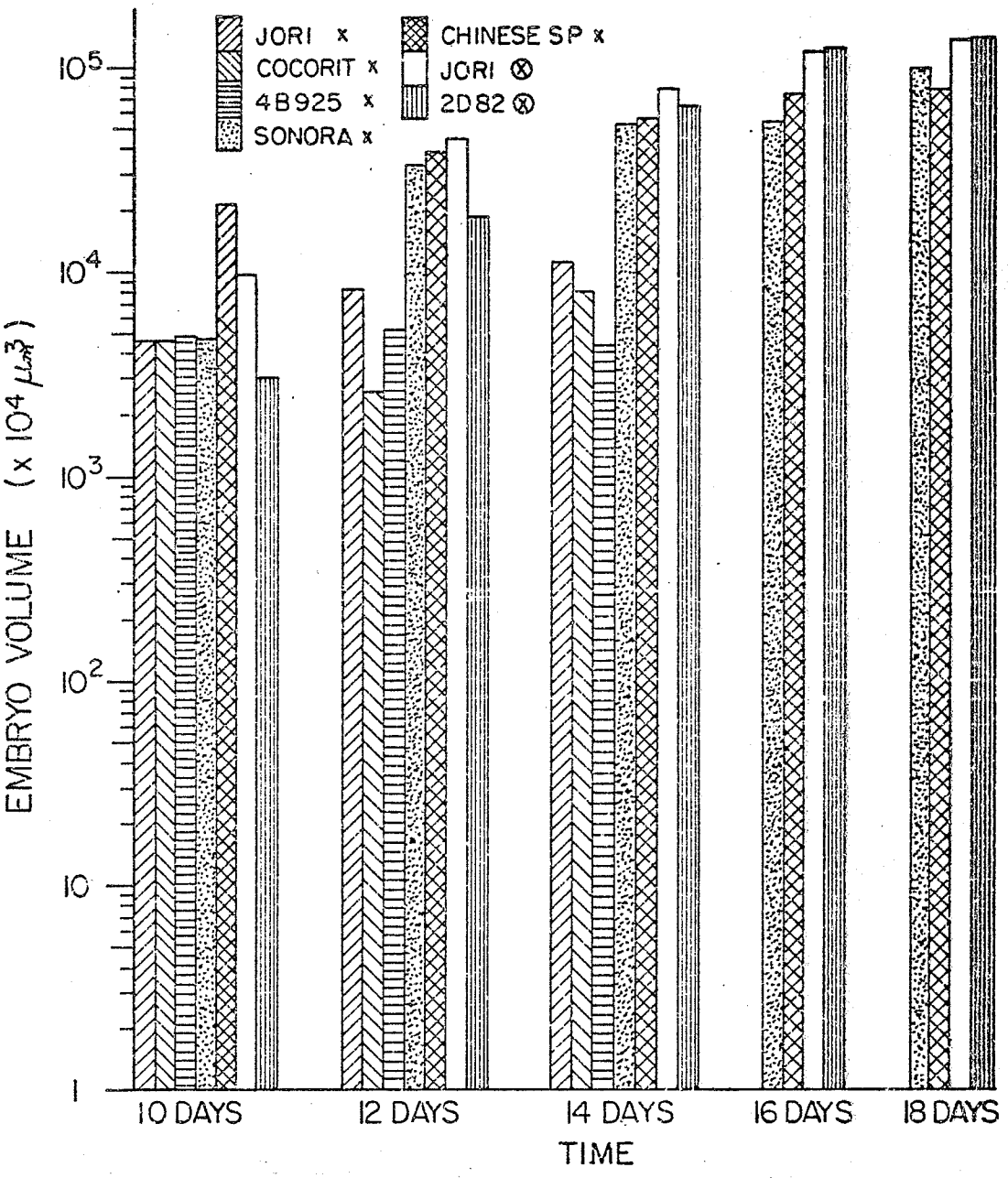
Comparing all genotypes, the hexaploid wheat-rye embryo cell volumes were the most variable. Son X rye embryo cells exhibited the largest initial cell volume and the most rapid rate of decline during the first two-day period. The C.S. X rye embryo cells had the smallest initial volume and the earliest levelling off time. Embryo cells of the inbred rye control had the smallest final volume of all material tested.

Embryo Volume versus Time

Embryo volume provided the most continuous measure of embryo growth over time. All genotypes showed a sigmoid

FIGURE 20. Mean embryo volume ($X10^6 \mu m^3$) versus days after pollination


JORI x
COCORIT x
4B925 x
SONORA x
CHINESE SP x
JORI ⊗
2D82 ⊗
EMBRYO VOLUME (x 10⁴ μm³)
10
1
1 DAY
2 DAYS
3 DAYS
10³
10²
10
1
4 DAYS
6 DAYS
8 DAYS
TIME


JORI x
COCORIT x
4B925 x
SONORA x
CHINESE SP x
JORI ⊗
2D82 ⊗
EMBRYO VOLUME (x 10⁴ μm³)
10⁵
10⁴
10³
10²
10
1
10 DAYS
12 DAYS
14 DAYS
16 DAYS
18 DAYS
TIME

growth curve with a levelling off of embryo volume at the later stage (Fig. 20). The slow increase in embryo volume for the 1 to 4-day period reflects the decreasing embryo cell volume. Bennett et al.(1975) found a similar initial embryo volume pattern.

In the tetraploid wheat-rye crosses, the Jori X rye embryos exhibited a larger volume than embryos of both the Coco X rye and the 4B X rye hybrids at most observation times. The Coco-rye embryos were generally the smallest, particularly during the 3-9 day period. At 10 days the Jori X rye embryos appeared to be still slowly increasing in volume but those of both the Coco X rye and the 4B X rye hybrids had ceased (Fig. 20). However, beyond 10 days the standard deviations for the tetraploid wheat-rye embryo volume data were very high which made any conclusion regarding their growth patterns beyond the 10-day stage unreliable.

Of the two hexaploid wheat-rye crosses, the C.S. X rye embryos had the larger volumes for all time observations except at the 1st and 18th day. The Son X rye embryos' larger initial volume and slower growth in the first four days compared to the C.S. X rye embryos, reflects the decreasing embryo cell volume patterns previously described. From 12 to 18 days the differences in embryo volume between the two hexaploid wheat-rye hybrids were small (Fig. 20).

In summary, the 7 genotypes exhibited variation in their embryo volume growth patterns. The C.S. X rye embryos were

the largest from 2 until 10 days while those of the rye control were the smallest for the same period. By 16 days, however, the 2D82 (selfed) embryos had exceeded the volume of those of the other genotypes. The tetraploid wheat-rye embryos generally stabilized at about 10 days. In contrast, the hexaploid wheat-rye embryos were roughly equal in size to those of the controls up to 14 days but remained slightly smaller thereafter. Boyes and Thompson (1937) also found the growth of C.S. X rye embryos to parallel those of the maternal parent but to be slightly smaller at the 21 day stage.

Endosperm Condition

In some of the material examined, aberrant endosperm nuclei appeared at the 4^{th} and 6^{th}-day post-fertilization periods. Giant polyploid, dumbbell, star-shaped, and necrotic nuclei were observed which were found most frequently proximal to the embryo (Fig. 17; Kaltsikes *et al*, 1975; Moss, 1970; Bennett, 1973). In the lines exhibiting a high degree of aberrant nuclei there followed a degeneration of the endosperm tissue.

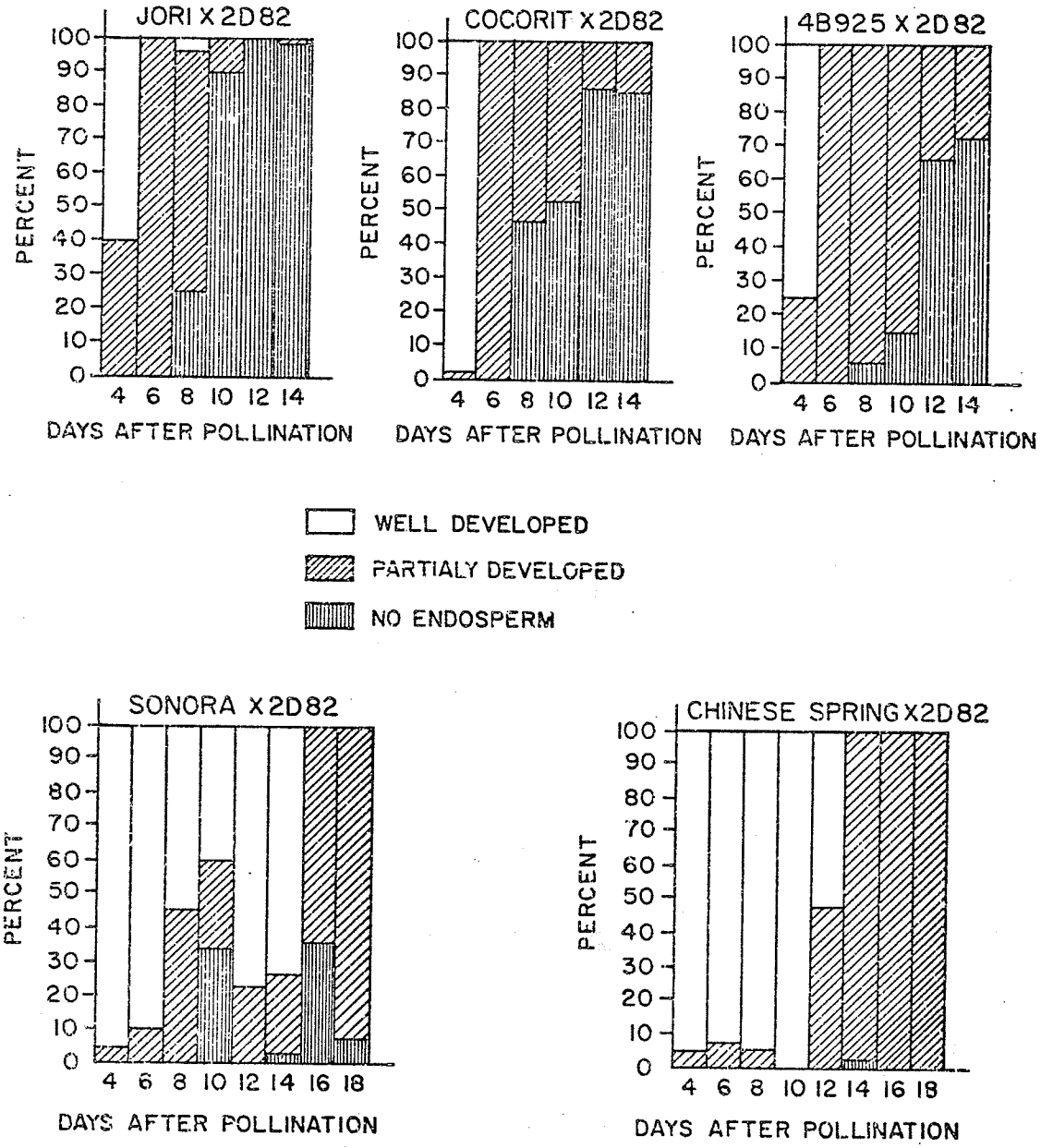
The tetraploid wheat-rye hybrids exhibited endosperm failure. In all three lines over 50% of those endosperms observed at 4 and 6-day stage had some aberrant nuclei. At 6 days the endosperms were only partially present, existing as either fragments or thin membranes. By 14 days, 98% of the fertilized ovules of Jori X rye contained no

FIGURE 21. Endosperm condition expressed as a percentage of number observed versus days after pollination

Endosperm conditions:

1) well developed
2) partially developed
3) no endosperm

(based on Figures 3-5)


JORI X 2D82
COCORIT X 2D82
4B925 X 2D82
PERCENT
DAYS AFTER POLLINATION
WELL DEVELOPED
PARTIALY DEVELOPED
NO ENDOSPERM
SONORA X 2D82
CHINESE SPRING X 2D82

endosperm. Similarly, 84 and 72% of the Coco X rye and 4B X rye ovules, respectively, were without endosperms. No complete endosperms were observed in these genotypes after 10 days (Fig. 21). Taira and Larter (1977a) and May (1977) found similar endosperm deterioration patterns in tetraploid wheat-rye hybrids.

In the hexaploid 4-day-old wheat-rye hybrids, one third of the Son X rye hybrids had aberrant endosperm nuclei whereas only one individual hybrid in the C.S. X rye line was found to have aberrant nuclei. Most of the C.S. X rye hybrids exhibited good endosperm development up to the 12-day stage. Both C.S. X rye and Son X rye hybrid ovules exhibited a "milky" condition in the endosperm at approximately the 12 to 18-day period. In this "milky" condition the cells of the endosperm tissue seemed unconnected and able to flow out individually when dissected (Fig. 10). Observations of this condition were scored in the partial endosperm category because the endosperms, even though they were complete, were not normal.

Of the 7 genotypes studied, fertilized ovules of the 3 tetraploid wheat-rye hybrids showed the most severe endosperm failure. From the initial occurrence of aberrant nuclei, the endosperm in these hybrids progressively deteriated until most ovules were completely devoid of endosperm tissue. These results confirm those of Moss (1970), May (1977), and Taira and Larter (1977a). In the hexaploid

wheat-rye hybrid endosperms development was more nearly normal and similar to that of the controls. Moss (1970) also reported a lower level of aberrant endosperm nuclei and less endosperm deterioration in hexaploid wheat-rye ovules compared to those from tetraploid wheat-rye crosses.

Level of Embryo Differentiation

There were large differences among genotypes in the ability of their embryos to differentiate normally. As was pointed out by Taira and Larter (1977a), the level of differentiation of an embryo is a good indicator of the embryo's viability *in vitro*.

The tetraploid wheat-rye embryos exhibited poor differentiation (Fig. 22). At 8 days, all three tetraploid hybrids had some slightly differentiated embryos with 4B X rye hybrids having the highest frequency (51%). From 10 to 14 days of age no further differentiated embryos appeared indicating a cessation of development. In addition, several embryos with yellow and brown patches were observed which suggested the occurrence of deterioration (Fig. 11). The Jori X rye cross still retained a small percentage of slightly differentiated embryos at the 14 day-stage. Taira and Larter (1977a) also studying Jori X rye hybrids, found a small percentage (8.6%) of normal, differentiated embryos at 15 days post-fertilization. Since a different scale of differentiation was used in their study relative to the present investigation, a direct comparison is not possible.

FIGURES 10-13. Fig. 10. Milky condition of the endosperm of Chinese Spring X 2D82, 14 days

Bar represents approximately 1600 μm

Fig. 11. Dark deteriorating patches in the embryo of 4B925 X 2D82, 10 days

Bar represents approximately 200 μm

Fig. 12. Small fluted scutellum of Chinese Spring X 2D82 embryo, 12 days

Bar represents approximately 200 μm

Fig. 13. Twisted embryo in 2D82 rye, 14 days

Bar represents approximately 800 μm

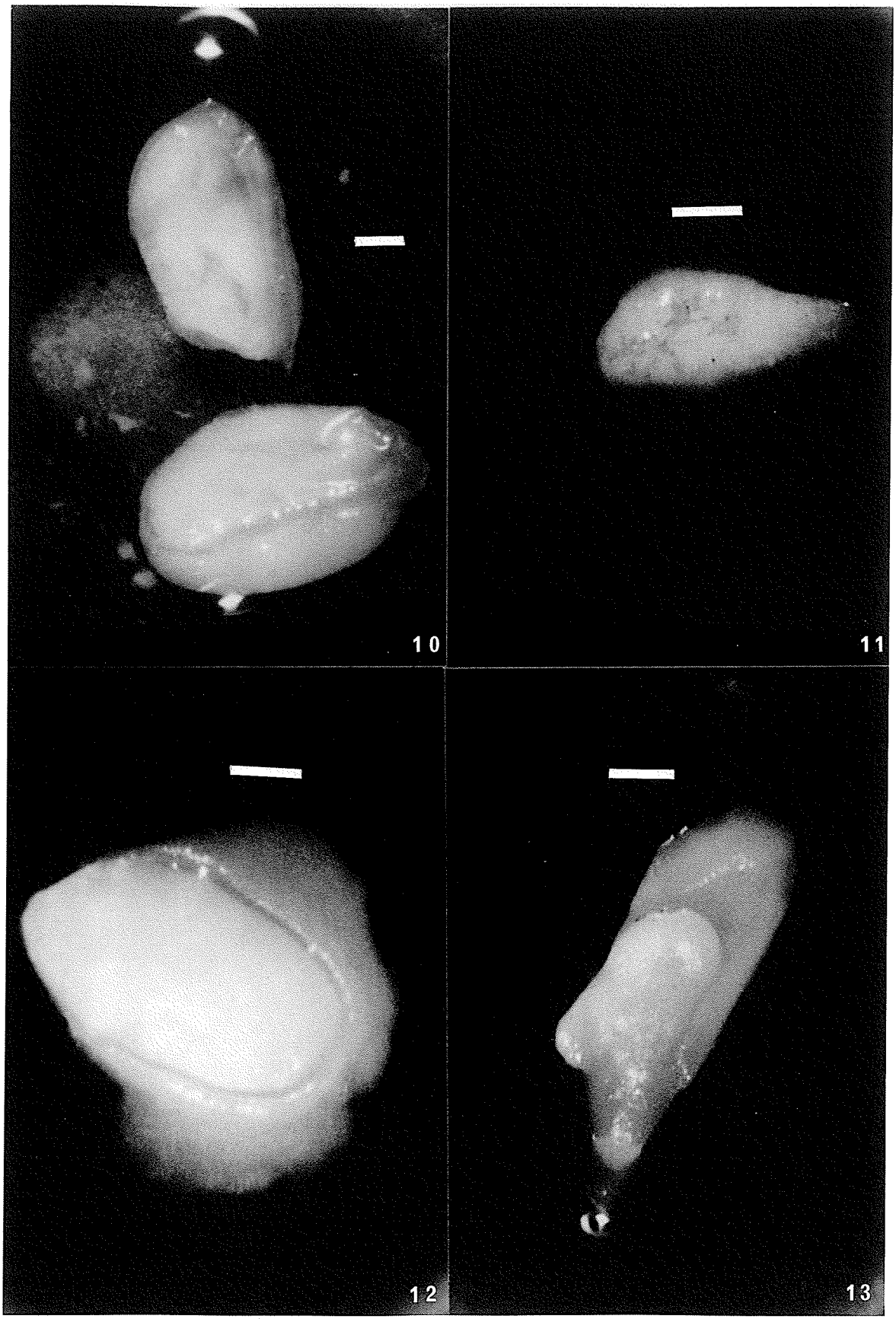
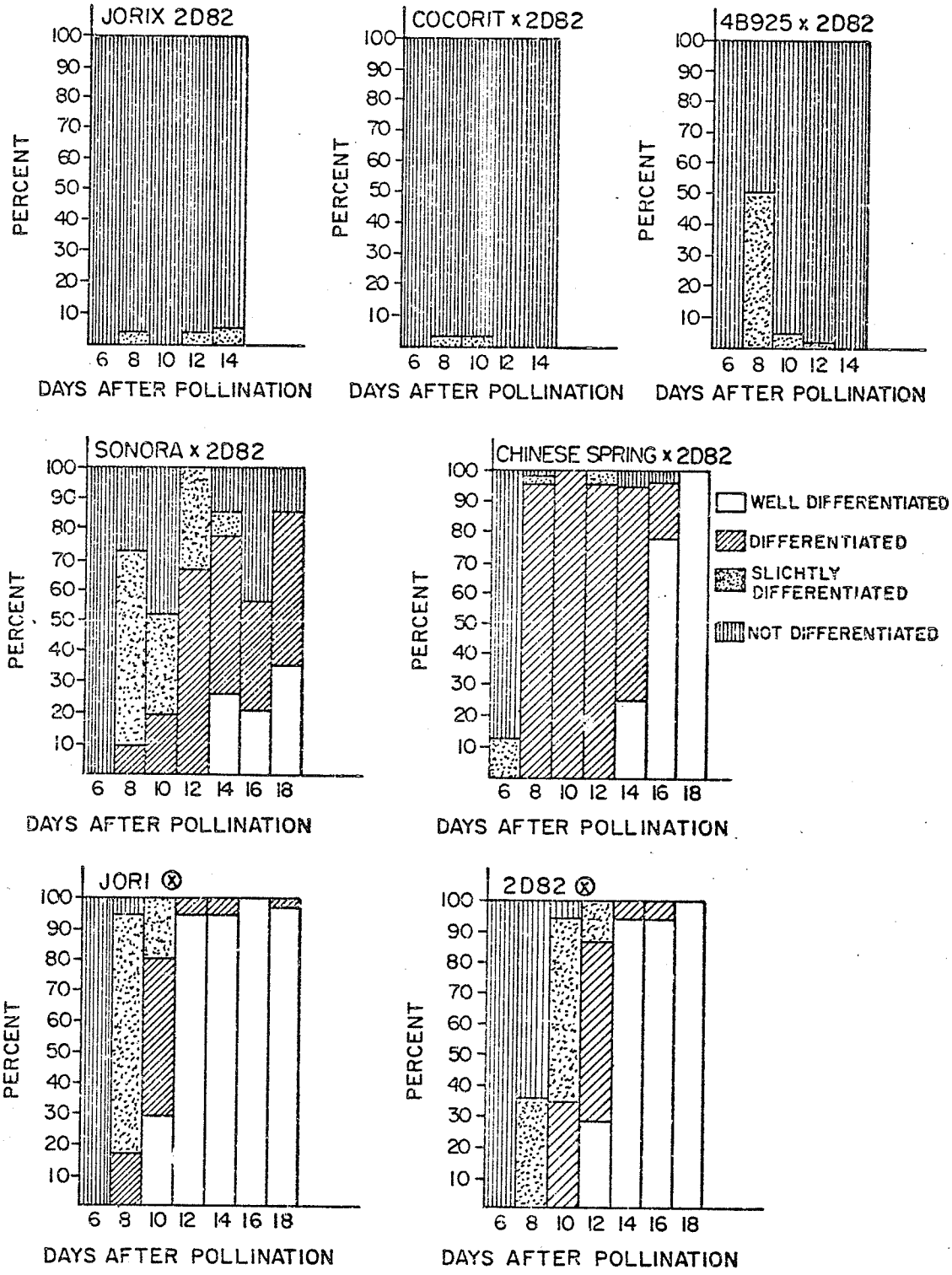

10
11
12
13

FIGURE 22. Level of differentiation in embryos expressed as a percentage of number observed versus days after pollination

Embryo conditions:

1) well differentiated
2) differentiated
3) slightly differentiated
4) not differentiated

(based on Figures 6-9)


JORIX 2D82
COCORIT x 2D82
4B925 x 2D82
SONORA x 2D82
CHINESE SPRING x 2D82
JORI ⊗
2D82 ⊗
PERCENT
DAYS AFTER POLLINATION
WELL DIFFERENTIATED
DIFFERENTIATED
SLICHTLY DIFFERENTIATED
NOT DIFFERENTIATED

Embryo differentiation in hexaploid wheat-rye crosses was nearly normal. C.S. X rye embryos showed the earliest initiation of differentiation (6 days) and developed rapidly thereafter. Son X rye embryos were slower differentiating than those from the C.S. X rye cross. A small percentage of undifferentiated embryos remained in both the C.S. X rye and the Son X rye hybrids. Although both hybrids contained some abnormally differentiated embryos, the C.S. X rye had a reoccurring abnormal condition in which the scutellum was small and fluted (Fig. 12).

Differentiation of the embryos in Jori wheat and 2D82 rye controls began at 8 days and progressed normally through until 18 days. Undifferentiated embryos did not persist past 10 days and the only abnormalities seen were a few twisted embryos in 2D82 (Fig. 13).

In summary, the control embryos were well differentiated as were most of the hexaploid wheat-rye embryos. In contrast, differentiation within tetraploid embryos failed at an early age. May's (1977) results show that embryos from the hexaploid wheat (cultivar Sonora 64)-rye cross were better developed than those of the tetraploid (cultivar Jori) wheat-rye cross. The C.S. X rye embryos were the first to undergo differentiation. Hexaploid wheat-rye embryos, although similar in development to the control embryos, exhibited more abnormalities.

Crossability Barriers

1. Seedset

Seedset expressed as a percentage of florets pollinated indicated in which hybrids the pre-fertilization barrier occurred.

All three tetraploid wheat-rye hybrids exhibited similar mean seedset of 75.4, 71.6 and 67.1% for Jori X rye, 4B X rye and Coco X rye respectively (Table 2). These values are slightly higher than those obtained by Krolow (1970), and Pienaar and Marais (1976) for other tetraploid wheat-rye crosses.

In the hexaploid wheat-rye crosses the seedset varied greatly between the combinations C.S. X rye and Son X rye. The C.S. X rye had a mean seedset of 80.1% which is slightly higher value than that reported by Lange and Wojciechowska (1976) for Chinese Spring crossed with rye. The Son X rye had a low seedset of 10.9% (Table 2) which is a little higher value than that reported by Pienaar and Marais (1976) for other T. aestivum cultivars crossed with rye. The present results, however, are in agreement with the general poor combining ability of the "vulgares" with rye found by Riley and Chapman (1967), Pienaar and Marais (1976), and Prabhahara Rao (1968). The excellent combining ability of the C.S. X rye is due to both of the crossability genes (Kr1 and Kr2) being in the recessive form in this cultivar (Riley and Chapman 1967; Lange and Wojciechowska, 1976).

TABLE 2. Seedset as a percentage of florets pollinated

Days	Genotypes													
	JoriX	(No. (spikes)	CocoritX	(No. (spikes)	4B925X	(No. (spikes)	SonoraX	(No. (spikes)	Chinese SpX	(No. (spikes)	Jori⊗	(No. (spikes)	2D82⊗	(No. (spikes)
1.	-	(7)	-	(7)	-	(7)	-	(6)	-	(6)	-	(6)	-	(5)
2.	76.56	(7)	60.24	(8)	72.44	(8)	4.51	(6)	36.96	(4)	9.82	(6)	42.74	(6)
3.	80.63	(7)	78.74	(8)	56.74	(9)	8.30	(20)	91.09	(4)	78.08	(7)	61.38	(6)
4.	78.87	(7)	79.35	(4)	80.68	(4)	31.06	(6)	91.71	(6)	65.08	(6)	75.47	(6)
6.	78.89	(4)	69.83	(4)	75.68	(3)	8.96	(6)	78.89	(3)	39.77	(5)	50.57	(5)
8.	25.00	(5)	50.00	(4)	50.00	(4)	7.25	(8)	91.94	(3)	64.75	(6)	61.76	(3)
10.	75.00	(4)	46.25	(4)	68.29	(4)	19.18	(8)	88.33	(2)	41.67	(6)	55.26	(3)
12.	95.16	(3)	79.69	(3)	87.10	(3)	5.84	(7)	80.00	(2)	80.00	(3)	57.69	(3)
14.	93.10	(3)	72.50	(2)	81.58	(2)	13.48	(10)	64.86	(3)	83.75	(4)	56.60	(3)
16.	-	-	-	-	-	-	5.51	(13)	87.14	(3)	86.96	(2)	54.55	(2)
18.	-	-	-	-	-	-	5.26	(13)	90.38	(2)	71.67	(3)	71.21	(2)
Mean	75.40		67.08		71.56		10.94.		80.13		62.16		58.72	

The control lines produced reasonably good seedset. The Jori selfed line (62.2%) was somewhat lower in fertility than the Jori X rye line (75.4%). This may be partially accounted for by the difficulty in obtaining sufficient Jori pollen for hand-pollinating the Jori maternal parent.

The present results indicated that only the hexaploid wheat Sonora exhibited any major crossability barrier with rye prior to fertilization. Lange and Wojciechowska (1976) associated the poor seedset found in most hexaploid wheats with the presence of Kr genes in the dominant form resulting in retardation pollen tube growth and eventual inhibition in the stylar tissue of the ovule. Confirming this, very few of the Son X rye embryo sacs at 24 hours after pollination exhibited any signs of sperm nuclei or fertilization. That the crossability barrier expressed itself prior to fertilization in hexaploid wheat-rye crossed and not in the tetraploid wheat-rye crosses agrees with the conclusions of Moss (1970).

2. Seed Viability

Assuming seed viability is a reflection of normal embryo differentiation and growth, together with a normally-developed endosperm, the material studied can be evaluated as to its potential seed viability.

In the tetraploid wheat-rye hybrids, inhibition of

embryo differentiation, cessation of embryo growth, and endosperm failure occur collectively at 10 to 14 days post-fertilization. It appeared that most embryos of these hybrids would not be viable *in vivo*. This conforms to Moss's (1970) findings of a crossability barrier in tetraploid wheat-rye hybrids which expresses itself in seed inviability. A further study of seed viability *in vivo* and *in vitro* using the same three tetraploid wheat-rye hybrids may have yielded better evidence of a crossability barrier existing at this level.

In most ovules of the hexaploid wheat-rye hybrid, embryo and endosperm development proceeded normally up to the 18^{th} day. A few abnormal embryos were present in addition to some embryos and endosperm tissue which had ceased to develop. However, no major crossability barrier expressed in terms of seed inviability appeared in the hexaploid wheat-rye hybrids. Further tests, using the same material grown *in vivo* and *in vitro*, might better substantiate the absence of this barrier in hexaploid wheat-rye hybrids.

In the control ovules, all three indicators of good seed viability showed normal development.

Thus, of the genotypes studied, seed inviability as a barrier to crossing wheat with rye appeared most prevalent in the tetraploid wheats. This agrees with Krolow (1970), Pienaar and Marais (1976), Pienaar (1973) and Moss (1970)

who all found the tetraploid wheat-rye hybrids to be less able to produce viable seeds than the hexaploid wheat-rye hybrids. The cause of the malfunctioning of embryo and endosperm development resulting in a crossability barrier is not completely understood.

The study was able to relate early embryo development with later observations of endosperm failure and embryo differentation. This association has not been previously examined in wheat-rye hybrids to any extent. Unfortunately, due to the nature of the material, the only continuous measurement of embryo growth which could be used for the 18 days was embryo volume. Measurements of embryo growth over time by other more accurate methods would be beneficial in identifying the exact times and patterns of hybrid embryo failure.

The choice of tetraploid wheats was based on their previously reported differential crossability with rye. For all the parameters measured, however, the three tetraploid wheat-rye hybrids exhibited similar behavior. Since the success of crossing durum wheat with rye is known to vary with the wheat cultivar used, a better selection of good and poor combining wheats would have yielded more information as to the cause of this difference. In addition, the inclusion of a selfed hexaploid wheat control would have facilitated comparisons with the crossed hexaploid wheat-rye material.

SUMMARY AND CONCLUSIONS

This study was undertaken to gain information on embryo growth and seed failure in primary wheat-rye hybrids. Embryo growth was measured by embryo volume, embryo cell volume, and embryo cell numbers versus time. The locations of crossability barriers were indicated in the hexaploid wheat-rye hybrids studied by seedset values and in the tetraploid wheat-rye hybrids, by seed inviability. The following conclusions were drawn:

Two barriers seemed to be operating to prevent natural hybridization of wheat and rye. In the hexaploid wheat-rye material the major barrier appeared prior to fertilization and was reflected in the percentage seedset. Sonora, a poor combiner with rye, produced a low percentage seedset while Chinese Spring, a good combiner, had a high percentage. This barrier is known to be controlled by two dominant genes, Kr1 and Kr2. Chinese Spring has both Kr genes in the recessive condition while Sonora is believed to possess them in the dominant form.

In the tetraploid wheat-rye hybrids of the present study, a crossability barrier appeared at the 4 to 14-day stage of development. Aberrant endosperm nuclei were observed at 4 and 6 days in all three tetraploid wheat-rye hybrids.

Gradual endosperm failure followed until 14 days when most ovules were devoid of endosperm tissue. In these lines embryo differentiation, which started at 8 days, ceased approximately 2 days later after which the embryo deteriorated. Embryo volume growth also seemed to stop by the tenth day post-fertilization; the only exception seemed to be a small number of Jori-rye hybrid embryos which continued to grow. The majority of hexaploid wheat-rye crosses developed normally although endosperm abnormalities, aberrant nuclei, and embryo abnormalities occasionally appeared. The crossability barrier arising at the embryo and endosperm differentiation level was obviously more significant in the tetraploid wheat-rye hybrids than in those involving hexaploid wheat parents.

Fertilization was complete in all lines by 24 hours after pollination. With the exception of rye, first mitosis was in progress in all lines at this time. A high incidence of Son X rye ovules showed no signs of fertilization at this time or later.

Embryo cell numbers increased over time for all lines. Rye embryos exhibited the slowest cell number increase and lagged behind those of the wheat and the wheat-rye hybrids. No clear difference in growth rate could be discerned between the Jori X rye hybrid and the Jori wheat control.

Embryo cell volume of all hybrids decreased over time for the first 3 days and then levelled off. The C.S. X rye

cell volume stabilized earliest at 2 days and the 2D82 rye control had the smallest final cell volume. The Son X rye embryo had the largest initial cell volume and the fastest 1 to 2-day period decrease in cell volume.

In general, embryo volume had a sigmoidal growth curve over time. The tetraploid wheat-rye embryos levelled off at 10 days. The hexaploid wheat-rye hybrids and the control rye and wheat, however, were still slowly increasing in cell volume at 18 days.

Thus, in the seven genotypes studied embryo growth was approximately uniform for the first 4 days. At 8 to 10 days, the time of embryo differentiation and endosperm cellularization, embryo growth in the tetraploid wheat-rye hybrids seemed to stop. Embryo growth in the hexaploid wheat-rye hybrids and the Jori and 2D82 control material continued to increase for at least 18 days, the period of observation of this study.

TABLE 3. Summary

Fertilization Seed-Set	-hexaploid wheat-rye crosses → 1st barrier -Sonora X rye low seed-set -Chinese Spring X rye high seed-set	
Early Seed Development 1) Embryo cell number) 2) Embryo cell volume) 3) Embryo volume) 4) Endosperm aberrant nuclei	All genotypes similar -tetraploid wheat-rye crosses → Beginning of barrier?	
Later Seed Development 1) Embryo volume 2) Embryo differentiation 3) Endosperm condition	-tetraploid wheat-rye crosses → 2nd barrier -all tetraploid wheat rye crosses appear to cease development at 10-14 days post fertilization	

LIST OF REFERENCES

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APPENDICES

TABLE 1. Mean embryo cell number and standard deviation versus time after pollination

Days	Genotypes						
	JoriX CocoritX		4B925X SonoraX		Chinese SpX Jori⊗		2D82⊗
1. Mean	1.54	1.63	1.69	1.33	1.91	1.67	1.00
St.Dev.	.49	.50	.44	.50	.29	.50	.00
No.Obs.	20	20	13	9	22	9	13
2. Mean	6.45	4.84	8.00	8.67	9.45	5.14	5.71
St.Dev.	1.15	.80	1.04	1.51	1.75	.69	1.23
No.Obs.	20	25	29	6	31	7	21
3. Mean	19.10	11.52	14.21	17.52	25.84	20.71	14.44
St.Dev.	3.06	2.00	3.19	3.89	2.57	3.81	2.47
No.Obs.	20	48	47	25	50	21	25
4. Mean	51.34	33.20	40.60	42.88	46.21	55.78	26.28
St.Dev.	9.12	7.37	6.22	5.54	9.49	9.53	4.12
No.Obs.	44	50	50	84	84	18	25
5. Mean	-	-	-	-	-	-	77.55
St.Dev.	-	-	-	-	-	-	16.21
No.Obs.	-	-	-	-	-	-	12

TABLE 2. Mean embryo cell volume ($X10^3 \mu m^3$) and standard deviation versus time after pollination

Days	Genotypes						
	JoriX CocoritX		4B925X SonoraX		Chinese SpX Jori⊗		2D82⊗
1. Mean	32.71	31.79	27.17	49.07	22.19	28.93	36.19
St.Dev.	17.68	14.69	14.19	24.39	10.15	13.40	5.00
2. Mean	11.26	12.06	7.87	8.68	9.74	12.60	7.60
St.Dev.	4.11	4.87	2.77	4.85	4.34	3.84	4.42
3. Mean	7.28	8.11	6.40	6.14	9.28	8.70	5.06
St.Dev.	4.20	3.57	3.97	2.45	2.78	4.50	2.60
4. Mean	5.70	5.10	5.16	5.31	9.27	7.01	3.38
St.Dev.	3.00	3.09	2.22	2.16	3.37	3.38	1.37
5. Mean	-	-	-	-	-	-	4.47
St.Dev.	-	-	-	-	-	-	2.85

TABLE 3. Mean embryo volume ($X10^6 \mu m^3$) and standard deviation versus time after pollination.

Days		Genotypes						
		JoriX	CocoritX	4B925X	SonoraX	Chinese SpX	Jori♀	2D82♀
1.	Mean	.051	.052	.046	.065	.042	.048	.036
	St.Dev.	.011	.008	.012	.008	.013	.008	.005
	No.Obs.	20	20	13	9	22	9	13
2.	Mean	.070	.058	.063	.075	.092	.065	.043
	St.Dev.	.014	.014	.014	.029	.024	.011	.016
	No.Obs.	20	25	29	6	31	7	21
3.	Mean	.139	.093	.091	.108	.240	.180	.073
	St.Dev.	.058	.025	.036	.019	.048	.060	.025
	No.Obs.	20	48	47	25	50	21	25
4.	Mean	.292	.169	.209	.228	.428	.391	.089
	St.Dev.	.102	.065	.058	.063	.086	.122	.022
	No.Obs.	44	50	50	24	84	18	25
6.	Mean	4.671	1.316	4.607	4.878	30.186	6.236	.346
	St.Dev.	1.887	.804	3.987	3.219	18.279	2.896	.149
	No.Obs.	50	50	50	11	50	48	12
8.	Mean	26.19	7.36	13.21	11.55	66.64	24.75	7.80
	St.Dev.	16.73	3.28	4.76	7.54	25.73	7.16	4.39
	No.Obs.	25	37	39	11	50	36	40
10.	Mean	46.29	45.08	48.59	46.93	212.13	97.14	30.51
	St.Dev.	28.06	27.35	32.30	32.02	40.55	4.19	16.90
	No.Obs.	50	33	50	27	50	49	50
12.	Mean	84.93	26.34	51.92	332.59	389.42	454.16	189.27
	St.Dev.	55.72	18.33	49.36	172.97	103.78	144.30	97.85
	No.Obs.	50	47	50	9	50	49	50
14.	Mean	113.17	81.05	44.48	525.82	565.84	802.56	650.84
	St.Dev.	84.01	87.47	48.92	254.51	190.26	235.23	196.59
	No.Obs.	50	25	25	31	42	35	35
16.	Mean	-	-	-	543.61	750.18	1202.85	1244.36
	St.Dev.	-	-	-	431.43	225.84	241.66	261.92
	No.Obs.	-	-	-	14	43	35	35
18.	Mean	-	-	-	978.00	783.29	1369.89	1403.99
	St.Dev.	-	-	-	578.69	142.33	226.34	355.32
	No.Obs.	-	-	-	14	50	35	35

TABLE 4. Endosperm condition; 1) well developed, 2) partially developed, 3) no endosperm, versus time after pollination expressed as percentage of number observed

Days	Condition	Genotypes						
		JoriX CocoritX		4B925X SonoraX		Chinese SpX	JoriX	2D82X
4.	1	60%	98%	76%	96%	96%	100%	100%
	2	40	2	24	4	4	0	0
	3	0	0	0	0	0	0	0
6.	1	0	0	0	90	94	100	100
	2	100	100	100	10	6	0	0
	3	0	0	0	0	0	0	0
8.	1	4	0	0	55	96	100	100
	2	72	54	95	45	4	0	0
	3	24	46	5	0	0	0	0
10.	1	0	0	0	41	100	100	98
	2	10	48	86	26	0	0	2
	3	90	52	14	33	0	0	0
12.	1	0	0	0	78	52	100	100
	2	0	14	34	22	48*	0	0
	3	100	86	66	0	0	0	0
14.	1	0	0	0	81	0	100	100
	2	2	16	28	23	98*	0	0
	3	98	84	72	3	2	0	0
16.	1	-	-	-	0	0	100	100
	2	-	-	-	64*	100*	0	0
	3	-	-	-	36	0	0	0
18.	1	-	-	-	0	0	100	100
	2	-	-	-	93*	100*	0	0
	3	-	-	-	7	0	0	0

*Endosperms which appeared well developed but were "milky" were included in the 2) partially developed category.

TABLE 5. Level of differentiation in embryo expressed as percentage of number observed; 1)well differentiated, 2) differentiated, 3) slightly differentiated, 4) not differentiated, versus time after pollination

Days	Level	Genotypes						
		JoriX CocoritX		4B925X SonoraX		Chinese SpX Jori⊗		2D82⊗
6.	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	12	0	0
	4	100	100	100	100	88	100	100
8.	1	0	0	0	0	0	0	0
	2	0	0	0	9	96	17	0
	3	4	3	51	64	2	77	35
	4	96	97	49	27	2	6	65
10.	1	0	0	0	0	0	29	0
	2	0	0	0	19	100	51	34
	3	0	3	4	33	0	20	60
	4	100	97	96	48	0	0	6
12.	1	0	0	0	0	0	94	28
	2	0	0	0	67	96	6	58
	3	4	0	2	33	4	0	14
	4	96	100	98	0	0	0	0
14.	1	0	0	0	26	24	94	94
	2	0	0	0	52	71	6	6
	3	6	0	0	6	0	0	0
	4	94	100	100	16	5	0	0
16.	1	-	-	-	21	78	100	94
	2	-	-	-	36	18	0	6
	3	-	-	-	0	0	0	0
	4	-	-	-	43	4	0	0
18.	1	-	-	-	36	100	97	100
	2	-	-	-	50	0	3	0
	3	-	-	-	0	0	0	0
	4	-	-	-	14	0	0	0