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Short Communication

A cytological study on early development of endosperm in maize (*Zea mays* L.)

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The importance of maize (*Zea mays* L.) endosperm can not be overemphasized as it constitutes the bulk of economic yield of this crop and provides nutritive support to the developing embryo. Endosperm originates from the double fertilization phenomenon where the two haploid polar nuclei of the embryo sac get fertilized by one of the sperms to produce the triploid initial material [1]. The transfer of apomixis gene(s) from wild species into cultivated crop plants may open up the possibilities for preservation of hybrid vigor over generation [2]. However, inter-ploidy crosses in maize invariably lead to failure of endosperm development and seed abortion [3, 4]. Also, not much is known about endosperm development and the role of varying endosperm ploidy levels on normal endosperm development. In case the apomixis trait is introduced into maize, the endosperm ploidy levels would be diploid, triploid, tetraploid, pentaploid depending on whether the embryo sac is monosporic, bisporic or tetrasporic and whether the endosperm development is pseudogamous or autonomous. Precise knowledge about the endosperm development and its cytogenetic basis is important for successful transfer of apomictic gene(s). The present study was undertaken to analysed the initial stages of endosperm development in diploid, tetraploid and interploidy crosses, like 2n × 4n, 4n × 2n, 3n × 2n and (2n + 1) × 2n to get an insight into the role of varying ploidy levels on endosperm development in maize. The materials used in this study included two diploid inbred lines (CM 105 and CM111), one tetraploid (derivative of Alexanders Synthetic B tetraploid), two sets of triploids (obtained by crossing the tetraploid stock by two diploid inbred lines, CM105, CM111) and one trisomic stock (primary trisomy for chromosome 10).

Kernels were classified from different crosses according to the extent of endosperm development, as follows: i) plump (smooth pericarp, complete endosperm development), ii) nearly plump (wrinkled pericarp, rough), iii) intermediate type (pericarp folded in crown region, endosperm almost shriveled), and iv) shrivelled type (no endosperm development). Microtomic study was done to trace the events of double fertilization and early stages of development of endosperm. Three sets of materials were used namely, ovules from i) self-pollinated ears, ii) interploidy crosses and iii) unpollinated ears. The developing ears were collected one to eight days after pollination and the unpollinated ears were collected from two to six days after silk emergence. The ears were fixed in 1:1:18 formalin: glacial acetic acid: alcohol solution. The embryo sacs were gradually dehydrated by using Tertiary butyl alcohol (TBA), embedded in paraffin wax, sectioned maintaining 15µ, thickness in a rotary microtome (Spencer) and stained with Iron hematoxylin following the method of Sass [5] and its modification [6].

Unlike homoploidy crosses, in interploidy crosses, the majority of seeds were found shriveled or chaffy with very little endosperm development. Seeds from 20 randomly selected cobs from each cross combination were analyzed for their grain development. In 2n × 4n crosses, only 0.47% and in 4n × 2n crosses only 0.49% kernels were plump which may include mostly self or cross contaminants (Table 1). However, when the frequency of completely shriveled kernels were

Table 1. Classification of kernels from different inter-ploidy crosses in maize

Crosses	Plump (% age)	Nearly plump (% age)	Intermediate (% age)	Shriveled (% age)
2n × 4n	0.47	6.80	43.16	49.57
4n × 2n	0.49	1.54	11.15	86.82
3n × 2n	2.91	3.07	9.05	84.97
2n+1 × 2n	100	0.0	0.0	0.0
Mean	25.97	2.85	15.84	55.34
SE (mean)	10.43	6.0	0.92	3.76

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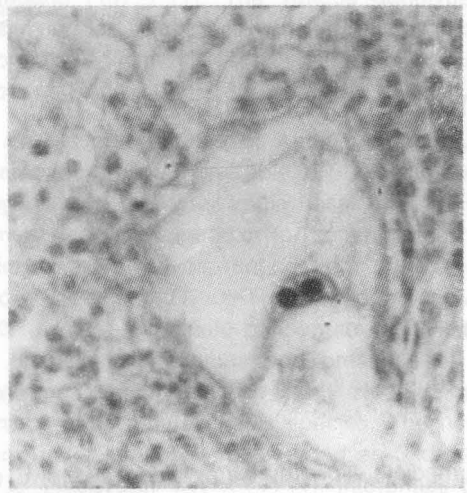

Fig. 1. In embryo sac two polar nuclei lie side by side in 2n ovule collected without pollination at 6 days after silk emergence, (at 400 x magnification)

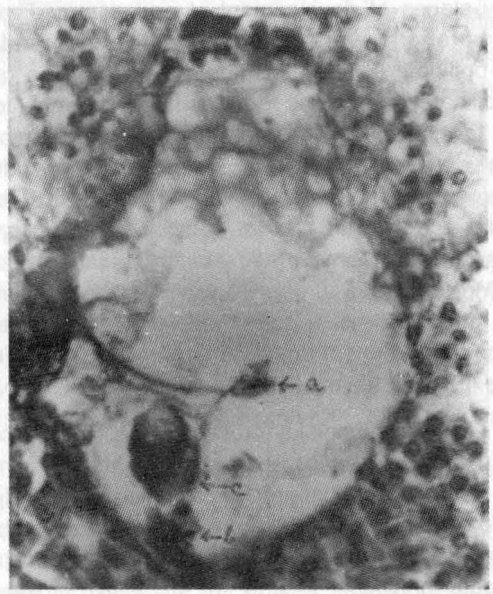

Fig. 2. Embryo sac in ovule from 2n selfed collected one day after pollination. One sperm is attached with the polar nuclei (a) and the other sperm is at the vicinity of the egg apparatus (b). Degenerated synergid is darkly stained (c), (at 400 x magnification)

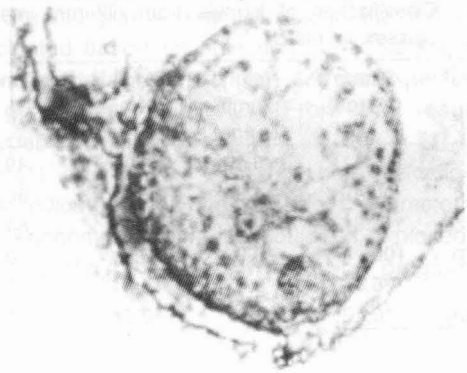

Fig. 3. Endosperm in 4n x 2n at six days after pollination. Embryo is in multicellular stage. (100 x magnification)

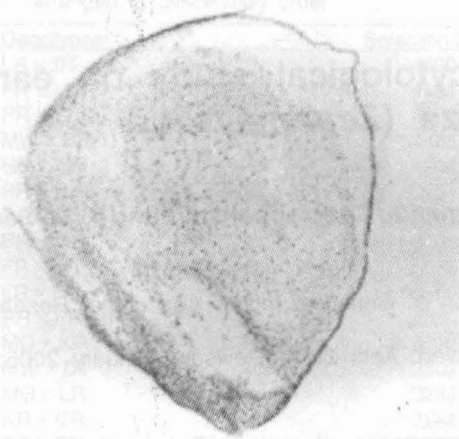

Fig. 4. Ovule from 4n x 2n collected 8 days after pollination showing advanced stages of degeneration in form of large gaps (at 40 x magnification)

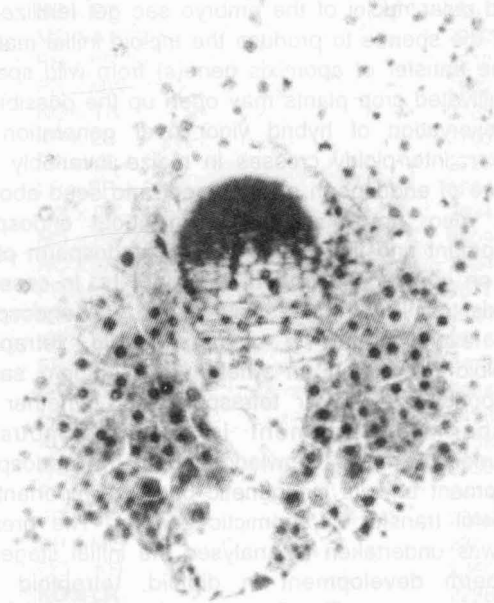

Fig. 5. Embryo and surrounding dividing endosperm cells in ovule from 2n x 4n collected 7 days after pollination (at 400 x magnification)

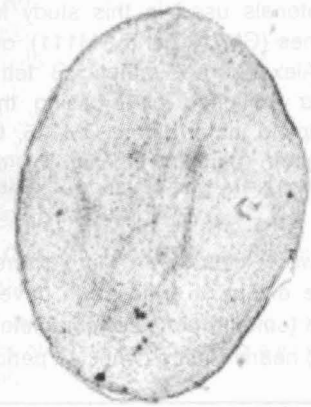

Fig. 6. Ovule from 2n x 4n collected 8 days after pollination showing folding at middle (at 40 x magnification)

compared, 4n × 2n crosses showed a much higher frequency (86.8%) than the 2n × 4n crosses (49.5%). The frequency of partially developed kernels (nearly plump and intermediate types) was more in 2n × 4n crosses (49.9%) than in the 4n × 2n crosses (12.6%).

When the unpollinated ears from 2n and 4n plants were subjected to microtomic studies, no indication of division of egg cell and polar nuclei was noticed. The embryo sac and its constituents did not start to degenerate even at six days after the stigma become receptive. The incidence of increased frequency of haploids in late pollination, reported by Aman *et al.* [7], may be explained by the occurrence of pseudogamy, i.e. single fertilization of the polar nuclei only. The two polar nuclei were found to remain separated at six days after silk emergence (Fig. 1) confirming the observation of Jensen [8] that the polar nuclei in maize do not fuse to form a secondary nucleus before fertilization. In the present study, it was noted that fertilization occurs at about 24 hours after pollination and the fusion of the sperm with the polar nuclei precedes fusion of the egg cell with the other sperm (Fig. 2). This observation was common to all interploidy and intraploidy crosses. It was observed that the sperm cells were discharged inside one of the two synergids leading to disintegration (darkly stained) of the synergid. This was a similar observation made earlier by Weatherwax [9], Jensen [10], Heslop-Harrison [11] and Mahendru [6] during their investigations of events of double fertilization.

Development of endosperm followed the same pattern in all the crosses. However, the speed of endosperm development was slower in 4n × 2n crosses than in 2n × 4n crosses. The sequence of division of endosperm nuclei and cellularization followed the same pattern upto 6 days after pollination in all the crosses. Abnormalities of development were observed in ovules collected at 6 days after pollination onwards. In 4n × 2n crosses there was no clear meristemetic layer above the embryo (Fig. 3). There was clear-cut indication of disintegration of endosperm with empty spaces at the peripheral and central region of the ovules collected 8 days after pollination (Fig. 4). In case of 2n × 4n crosses peripheral endosperm cells in the vicinity of the embryo showed high meristemetic activity in ovules at 7 days after pollination (Fig. 5). Here many layers of endosperm cells surrounded the embryo. By that time, the embryo becomes differentiated into primodial and radial axis in normal 2n ovule. But in 2n × 4n ovules such differentiation was not observed. In some ovules of 2n × 4n crosses, longitudinal cracks in the central portion of the endosperm were observed in ovules at 7 and 8 days after pollination. (Fig. 6). These are signs of beginning of the disintegration of endosperm. These observations together explain the collapse of the endosperm and formation of shriveled kernels in interploidy crosses. This type of anomalous endosperm development was noted earlier by Cooper [3] and Mathur [4]. This anomaly of endosperm development may not be due to increased number of chromosomes in endosperm as the chromosome number in endosperm in 4n × 4n cross is larger (6n). There was normal endosperm development and division of endosperm cells in 4n selfed kernels. There must be some inherent physiological conditions for collapse of 4n and 5n endosperm after normal developmental activities upto 6 days after pollination. Also, there was more shriveling (86.8%) in the endosperm from 4n × 2n crosses (50 chromosomes) than in endosperm (49.5%) from 2n × 4n crosses (40 chromosomes). In all the ovules of trisomy and diploid crosses (2n+1×2n) normal development were noticed. Here endosperm chromosome number varies from 30-32. All these observations reaffirm the hypothesis by Sarkar and Coe [12, 13] that 3n condition of the endosperm *per se* is important for normal endosperm development and as the number deviates from 3n and 6n condition, endosperm failure occurs and the extent of endosperm development depends on the quantum of deviation from 3n number.

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