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CHROMOSOME NUMBER IN RELATION TO ENDOSPERM DEVELOPMENT IN MAIZE (*ZEA MAYS* L.)

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ABSTRACT

To elucidate the role of chromosome number in endosperm development, the progeny from the 3n × 2n cross in maize was studied. The extent of endosperm development in such crosses varied from fully plump to shrivelled kernels. Endosperm chromosome number extrapolated from root tip chromosome counts of seedlings from eight different categories of kernels revealed that the extent of endosperm development depends on the chromosome number of the endosperm. The 3n or multiple of 3n condition of the endosperm is required for normal endosperm development. As the endosperm chromosome number increases from 30, more and more seed shrivelling is observed.

Key words: Endosperm, triploid, maize, chromosome number.

Double fertilization in angiosperms leads to the formation of a triploid endosperm and a diploid embryo. In crosses between plants of different ploidy levels, endosperm impairment frequently occurs, often preventing successful hybridization. The reasons for failure of proper seed development may be genetical and developmental conditions [1].

The endosperm has played a significant role in the evolution of angiosperms because of its physiological and genetic relationship with the embryo. One manifestation of this evolutionary role is its abnormal development in interploidy, both intraspecific and interspecific, crosses. In the former case, no qualitative genetic difference can be expected. The chromosome numbers of the endosperm, embryo and maternal tissue have been considered to play a vital role in proper seed development. Müntzing [2, 3] suggested that the genomic ratio among the embryo, endosperm and maternal tissue should be 2 : 3 : 2 for normal seed development. Watkins [4] proposed that a ratio of 2 : 3 between embryo and endosperm is essential while Valentine [5] considered a 3 : 2 ratio between endosperm and maternal tissue as necessary for normal endosperm development. Stephens [6] assigned different genetic strength or values to various cotton species in his studies on incompatibility. The hypothesis of genetic strength was supported by Howard [7] and Valentine and Woodell [8]. Wangenheim [9] could not confirm the influence of ploidy relationship between embryo, endosperm and maternal tissue in his investigations on potato where normal differentiation of hexaploid endosperm was observed despite the fact that the embryo was diploid, tetraploid or absent. Sarkar and Coe [10] also recovered monoploid embryos associated with normal triploid endosperm in 2n × 2n crosses. Studies on anomalous fertilization in diploid–tetraploid crosses in maize led them [11] to postulate that for proper kernel development an endosperm constitution of 3n or multiples of 3n is essential.

Nishiyama and Inomata [12] proposed a ratio of 2 : 1 between maternal and paternal genomes in endosperm. This was modified later as the endosperm balance number (EBN) hypothesis [13] to explain endosperm development in interploidy, interspecific and intraspecific crosses. Under this hypothesis, an EBN was arbitrarily assigned to one species and its crossing behaviour with other species helped in assigning the EBN to those species. Success of the cross depends on whether the cross had 2 female: 1 male ratio. The authors [13], however, indicated that a 2 : 1 EBN ratio is necessary but not sufficient for the success of a cross as seed abortion in a predicted successful cross with 2 : 1 EBN ratio was also reported.

However, none of these postulations have been experimentally verified to date and an attempt has been made in the present study to ascertain the role of chromosome number in maize endosperm development through manipulation of the chromosome number of endosperm tissue and correlating it with the extent of endosperm development and confirm the validity of the hypothesis [11] that the endosperm constitution of 3n or multiples of 3n is essential for normal seed development.

MATERIALS AND METHODS

The maize stocks used in this study included one diploid and two tetraploid strains. The diploid strain was a white semident inbred line developed from the commercial hybrid Ganga Safed 2. The tetraploid stocks were designated as purple 4n and yellow 4n. The triploid plants obtained from the 4n×2n cross were used as the female parent in the 3n×2n cross. The resultant ears were shelled and the kernels classified into eight categories (I-VIII) on the basis of the extent of endosperm development (Fig. 1). Mean kernel weight was determined for each category in three samples each. The results were analyzed by F and t tests of significance.

The endosperm cytology, to ascertain the chromosome number of the endosperm, is not possible in the developed kernels. Therefore, an indirect approach involving chromosome count in the root tip smears of progeny from the 3n×2n cross was followed. The diploid male parent contributes one set of chromosomes to the embryo and endosperm, while the contribution of the triploid female parent to the endosperm is double that to the embryo. Hence, the expected somatic number in the seedlings from the 3n×2n cross will vary from 20 to 30 and the corresponding range of endosperm chromosome number will be 30-50. Thus, if a kernel yields seedling with a somatic chromosome number of 21, the endosperm chromosome number would be 32(11+11+10).

The kernel categories obtained from the 3n×2n cross were germinated at 28°C in moist vermiculite and root tips were collected and pretreated with cyclohexamide and 8-hydroxyquinoline solution [14]. The root tips were fixed in 1 : 3 acetic alcohol and stored in 70% alcohol. The stored root tips were washed and hydrolysed in

1N HCl at 60°C for 8–10 min. Schiff's reagent was used for staining and 2.5% solution of pectinase and cellulase for softening, prior to squashing in 1% aceto-orcein. The chromosomes were counted at metaphase.

RESULTS AND DISCUSSION

The mean kernel weight for different categories ranged from 257 mg (cat. I) to 14 mg (cat. VIII) (Table 1). The decrease in mean weight among different categories was gradual and significant, except between categories VII and VIII.

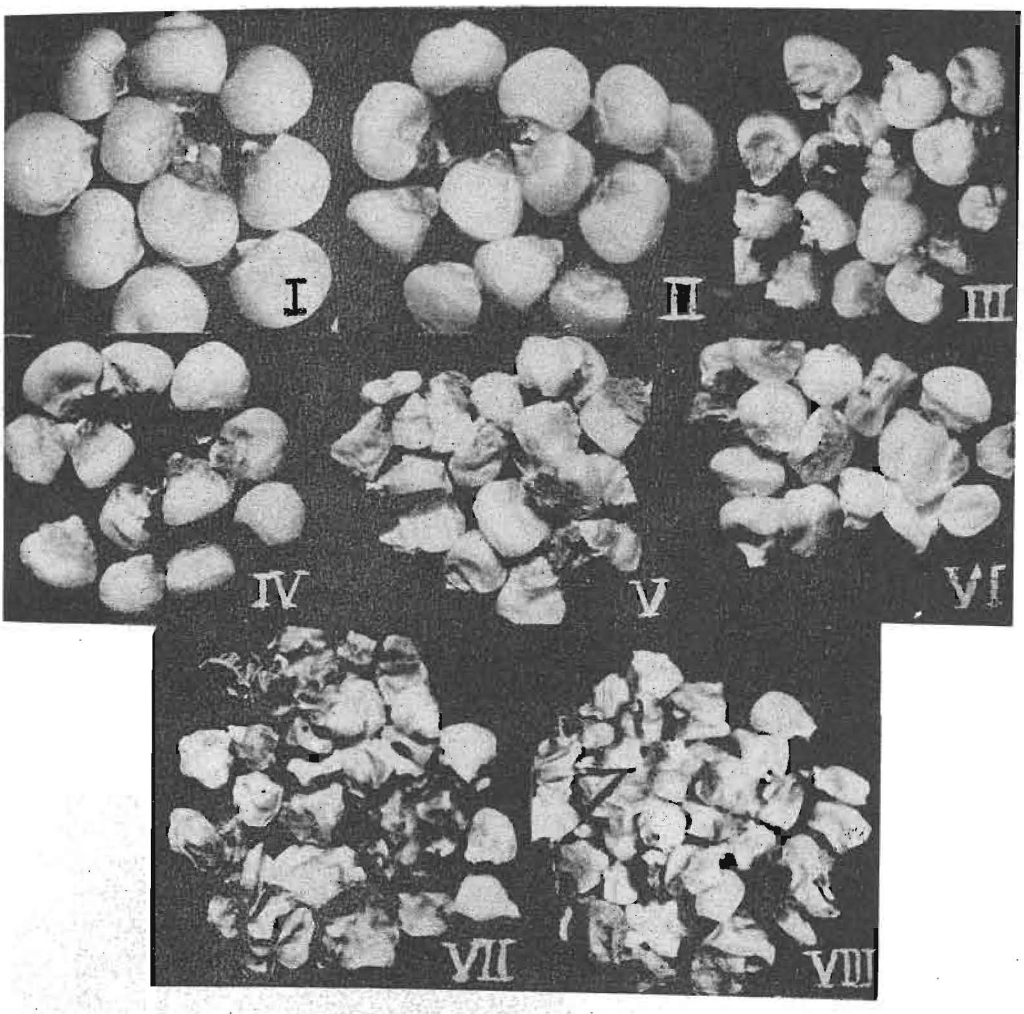

Fig. 1. Classification of kernels obtained from the 3n×2n cross into eight categories (I-VIII) on the basis of extent of endosperm development.

Table 1. Chromosome number in the endosperm extrapolated for each category from 3n×2n crosses

Category of endosperm development	Mean kernel weight (mg)	Percentage of kernels with different endosperm chromosome numbers								Total kernels used in root tip count	Mean chromosome No. of endosperm
		30	32	34	36	38	40	44	46		
I	257	69.05	9.52	9.52	11.90	—	—	—	—	42	31.29
II	200	51.92	26.92	13.46	7.69	—	—	—	—	52	31.54
III	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IV	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	37	[illegible]
V	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]
VI	[illegible]	[illegible]	[illegible]	[illegible]	23.26	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]
VII	[illegible]	[illegible]	[illegible]	26.67	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	60	[illegible]
VIII	14	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	45	37.33
C	31	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

The extrap[illegible] ch[illegible]me [illegible]mbers o[illegible] are pr[illegible]nte[illegible]n Ta[illegible] T[illegible]nge [illegible] 30-36 [illegible]s in the endosp[illegible] [illegible]bse[illegible]d in th[illegible] ca[illegible] 30 [illegible]in cate[illegible] a[illegible] category V[illegible] freque[illegible]perm chromoso[illegible] n[illegible]ber of 30 was highe[illegible] [illegible] and lowest in [illegible]VII. The endosperm cl[illegible]ses carrying 44 and 46 c[illegible]somes were observed in [illegible]y [illegible] frequencies of 1.67 and [illegible]%, respe[illegible]

The mean chromos[illegible]ulat[illegible]egory show[illegible]d a co[illegible]elation [illegible] the [illegible] endosperm develop[illegible]d c[illegible] number. T[illegible] mean [illegible] which is v[illegible] close to [illegible] othe[illegible] ke[illegible] category VII [illegible]

The t v[illegible]nifi[illegible]mber among different cat[illegible] a[illegible] presented [illegible] The t v[illegible]nsignificant between cate[illegible] I, II and III and [illegible] classes could be pooled [illegible] one group. Similar nonsi[illegible] values were observed among categories [illegible] and [illegible]I. However, the mean chromosome numbers of categor[illegible] were si[illegible]tly different from others but not between themselves at [illegible]ce. Accordingly, the eight categories may be reclassified [illegible] groups [illegible] comprising categories I, II and III (plump [illegible]r-plump kernels), [illegible]d including categories IV [illegible] (intermedia[illegible]) [illegible] third of shrivelled [illegible]ls including categories V[illegible] VIII. [illegible] reconstituted endosperm gr[illegible] The plump kernel group had a mean endosperm chromosome number of 31.61, the intermediate 33.92, and the shrivelled group 36.78, revealing that as the chromosome number in the endosperm increases there is more and more shrivelling.

The present studies on manipulation of the endosperm chromosome number in the 3n×2n cross were designed to verify the hypothesis [11] that triploidy condition per se is an essential condition for normal endosperm development. The majority of kernels of the plump class (55%) showed a triploid (3n=30) endosperm constitution,

Table 2. Significance of difference (t values) in chromosome numbers among different kernel categories

Categories	I	II	III	IV	V	VI	VII	VIII
II	0.49							
III	1.33	1.29						
IV	4.00**	3.78**	2.80**					
V	4.07**	3.80**	6.21**	0.44				
VI	5.47**	5.27**	4.26**	1.28	0.71			
VII	9.98**	9.74**	8.48**	5.03**	3.40**	3.82**		
VIII	11.17**	11.29**	7.18**	6.78**	5.66**	5.71**	2.30*	

Table values of t at 5% = 1.96, 1% = 2.33.

while the percentages of endosperms with 3n+2, 3n+4 and 3n+6 chromosomes were 22, 12 and 11, respectively. No plump kernel had a chromosome number beyond 36. Only 11% intermediate type of kernels had 30-chromosome endosperm, while the shrivelled group showed a near normal distribution of chromosome numbers with the mode at the endosperm class of 3n+8 chromosomes. Endosperms carrying 44 (3n+14) and 46 (3n+16) chromosomes were observed with the frequencies of 0.95 and 1.90%, respectively. The mean chromosome numbers in the three broad classes of kernels–plump, intermediate and shrivelled–show a distinct relationship between the endosperm chromosome number and extent of seed shrivelling.

The classification of endosperms carrying 30, 32, 34, 36, 38, 40, 44, and 46 chromosomes into the three broad classes of kernel phenotypes is presented in Table 4. It may be seen that the 30-chromosome endosperms mostly developed into plump

Table 3. Distribution of chromosome numbers in the three broad classes of kernels obtained from 3n×2n cross

Kernel pheno-type	Original cate-gories	Kernels with different chromosome Nos. in endosperm								Total No. of kernels	Mean chromo-some number
		30	32	34	36	38	40	44	46		
Plump and near-plump	I, II, III	76* (54.7)	30 (21.6)	17 (12.2)	16 (11.5)	0	0	0	0	139 (100)	31.61
Interme-diate	IV, V, VI	12 (11.3)	30 (28.3)	30 (28.3)	22 (20.8)	8 (7.6)	4 (3.8)	0	0	106 (100)	33.92
Shri-velled	VII, VIII	1 (1.0)	11 (10.5)	20 (19.1)	23 (21.9)	27 (25.7)	20 (19.1)	1 (1.0)	2 (1.9)	105 (100)	36.78
Total		89 (25.4)	71 (20.2)	67 (19.1)	61 (17.4)	35 (10.0)	24 (6.9)	1 (0.3)	2 (0.6)	350 (100)	—

*Percentage of each class is given in parentheses.

kernels. As the chromosome number increased, the percentage of plump kernels decreased and there was no development of plump kernels with 38 or more chromosomes in the endosperm. The percentage of shrivelled kernels, on the other hand, increased with the rising chromosome number. The 44 and 46-chromosome classes had only shrivelled kernels. These results clearly indicate that 3n condition of endosperm is ideal for proper grain development and increase in the number of chromosomes causes more and more disturbances in endosperm development, ultimately leading to its near collapse, producing shrivelled types, thus, confirming the hypothesis of Sarkar and Coe [11].

Table 4. Extent of endosperm development in kernels (from 3n×2n cross) carrying different chromosome numbers in the endosperm

Chromosome No. in endosperm	Total No. of kernels	Frequency of different kernel phenotypes, %			Ratio between chromosome numbers of	
		plump	intermediate	shrivelled	endosperm and embryo	female and male contribution in endosperm
30	89	85.4	13.5	01.1	1.50:1	2.0:1
32	71	42.3	42.3	15.5	1.52:1	2.2:1
34	67	25.4	44.8	29.8	1.54:1	2.4:1
36	61	26.2	36.1	37.7	1.57:1	2.6:1
38	35	0.0	22.9	77.1	1.58:1	2.8:1
40	24	0.0	16.7	83.3	1.60:1	3.0:1
42	0	0.0	0.0	100.0	1.62:1	3.2:1
44	1	0.0	0.0	100.0	1.63:1	3.4:1
46	2	0.0	0.0	100.0	1.65:1	3.6:1

These findings, however, do not support or contradict the hypothesis [15] that the 2 : 1 ratio of female : male genomes is required for proper seed development. This hypothesis is basically not different from that of [11], as it recognises that 3n or multiples of 3n condition of the endosperm is essential for normal endosperm development. However, it further emphasises that this 3n condition alone is not sufficient for normal endosperm development. In the present study, we do not find sufficient evidence to support the hypothesis involving balance between materna' and paternal genomes [12, 15]. As such, plump kernels were also obtained wher the genomic balance between maternal and paternal contribution increased from 2.(to 3.6 (Table 4).

From the present studies, it appears that the 3n condition of endosperm alone is important for normal development of endosperm. Increase in chromosome number beyond the normal of 30 for the endosperm of diploid maize disturbs endosperm development, leading to endosperm collapse sooner or later.

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