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Study of Possibility in Raising Maize Inbred Lines with two Embryos

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Summary. Ears having 1 to 3 kernels with two embryos were found in a synthetic and local maize population at the Maize Research Institute, Beograd-Zemun, in 1963-1964. From this material, using the method of individual kernels, selection was initiated and inbred lines with two embryo kernels were obtained.

The present paper gives the results of further breeding of maize lines having two embryo kernels, the frequency and variability of this occurrence within and among lines, and the results of some cytogenetic investigations of plants originating from two embryo kernels.

The frequency of two embryo kernels in ears of 12 selected lines in 1973 varied between 2.1% (the line IT) and 25.3% (the line Iab). The average for all lines was 11.8%. The best inbred lines have 8 times the number of kernels with two embryos found for the initial material (3.1%). Compared with normal kernels of the same lines, two-embryo kernels have a considerable increase in protein (4-6%), lysine g/100 g of dry matter (38-70.9%), lysine g/100 g of protein (21.3-34.0%) and oil (3.5-13.6%).

The presence of univalent chromosomes at metaphase I is not relatively high and in most cases it occurs in approximately 10-20% meiocytes, indicating partial desynapsis. No obvious differences in the frequency of univalent chromosomes at metaphase I and lagging chromosomes at anaphase I were found between plants of various height originating from the same kernel.

Introduction

The occurrence of maize kernels with two embryos is an interesting biological phenomenon, because, as found in several plant species (Nezhevenko and Shumny 1970), it is not uncommon that the two embryos differ in chromosome number. As a rule one of the embryos has the characteristic number of chromosomes for its species, while the other can be a haploid, diploid, polyploid or aneuploid. Haploid plants are important for breeding because of their use in obtaining highly homozygous lines relatively quickly.

It is well known that the maize germ contains the greatest portion of the kernel oil, so that better quality proteins are contained in the germ than in the endosperm. This is due to the considerable amounts of essential amino acids in the germ which are important for nutrition.

The present paper deals with the results of breeding maize inbred lines with two embryo kernels, the frequency and variability of this occurrence within and among lines, and with some cytogenetic investigations of plants originating from kernels with two embryos.

Material and Methods

We have found ears with 8.2, 0.5 and 0.4% of kernels with two embryos at the Maize Research Institute, Beograd-Zemun, in 1963 and 1964 in a synthetic and a local maize population. From this material, using the single kernels and ear to row method, we obtained inbred lines. In 1973 we studied the frequency and variability of kernels with two embryos in selfed ears of 14 line genotypes and the F_2 generation.

Protein content (nitrogen × 6.25) was analyzed by the micro-Kjeldahl method. The amino acids in 100 mg of whole kernels were determined by the Beckmann aminoacid analyzer. Oil content was determined by the Soxlet method.

The procedure described by Zečević and Paunović (1967) was used for determination of the somatic chromosome number in the analyzed inbred lines.

For meiotic studies, the sporocytes were collected from immature tassels of the plants. The sporocytes were fixed in Carnoy's fluid for 24 hours, after which they were fixed in 70% alcohol (twice), conserved in the same concentration of alcohol and kept in a refrigerator until analysis. The slides for cytological analysis were made by the standard acetocarmine smear method, as described (Zečević 1966). The subject of the study was the behaviour of chromosomes at the first meiotic division.

Results and Discussion

Frequency of Kernels with two Embryos in Analyzed Genotypes

Several authors designate the occurrence of maize kernels with two embryos as polyembryony (Ran-

Table 1. The occurrence of two embryo kernels among various genotypes of maize

Genotype label	No. of ears	No. of kernels total	No. of kernels with two embryos	Two embryo kernels %
IT	3	323	7	2,1 (0,0 - 3,4)
SP-650	8	2214	54	2,4 (0,9 - 4,1)
5-2	4	1670	79	4,7 (1,3 - 7,4)
5-b	5	334	20	5,9 (3,7 - 8,5)
SP 671-3	2	208	14	6,7 (2,5 - 9,3)
5-2d	5	682	109	7,4 (3,0 - 12,0)
20/1	6	839	89	10,6 (3,1 - 16,0)
SP 669	7	932	120	12,8 (7,6 - 18,2)
SP 670 × IF-F2	4	310	46	14,8 (9,6 - 24,5)
5-2F	7	870	137	15,7 (7,4 - 29,6)
SP 669a	8	352	58	16,4 (4,8 - 42,1)
20/1 × SP 670-F2	9	1973	352	17,8 (9,0 - 30,0)
SP 670a	10	1196	285	23,8 (14,5 - 34,1)
I ab	4	290	66	25,3 (18,3 - 32,7)
Total:	82	12193	1436	11,8

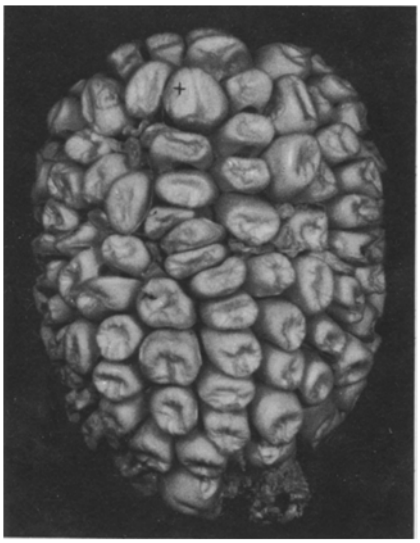

Fig. 1. Appearance and location of grain with two embryos (marked with +) on ear of basic material used for obtaining inbred lines

dolph 1936; Morgan and Rappleye 1951; Yudin and and Hvatova 1967; Tyrnov and Zavashin 1973) and the respective plants as twins (Skovsted 1939; Zimmerman 1953; Nezhevenko and Shumny 1970).

As a rule, embryos in two-embryo kernels are located on opposite sides (Fig. 1). The connection of the common pericarp is in the form of a thickened line. From a kernel of this type two plants germinate and develop, one of which is usually less tall than the other in the early stages of growth, as well as at full maturity. In just a few cases, both plants are of equal height.

Table 1 gives data on the frequency of kernels with two embryos in selfed ears of 12 inbred lines and two F_2 generations. In 1973, in each selected line, kernels with two embryos were found, on average, from 2.1% (with a variation of 0.0 to 3.4%) in the line IT

Table 2. Average dimensions of normal and two embryo kernels

Kernel characteristics	Kernel dimensions in mm		
	length	width	thickness
Normal kernels	95 (83 - 102)	60 (45 - 76)	38 (29 - 56)
Two embryo kernels	98 (88 - 108)	70 (59 - 81)	43 (32 - 54)
Relative to normal kernels (%)	+ 3,2	+ 16,7	+ 13,2

to 25.3% (with a variation of 18.3 to 32.7%) in the line Iab. For the 14 genotypes, a total of 82 ears with 12193 kernels were analyzed, and 1436 kernels with two embryos were found, so that for all genotypes the frequency of kernels with two embryos was determined to be 11.8%.

According to Zimmerman (1953) the frequency of maize twins is 0.04%, while Yudin (1967) gives the frequency of polyembryony in diploid maize as a ratio of 1: 4480; Sarkar and Coe (1966) found twins in the ratio of 1:1040, and Hvatova, 1968 (cited by Nezhevenko and Shumny 1970) obtained haploids in 17% cases of twin maize, using Chaise's monoploids for crossing.

According to the percentage of kernels with two embryos in the ear, the obtained inbred lines were divided into three groups:

1) Low up to 10%;
2) Medium 11 to 20%; and
3) High frequency of over 20% of kernels with two embryos.

The first group includes 5, the second 3 and the third only 2 lines.

The initial material used for raising these inbred lines contained 0.4-8.2% kernels with two embryos. After 10 years we have created inbred lines with 2.1-25.3% of kernels with two embryos (Table 1). This means that the best inbred lines have 8 times the number of kernels with two embryos found on average in the initial material (3.1%).

On the basis of the results, it can be stated that the occurrence of kernels with two embryos is a hereditary characteristic, concurring with Lorenzoni et al. (1971).

The variation in the number of kernels with two embryos per ear for all analyzed inbred lines ranged from 2.1 to 25.3%. However, within lines, the variability in kernels with two embryos ranged from 0.0-3.4% in the line IT to 4.8 - 42.1% in the line SP 669a. Such a frequency probably results from the nature of polyembryony in maize, about which data has already been cited from the literature. Such variability indicates the possibility of increasing the frequency of kernels with two embryos by continued selection in hybrids and other material.

In relation to normal kernels of the same lines, kernels with two embryos show an average increase in kernel dimension (computed for all analyzed lines) of 3.2% in length, 16.7% in width and 13.2% in thickness (Tab.2). They have a considerable increase in protein content (4.0 - 6.0%), lysine g/100 g of dry matter (38.0 - 70.9%), lysine g/100 g of protein, (21.3 - 34.0%) and oil (3.5 - 13.6%)(Tab.3). Opaque-2

Table 3. Chemical composition of normal and two embryo kernels in 2 examined maize lines

Line	Content			
	protein %	lysine g/100 g of dry substance	lysine g/100 g of protein	oil %
SP 650				
Two embryo kernels	14,17	0,637	3,172	5,25
Normal kernels	13,62	0,356	2,614	5,07
Relative to normal kernels (%)	104,0	170,9	121,3	103,5
Iab				
Two embryo kernels	14,77	0,421	2,971	5,65
Normal kernels	13,85	0,305	2,209	4,97
Relative to normal kernels (%)	106,0	138,0	134,0	113,6

Table 4. Metaphase I frequency of aberrations and average number of univalent chromosomes per PMC

Type and plant label	% PMC's with aberrations								Average univalent chromosomes per PMC							
	0-10	15	20	25	30	35	40	45	0,0-0,2	0,4	0,6	0,8	1,0	1,2	1,4	
Higher plant (22)	5	9	5	1	1	0	1	0	5	11	4	1	0	1	0	
Lower plant (22)	1	8	5	3	2	0	1	2	1	10	5	2	2	1	1	
Equal plant height (10)	1	3	2	2	2	0	0	0	0	5	4	1	0	0	0	

Table 5. Anaphase I frequency of aberrations and average number of lagging chromosomes per PMC

Type and plant label	% PMC's with aberrations							% PMC's with lagging univ. chromosomes							
	0-5	10	15	20	25	30		0,01-0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8
Higher (22)	15	4	1	1	0	1		19	1	0	1	0	0	1	
Lower (22)	12	7	2	0	1	0		19	1	1	0	0	0		1
Equal height (22)	2	1	2	1	3	1		4	2	2	2				

corn, with 9.0 - 10% of protein in the endosperm, contains 63 to 122 percent more lysine than do normal kernels of the same line (Pešev et al. 1973).

Cytological Investigations

The behaviour of chromosomes at the first meiotic division has been studied in 80 plants, mostly representing both analyzed plants originating from the same kernel.

In all plants for which meiosis was studied, as well as in the meristem root tissue of 200 selected kernels with two embryos, it was found that the number of chromosomes was normal, 2n = 20.

The presence of univalent chromosomes was noticed at metaphase I (Tab.4); their number per cell ranged from 2 to 8, but the majority contained 2 to 4. Of 68 plants in which metaphase I was analyzed, only one lacked univalent chromosomes. Otherwise, the frequency of univalent chromosomes per plant ranged between 5% and 45%.

At anaphase I (Tab.5), besides lagging univalent chromosomes, the formation of bridges with or without fragments and early passing of individual chromosomes to the poles, preceding the anaphase groups, were observed. Aberrant anaphases were not found in 3 out of 68 analyzed plants. The frequency of aberrant anaphases I ranged from 1% to 26%. The number of lagging univalent chromosomes at anaphase I ranged between 1 and 10, and mostly from 1 to 4. Of 68 plants analyzed at anaphase I, lagging univalent chromosomes were not found in 32 plants (47%).

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