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# Pseudogamous Apomixis in Maize and Sorghum in Diploid-Tetraploid Crosses

**Marina Tsvetova[1], Lev Elkonin[1,*] and Yulia Italianskaya[1]**

[1]Agricultural Research Institute of South-East Region, Department of Biotechnology, Saratov, 410010, Russia.

[*]Corresponding Author: Lev Elkonin. Email: lelekonin@gmail.com.

**Abstract:** Apomictic seed development is a complex process including formation of unreduced embryo sac, parthenogenetic embryo development from the egg cell, and endosperm formation either autonomously, or due to fertilization of polar nuclei by the sperm (under pseudogamous form of apomixis). In the latter case, an obstacle to the normal endosperm development is disturbance of maternal (m) -to-paternal (p) genomic ratio 2m: 1p that occurs in the cases of pollination of unreduced embryo sac with haploid sperms. Usage of tetraploid pollinators can overcome this problem because in such crosses maternal-to-paternal genomic ratio is 4m: 2p that provides formation of kernels with plump endosperm. Using tetraploid lines as pollen parents we observed formation of plump kernels on the ears and panicles of diploid maize and sorghum accessions. These kernels had hybrid endosperm and diploid maternal-type embryo or hybrid embryo with different ploidy level (2n, 3n, 4n). The frequencies of plump kernels on the ear ranged from 0.2-0.3% to 5.7-6.2% counting from the number of ovaries. Maternal-type plants were found in two maize lines, their frequency varying from 10.7 to 37.5% of the progeny plants. In CMS-lines of sorghum pollinated with tetraploid sorghum accessions, the frequency of plump kernels ranged from 0.6 to 14.0% counting from the number of ovaries; the frequency of maternal-type plants varied from 33.0 up to 96.1%. The hybrid nature of endosperm of the kernels that gave rise to maternal-type plants has been proved by marker gene expression and by SDS-electrophoresis of endosperm proteins. These data testify to variable modes of seed formation under diploid × tetraploid crosses in maize and sorghum both by amphi- and by apomixis. Therefore, usage of tetraploid pollinators might be a promising approach for isolation of apomixis in maize and sorghum accessions.

**Keywords:** Interploidy crosses; genomic balance; unreduced embryo sac; pseudogamous apomixis; *Zea mays* L; *Sorghum bicolor* (L.) Moench

## 1 Introduction

Apomixis is the mode of reproduction in which a seed develops from an unreduced embryo sac (ES), and endosperm arising from fertilized (pseudogamous apomixis) or unfertilized nuclei of a central cell of ES (autonomous apomixis). Research on this type of reproduction is closely related to questions of evolution, biosystematics, speciation, gene drift, and plant developmental biology [1-4]. Apomixis may be of great importance for plant breeding, as it makes it possible to preserve heterotic or another superior genotypes of agriculturally valuable plants through multiple generations without loss of vigor or genotypic segregation [5-8]. Over the last 20 years, interests of researchers have been focused on the molecular mechanisms of apomixis. These studies identified genes regulating development of ovule, embryo sac, embryo, and endosperm [9]. Candidate genes for different components of apomixis have been identified in a number of species [10-13]. However, in spite of the attention to this problem and numerous attempts to construct apomixis *de novo* in agriculturally important crops, significant progress has not been achieved [7,14-16]. Therefore, investigations on apomixis are relevant, especially since natural apomictic systems can validate the function of candidate genes for apomixis [14].

Despite many efforts, natural unreduced gametophytic apomixis has not been detected or introduced in an important crop such as maize (*Zea mays* L.). Traditionally, the technique used to create apomictic accessions is introgressing apomixis into sexual species from apomictic wild relatives. In maize, the tetraploid species *Tripsacum dactyloides* L. was used as the donor of apomixis [17-19]. Apomictic hybrids obtained in these crosses contained the *Tripsacum* chromosomes that negatively impacted agronomically important traits tightly linked to the trait. Therefore, producing of economically valuable agamic maize has failed [20,21]. It must be noted, that in contrast to the numerous efforts directed to studying apomixis in maize × gamagrass hybrids, fewer resources have been directed towards investigation of diploid apomixis in *Zea mays* L.

The prerequisites of apomixis in maize have been demonstrated in different studies. Cytoembryological investigation showed a diplosporous mode of unreduced embryo sac formation [22]. Mutants were obtained with disturbed chromosome segregation during I meiotic division resulting in a mitosis-like division and subsequent formation of unreduced egg cells [23,24]. These phenotypes resembled diplospory. Additionally, it has been demonstrated that down-regulation of two DNA methyltransferase genes *dmt*102 and *dmt*103 in maize resulted in formation of multiple ESs in the ovule, ESs with supernumerary gametes, and, apparently, unreduced egg cells [25], that are important prerequisites of apomixis.

In sorghum, the presence of aposporous and, rarely, diplosporous ESs have been described [26-29]. In the progeny of lines selected for a high frequency of aposporous ESs, parthenogenetic embryos and maternal-type plants were found [30-32].

In the majority of higher plants, the absolute requirement for normal seed development is a maternal: paternal genome ratio (2m:1p) in the endosperm, due to specific imprinting of gametic nuclei [33-36]. Deviations from this ratio occurring as a result of interploid crosses, lead to embryo or seed abortion. This phenomenon has been well studied in *Zea mays* L., which belongs to species highly sensitive to departure from this genome ratio [37-39]. Fertilization of reduced (haploid) ES containing two haploid polar nuclei, with diploid sperm cells results in triploid embryo and tetraploid endosperm. Such endosperm degenerates because of the disturbed genome ratio. However, fertilization of diploid polar nuclei of unreduced ES by diploid pollen of tetraploid may result in emergence of hexaploid endosperm with the 2m:1p ratio, that provides the normal seed development. Apparently, this phenomenon takes place in case of pseudogamous apomixis when unreduced ES with two polar nuclei is fertilized by diploid sperms, and an embryo develops as a result of parthenogenesis of an unfertilized diploid egg cell.

Diploid-tetraploid crosses were used as a tool for identification of unreduced ESs, which are the important prerequisite of apomixis, in several plant species [40-43]. We performed a series of these crosses to identify gametophytic apomixis in inbred lines of maize. In literature, there are only few reports, in which tetraploids were used as pollen parents with the aim to reveal apomictic maize accessions [17,44,45]. However, in these studies, maternal plants used in crosses derived from maize-*Tripsacum* hybrids. Previously, we have briefly described obtaining of diploid maternal-type plants in the progeny of diploid maize plants pollinated by tetraploid maize accessions [46]. In this paper, we present detailed description of our experiments on diploid-tetraploid crosses in maize, and present additional experimental data on interploid crosses in the grain sorghum lines, which suggest the existence of unreduced pseudogamous apomixis in these economically important crops.

## 2 Material and Methods

### *2.1 Plant Material and Crossings*

Three inbred maize lines (KM, V47 and HLG 1258) were used in crosses as maternal parents. Among these lines, KM contains specific genetic markers *a B Pl R* that cause anthocyanin coloration of aleurone, leaf sheath and other organs of the $F_1$ hybrid plants (auricles, blades, culm, ear, husks, glumes, anthers, coleoptile, brace roots, and grains) obtained with the lines possessing the dominant *A1* gene [47]. By themselves, the plants of this line have light brown coloration of stem, glumes, husks of ears, yellow

anthers, and yellow medium-sized kernels. Seeds of this line were provided by Dr. V.S. Tyrnov (Saratov State University, Saratov, Russia). V47 and HLG 1258 also do not contain genes determining anthocyanin coloration. Seeds of these lines were provided by Dr. V.I. Zhuzhukin (Agricultural Research Institute of South-East Region, Saratov, Russia). Plants of these lines have green coloration of stem, leaf blade, auricle, tassel, husks, white midrib and clearly distinguished from $F_1$ hybrids, if these hybrids were obtained with the lines possessing the dominant *A1* gene.

As paternal parents, tetraploid lines Tetra-Paryi (TP) (provided by Dr. A.N. Zavalishina from Saratov State University, Russia) and Chernaya Tetra (ChT) (provided by Dr. E.B. Hatefof from Agricultural Research Institute of Kabardino-Balkarya, Russia) were used. These lines have green coloration of stem, glumes, and husks of ears. Anthers of TP have weak anthocyanin coloration; anthers of ChT are yellow. TP has large yellow kernels. ChT has large black or dark maroon kernels. Both paternal lines have dominant *A1* gene, and $F_1$ hybrids of these lines with KM as maternal parent are characterized by intensive purple coloration.

All maternal lines and pollinators were grown in the experimental field of Agricultural Research Institute of South-East Region in the plots isolated from other maize sowings. In all maternal plants, the tassels were carefully removed before anthesis, and additionally all the ears were bagged to prevent contamination.

In experiments with sorghum (*Sorghum bicolor* (L.) Moench), the lines with stable cytoplasmic male sterility (CMS) were used as female parents: $A_3$ Feterita-14, M35-1A Karlikovoye beloye (M35-1A KB), $A_2$ KVV-181, and iso-nuclear lines with Zheltozyornoe-10 (Zh10) genome in the $A_3$ and 9E CMS-inducing cytoplasms. These lines were previously obtained by us and were taken from our laboratory collection. The tetraploid lines NT originated from the line Negrityanskoye 3366/2, and AST, originated from the line AS-1-30, were used as paternal parents. Tetraploids were obtained by us previously by shoot meristem treatment with 0.2% colchicine solution. Panicles of maternal sorghum plants were carefully bagged before anthesis.

Plump kernels, obtained from diploid-tetraploid crosses were cut into two parts. The embryo with the part of endosperm was placed on the moister filter paper for germination, while the other part of the kernel was used to study endosperm proteins by SDS-electrophoresis. Seedlings were planted in plastic pots containing garden soil, and after formation of well-developed root system, were transplanted into the field where the phenotypes were evaluated.

### *2.2 Endosperm Protein Analysis*

To confirm hybridity, the analysis of endosperm proteins by SDS-PAG electrophoresis (SDS-PAGE) in reducing conditions was performed. The part of kernel without embryo was ground in the sample buffer (62.5 mM TRIS_HCl, pH 6.8, 20% glycerol, 2% SDS, 5% β-mercaptoethanol). SDS-PAGE was carried out in the 12.5 % (w/v) acrylamide separating gel (0.375 M TRIS_HCl, pH 8.8) and 4% stacking gel (0.125 M TRIS, pH 6.8) according to modified Laemmli method [48]. The gels were stained with Coomassie Brilliant Blue G-250.

### *2.3 Chromosome Counts*

For determination of seedling ploidy the root tips were fixed in 3:1 ethanol: acetic acid, then were treated with HCl (3.5% for 15 min, then 50% for 20 min), washed with distilled water for 2 min, then they were treated with acetic acid (45%) for 20 min, and stained with acetohematoxiline (2%) for 30-45 min. Squashes were prepared using mixture of 45% acetic acid with 70% chloral-hydrate (1:1) colored with some drops of acetohematoxiline.

### *2.4 Analysis of Pollen*

Spikelets from different branches of panicles were fixed in acetic alcohol (1:3), and then stored in 75% alcohol. Pollen grains were stained with 1% $I_2$-KI. Squashes for analysis of pollen were prepared

from the anthers evoked from several individual flowers from different branches. Black pollen grains were presumed to be fertile. To analyze pollen from the male flowers developed on the ears, the anthers were evoked from the dried-up flowers and squashes were prepared and stained similar to the ones prepared from the fixed material. Pollen grain measurements were made with the usage of the A1 Axioscope coupled with AxioCam camera and Axiovision software (Carl Zeiss, Germany).

### *2.5 Analysis of Phenotypes*

Plant traits were evaluated starting from the seedling stage up to full maturity. Anthocyanin coloration was recorded in seedlings, stem, nodes and internodes, leaf blades, ligule, and leaf midrib. A shape, length and density of tassel, coloration of glumes, anthers, and silks were noted at blooming. At full maturity the coloration of husks of ears, kernels and ears was noted. The proportions of maternal-type plants in different progenies were compared by Fisher's method using *F*-criterion [49].

## 3 Results

### *3.1 Maize*

Pollination of diploid plants by tetraploids resulted more often in shriveled kernels (Fig. 1). In addition, in all the lines the ears with plump kernels were observed, the frequency of such ears being from 43 to 67% counting from the number of pollinated ears. The frequencies of plump kernels on the ear varied from 0.2-6.2% counting from the number of ovaries. More often there were 1-5 plump kernels on the ear, while in KM line few ears with several dozen of kernels were observed (Tab. 1). Among the 45 control ears, which were bagged but not pollinated, no kernels were produced.

All the ears were carefully examined for presence of male flowers, which could be the reason of seed set on the bagged ears. Among 215 ears, six ears found in the lines KM and V47, had male flowers. However, the pollen of five of them was completely sterile (Fig. 2) and one had underdeveloped and shrunken anthers with no pollen.


**Figure 1:** Ears of diploid maize lines pollinated by tetraploid pollen parents. A -HLG 1258 × Tetra-Paryi; B -HLG 1258 × Chernaya Tetra; C -KM × Tetra-Paryi; D -KM × Chernaya Tetra. ↙ defective kernels, ⇨ plump kernels

**Table 1:** Frequency of plump kernels developed on ears of maize inbred lines pollinated by tetraploid lines Terta-Paryi (TP) and Chernaya Tetra (ChT)

| Cross combination | Number of pollinated ears | Number of Ears with Plump Kernels | | | | |
|---|---|---|---|---|---|---|
| | | Total | Number of kernels per ear | | | |
| | | | 1-5 | 6-15 | 16-30 | ≥30 |
| KM ×TP | 76 | 35 (46.0) [a] | 24 | 8 | 1 | 2 |
| KM × ChT | 43 | 29 (67.4) | 18 | 6 | 2 | 3 |
| V47×TP | 38 | 18(47.4) | 14 | 2 | 2 | - |
| V47 × ChT | 5 | 3 (60.0) | 2 | 1 | - | - |
| HLG 1258 × TP | 39 | 17 (43.6) | 17 | - | - | - |
| HLG 1258 × ChT | 14 | 6 (42.9) | 6 | - | - | - |
| Total in crosses with TP | 153 | 70 (45.8) | 55 | 10 | 3 | 2 |
| Total in crosses with ChT | 62 | 38 (61.3) | 26 | 7 | 2 | 3 |

[a] percentage of the number of pollinated panicles

The kernels set on the ears pollinated by the line ChT were dark-colored due to genes determining anthocyanin pigmentation of the aleurone layer (*A1, R*) [47] (Figs. 1(B), 1(D)). The kernels resulted from pollination by the line TP, lacking genes for anthocyanin coloration, were yellow resembling the maternal lines V47 and HLG 1258 (Fig. 1(A)). The kernels developed on the ears of the line KM had black coloration (Fig. 1(C)) because this line has genotype *aBPlR,* which condition anthocyanin coloration of aleurone layer in the presence of the dominant gene *A1* [47].

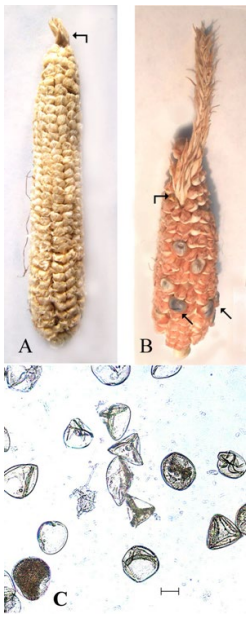


**Figure 2:** Ears of the maize with male flowers and its pollen. A -unpollinated ear of HLG 1258. No kernels were formed. B -Ear of V 47 pollinated by ChT. Plump and shriveled kernels have black coloration testifying to their hybrid origin; C -pollen from the male flower of HLG 1258; bar 50 μm; ↱ male flowers; ↖ plump kernels

Cytological and phenotypic analysis of the plants developed from the plump kernels showed that among them there were di-, tri-, and tetraploid hybrids, and diploid plants of the maternal type (Fig. 3, Tab. 2). Hybrid and maternal plants in the progeny of the line KM pollinated by tetraploids were easily distinguished

because both the diploid and tetraploid $F_1$ hybrids with this line (KM/TP, KM/ChT) had intensive anthocyanin coloration in roots, hypocotyls, and coleoptiles already at the seedling stage. Later this coloration appeared in the stem, midrib, glumes, anthers, and husks of the ears (Figs. 4(A), 4(C)). However, in some diploid plants, developed from the plump kernels, obtained in these crossings, this coloration was absent (Figs. 4(B), 4(D)), and their phenotypes did not differ from that of the maternal parent.

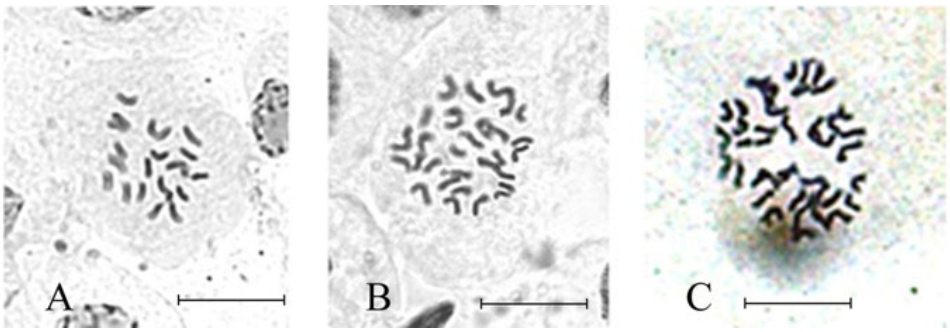


**Figure 3:** Metaphase plates of the $F_1$ hybrid plants KM/TP (**A**-2n, **B**-3n) and V 47/TP (**C**-4n); bar 20 μm

**Table 2:** Characterization of the progeny plants obtained from the plump kernels developed on the ears of the maize inbred lines pollinated by tetraploid lines Terta-Pariy (TP) and Chernaya Tetra (ChT)

| Cross combination | Number of progeny plants | | | | |
|---|---|---|---|---|---|
| | Total | Hybrids with ploidy | | | Maternal plants, 2n |
| | | 2n | 3n | 4n | |
| KM ×TP | 64 | 20 | 6 | 14 | 24 (37.5)[a] |
| KM × ChT | 53 | 16 | 17 | 15 | 5 (9.4) |
| V47×TP | 22 | 1 | 4 | 17 | 0.0 |
| V47 × ChT | 5 | 4 | 1 | 0 | 0.0 |
| HLG 1258 × TP | 28 | 2 | 7 | 16 | 3 (10.7) |
| Total in crosses with Tetra Paryi | 114 | 23 | 17 | 47 | 27 (23.7)** |
| Total in crosses with ChT | 58 | 20 | 18 | 15 | 5 (8.6) |

[a] Percentage of studied plants; ** $p \leq 0.01$ according to *F*-criterion, in comparison with crosses with ChT.

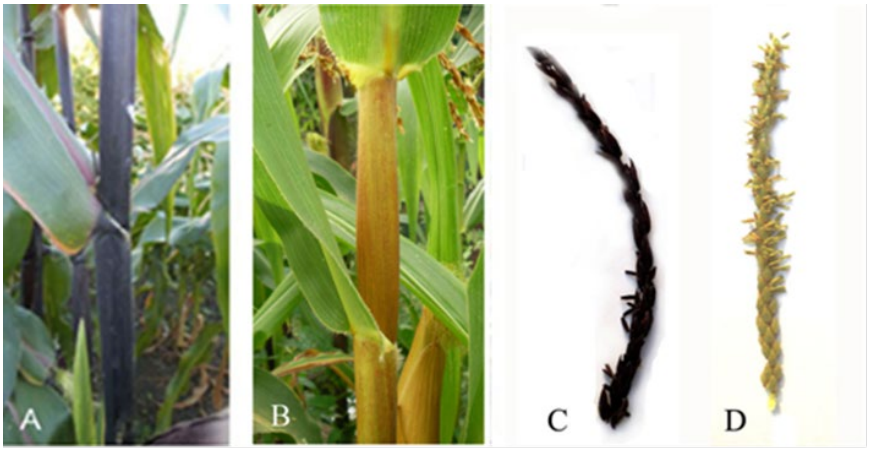


**Figure 4:** Phenotypes of hybrid (**A, C)** and maternal-type (**B, D)** plants in the progeny KM × ChT. Note anthocyanin coloration of leaf, sheath, and midrib (**A**), panicle branch and anthers (**C**) of hybrid plant; **B**, **D**-absence of anthocyanin coloration in maternal-type plant

Maternal-type plants have been identified also in the progenies, obtained from the crosses HLG 1258 × TP. Diploid hybrid plants differed from the maternal-type plants by the presence of anthocyanin in the seedlings, stems, leaves, while maternal-type plants had no this coloration. These data suggest that the kernels, which produced the maternal-type plants, formed on the basis of the unreduced embryo sacs, which were fertilized by diploid pollen of the tetraploid parent. Therefore, endosperm of the plump kernels should have a hybrid nature. Among the progenies obtained in the crosses of V47 with TP and ChT, maternal-type plants were not found.

To prove this hypothesis SDS-electrophoresis of endosperm proteins was performed to demonstrate the hybrid origin of the endosperm. As can be seen in Fig. 5, the electrophoretic spectrum of proteins isolated from the endosperm of the plump kernel formed on the ear of the KM line pollinated by the tetraploid line ChT produced maternal-type plant, clearly differed from the spectra of the maternal line. The pattern of protein bands in the endosperm of this kernel was characteristic to paternal line. These data verify the hybrid origin of endosperm in such kernels and convincingly prove that these kernels arose by pseudogamous apomixis, in which an embryo forms in result of parthenogenesis of unreduced egg-cell, and endosperm develops due to fertilization of polar nuclei.

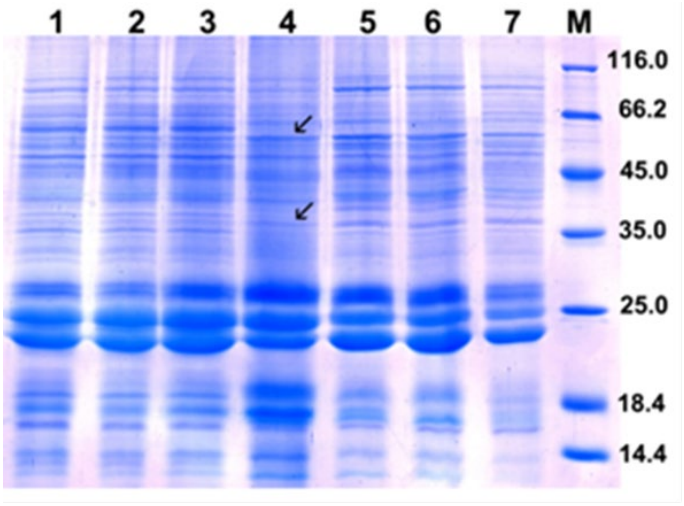


**Figure 5:** SDS-PAGE of endosperm proteins of the plump kernel developed on KM ears pollinated by tetraploid line ChT. 1-3 -KM; 4 -KM × ChT; 5-7 -ChT; M-molecular weight makers (kDa). Proteins characteristic to the paternal line are marked by arrows

### *3.2 Sorghum*

In CMS-lines $A_3$ Feterita 14 and M35-1A KB, no kernels were produced upon pollination by tetraploids. In other lines, the plump and shriveled kernels set on the panicles. In the panicles of $A_3$ Zh10 pollinated by tetraploids, the plump kernels were found that was never observed in unpollinated control panicles (Tab. 3). The number of panicles with plump kernels of CMS-line 9E Zh10 significantly increased compared with an unpollinated control where only 1-3 kernels were found on two among 30 bagged panicles (Tab. 3). In the line $A_2$ KVV-181, no kernels developed in the control unpollinated panicles, while in the pollinated panicles the plump kernels were found (Tab. 3). The frequencies of plump kernels on the pollinated panicles varied in $A_2$ KVV-181 from 1% to 3.3%; in the $A_3$ and 9E Zh10 -from 0.6% to 14% counting from the number of ovaries.

In the progeny obtained from the plump kernels, the diploid hybrid and maternal-type plants were observed (Tab. 4). The latter were identical in their phenotype to the maternal CMS-lines $A_2$ KVV-181 or $A_3$ or 9E Zh-10 in height, coloration of leaves, midribs, glumes, panicle shape, male sterility, presence of awns, etc. The shriveled kernels did not germinate.

To reveal the origin of plump kernels, the SDS-electrophoresis of endosperm proteins was performed

as it was done in maize. As can be seen in Fig. 6, in the electrophoretic spectrum of proteins isolated from the endosperm of the plump kernel formed on the 9E Zh10 panicle pollinated by the tetraploid line NT, from which the maternal-type plant was obtained, the proteins characteristic to the paternal line are clearly seen. In addition, the proteins that were not observed in the parental lines, which had arisen, possibly, as a result of the interaction of the parental genomes, are also visible. Therefore, the endosperm of this kernel developed as a result of fertilization. Assuming that this kernel produced maternal-type plant it is evident that this kernel originated by pseudogamous apomixis. At the same time, the electrophoretic spectrum of endosperm proteins of another kernel presented in the track 3 is completely identical to spectrum of maternal line (track 1). Such similarity allows to assume that this kernel developed by self-pollination due to occasional restoration of male fertility or, with caution, by autonomous apomixis.

**Table 3:** Frequency of plump kernels developed on panicles of CMS sorghum lines pollinated by tetraploid lines NT and AST

| Crossings | Number of pollinated panicles | Number of panicles with plump kernels | | | | |
|---|---|---|---|---|---|---|
| | | Total | 1-5 | 6-15 | 16-30 | ≥ 30 |
| $A_2$ KVV-181 × NT | 33 | 4 (12.1)[a] | 2 | 2 | - | - |
| $A_2$ KVV-181 × AST | 8 | 4 (50.0) | 4 | - | - | - |
| $A_2$ KVV-181, control | 30 | 0 | - | - | - | - |
| $A_3$ Zh10 × NT | 19 | 3 (15.8) | 3 | - | - | - |
| $A_3$ Zh10 × AST | 4 | 2 (50.0) | 2 | - | - | - |
| $A_3$ Zh10, control | 30 | 0 | - | - | - | - |
| 9E Zh10 × NT | 17 | 10 (58.8) | 7 | - | 3 | 1 |
| 9E Zh10 × AST | 8 | 8 (100.0) | 2 | 2 | 2 | 2 |
| 9E Zh10, control | 30 | 2 | 2 | - | - | - |
| Total in crosses with NT | 69 | 18 (26.1) | 12 | 2 | 3 | 1 |
| Total in crosses with AST | 20 | 14 (70.0)*** | 8 | 2 | 2 | 2 |

[a] percentage of the number of pollinated panicles; *** $p \leq 0.01$ according to *F*-criterion, in comparison with crosses with NT

**Table 4:** Characterization of the progenies obtained from the plump kernels developed on the panicles of the CMS sorghum lines pollinated by tetraploids

| Crossings | Number of diploid plants | | |
|---|---|---|---|
| | Total | Hybrid | Maternal |
| $A_2$ KVV-181 × NT | 10 | 2 | 8 (80.0)[a] |
| $A_2$ KVV-181 × AST | 9 | 9 | - |
| $A_3$ Zh10 × NT | 9 | 6 | 3 (33.3) |
| $A_3$ Zh10 × AST | 3 | 2 | 1 (33.3) |
| 9E Zh10 × NT | 51 | 2 | 49 (96.1) |
| 9E Zh10 × AST | 92 | 33 | 59 (64.1) |
| Total in crosses with NT | 70 | 10 (14.3)*** | 60 (85.7)*** |
| Total in crosses with AST | 104 | 44 (44.2) | 60 (57.7) |

[a] percentage of the number of progeny plants; *** $p \leq 0.001$, according to *F*-criterion, in comparison with crosses with AST

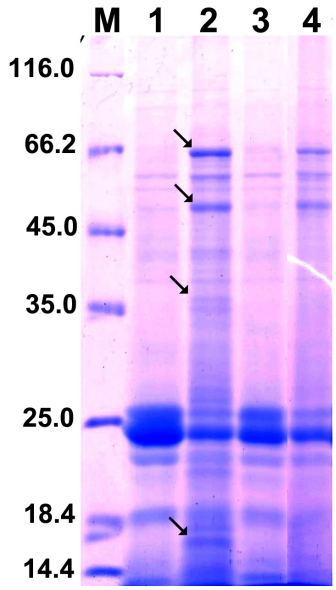


**Figure 6:** SDS-PAGE of endosperm proteins of the plump kernels developed in 9E Zh10 panicles pollinated by tetraploid line NT. 1 -9E Zh10; 2, 3 -9E Zh10 × NT; 4 -NT. M-molecular weight makers (kDa). In the track 2, the proteins of paternal line NT and the proteins which are absent in both parental lines, are indicated by an arrow (↘). In the track 3, the pattern of bands is identical to the maternal parent

## 4 Discussion

Diploid-tetraploid crosses are known to be an efficient tool for isolation of unreduced egg cells in a number of species [41-43]. Development of tetraploid hybrids originated as a result of such crosses verifies the presence of unreduced embryo sacs. In our experiments with maize, in all three lines pollinated by tetraploid parents, tetraploid hybrids were found. Therefore, formation of unreduced embryo sacs in inbred maize lines is a relatively frequent phenomenon. We also obtained tetraploid maize hybrids in eight other maize lines and three $F_1$ hybrids pollinated by the pollen of ChT and TP (data not shown here).

In addition, the results of our experiments demonstrate possibility to obtain maternal-type plants in such crosses, as well as diploid and triploid hybrids. Such diversity suggests different pathways of seed formation in such crosses (Fig. 7). In normal diploid × diploid crosses, haploid (reduced) sexual ES with two haploid polar nuclei is fertilized by two haploid sperms. As a result, triploid endosperm formed with maternal-to-paternal genomic ration 2m:1p and diploid embryo (Fig. 7(A)). When reduced sexual ES is fertilized by pollen grain with diploid sperms developed in tetraploid line, the seed is formed with a triploid embryo and tetraploid endosperm (Fig. 7(B)). Such endosperm has the maternal-to-paternal genomic ratio deviating from 2m:1p, which is necessary for normal endosperm formation in cereals. In result, the kernels with tetraploid endosperm are shriveled and have low germination capacity [37-39]. Such kernels were obtained in all combinations of crosses.

Fertilization of unreduced (sexual) ES by pollen grain with diploid sperms produces the seed with tetraploid embryo and hexaploid endosperm (Fig. 7(C)). Endosperm in such seed have balanced maternal-to-paternal genomic ratio (2m:1p = 4n:2n), and the seed is plump and viable.

Unreduced (apomictic) ES being fertilized by pollen grain with diploid sperms, produce the seed with diploid parthenogenetic embryo and hybrid hexaploid endosperm (pseudogamy, Fig. 7(D)). Endosperm in this seed have balanced maternal-to-paternal genomic ratio (2m:1p = 4n:2n) and such seed is plump and viable. The presence of maternal-type diploid plants in the progenies of crosses might suggest the existence of apomictic diploid ESs in studied lines of maize and sorghum.

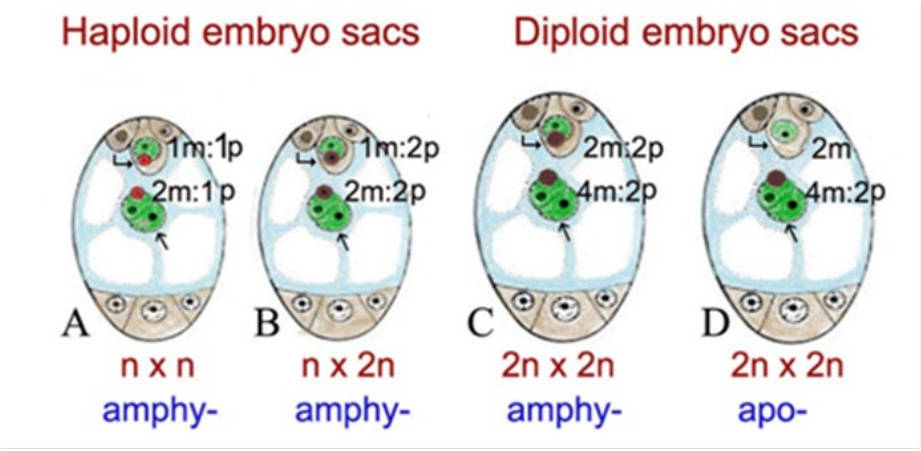


**Figure 7:** Modes of seed formation in diploid-tetraploid crossings. **A**-Reduced sexual embryo sac (ES) fertilized by pollen grain with haploid sperms produce the seed with diploid embryo and triploid endosperm, with maternal to paternal genomic ration 2m:1p; **B**-Reduced sexual ES fertilized by pollen grain with diploid sperms produce the seed with triploid embryo and tetraploid endosperm (2m:2p); **C**-Amphimictic unreduced sexual ES fertilized by pollen grain with diploid sperms produce the seed with tetraploid embryo and hexaploid endosperm with balanced maternal-to-paternal genomic ratio (2m:1p = 4n:2n), these seeds are plump and viable; **D**-Unreduced (apomictic) ES fertilized by pollen grain with diploid sperms produce the seed with diploid parthenogenetic embryo (2m) and hexaploid endosperm(2m:1p = 4n:2n) (pseudogamous apomixis). ● -haploid sperm, ● -diploid sperm. ↳ egg cell; ↖ polar nuclei

It should be noted that diploid parthenogenetic maize plants were obtained previously in diploid × tetraploid crosses with much lower frequency by Sarcar and Coe [41]. They explained this as doubling or by fusion of two reduced female nuclei, which are known to be rare phenomena. In our experiments, the frequency of these plants was much higher possibly because they developed by another mechanism, namely, by pseudogamous apomixis.

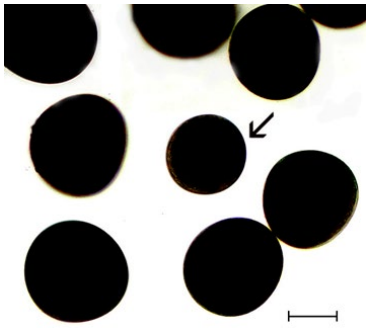

**Figure 8:** Pollen of the maize tetraploid line Chernaya Tetra. Haploid pollen grain is marked by an arrow. Bar 50 μm

Remarkably, in the progeny of diploid-tetraploid crosses, we found the diploid hybrids. These data indicate that in the pollen of tetraploid plants there were haploid pollen grains that produced diploid hybrids. There are reports on haploid pollen grain formation in tetraploids due to somatic reduction [50-53]. In this study, the analysis of pollen of tetraploid maize plants confirmed this possibility. In maize tetraploids, the diameter of diploid pollen grains usually ranges from 100 μm up to 126 μm, whereas in diploid plants haploid pollen grains range from 85 μm up to 106 μm. In the pollen of tetraploids used in our study, pollen grains with diameter characteristic to haploid ones have been revealed (Fig. 8). These data explain formation of diploid hybrids in our 2n × 4n crosses.

It should be noted that progeny of V47 maize line pollinated by tetraploids consisted only from 2n, 3n, and 4n hybrid plants, which have arisen by amphimixis. The line V47, most likely, has genetic factors providing relatively high frequency of sexual unreduced ESs. The mode of their formation is a subject of future research. The absence of maternal plants in crosses with this line could be explained by the absence of the genetic factor(s) governing parthenogenesis in the genome of V47.

Remarkably, in our experiments we observed both diploid maternal plants and tetraploid hybrids in the progeny of one and the same plant or even in the progeny of one and the same ear. Usually, at the facultative apomixis, in one and the same plant, apomictic progeny develops from unreduced ESs, while reduced ESs resulting from meiosis are involved in fertilization and give sexual progeny. In this regard, it should be noted that in our material, ESs of one and the same ploidy level (unreduced ESs) behaved in different manner: some of them expressed parthenogenetic ability while the others were fertilized. In this connection, important questions remain to be answered: whether the unreduced ESs of the studied lines have different pathways of formation and, as a consequence, were tended to amphi- or apomixis, or the same unreduced ESs were able both to parthenogenesis and fertilization.

Thus, experimental data reported in this paper indicate that the ability to create apomictic kernels is quite common to some maize and sorghum lines. Use of tetraploid pollinators is a promising approach for isolation of apomixis and can be used both in natural specimens and artificially genetically constructed plants.

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