

Chromosome Elimination in Intergeneric Hybrid of *Oryza sativa* × *Luziola peruviana*

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Received 7 October 2014; revised 6 November 2014; accepted 26 November 2014

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Abstract

Oryza sativa and *Luziola peruviana* present a diploid chromosome number of $2n = 24$ and basic number $x = 12$, confirmed by means metaphase chromosomes counts in young root tips of these species. Hybrid plants *O. sativa* × *L. peruviana*, with $2n = 24$ chromosomes are originated from simple crosses and present abnormalities in the meiotic behavior, chromosomal aberrations and cytological alteration. This genetic incompatibility is caused by different factors as absence of pairing and recombination, different spindles arrangements, cytoskeleton instability, apoptosis process and chromosomal elimination, leading to micronuclei formation, unbalanced gametes and sterile pollen grains. The chromosome elimination is established as a dynamic process of stabilization of the genome that occurs during hybridization. It is a common phenomenon among intergeneric crosses and corresponds to cytoplasmic and nuclear bodies that reflect chromosomal aberrations resulting from the combination of two genomes with high genetic distance. The genomic conflict occurs in meiosis, possibly by asynchronism and cell cycle length of the genomes involved, or by time differences in replication between parental species leading to strand breaks and genomic rearrangements.

Keywords

Rice, *Luziola*, Unbalanced Gametes, Meiosis

1. Introduction

Luziola genus presents unisexual flowers, the stamens and pistils in separate inflorescences [1]. *Luziola peruviana* presents a diploid chromosome number of $2n = 24$ [2]. The *Oryza* genus presents bisexual flowers, sta-

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How to cite this paper: Moreno, P.E., Caetano, C.M., Olaya, C.A., Agrono, T.C. and Torres, E.A. (2014) Chromosome Elimination in Intergeneric Hybrid of *Oryza sativa* × *Luziola peruviana*. *Agricultural Sciences*, 5, 1344-1350.
<http://dx.doi.org/10.4236/as.2014.513144>

mens and pistils in the same inflorescences, the floral structure only permit anthesis after it has been released pollen on the stigma [3] [4]. Mr. Kuwada in 1910 reported by first time the chromosome number in *O. sativa* diploid, $2n = 24$ chromosomes [5]. The metaphase chromosomes counts in young root tips of *O. sativa* and *L. peruviana* presented that are diploid species with a basic number $x = 12$, like other grass of the *Oryzaceae* tribe, except *Zizania*, which presents a basic number $x = 15$, 17.

The meiosis consists of two consecutive cell division (meiosis I and meiosis II), without DNA synthesis between them. The meiosis I is a specialized division whose purposed is generate a haploid gamete with reduced number of chromosomes, allowing the exchange of genetic material; the meiosis II is alike to a mitotic division, but the results are four haploid cells forming microspore tetrads [6]. The correct chromosome behavior during meiosis protects the structural integrity of the genome and the right segregation to daughter cells during the cell division [7]. Else, a species with sexual reproduction to be fertile must have a normal meiotic behavior and the degree of meiotic pairing in intergeneric hybrids is a measure of the homology between chromosomes of both species involved.

The intergeneric hybrid F1 between different genomes of rice presents a chromosome pairing level lower that in the parental species, these show abnormalities in the meiotic behavior, chromosomal aberrations, cytological alteration, degeneration of the pollen mother cell (PMC) and sterility in pollen grains [8] [9]. Interspecific crosses in other grasses also present abnormalities in the development of the PMC producing male sterile. These hybrids permit allow deeper understanding of process of the meiosis, and fundamental for promote research over reproduction, fertility, genetic, evolution, phylogenetic relationships and plant breeding [10]. In addition, interspecific hybrids have been used as the main tool for transferring characters of agronomic interest from wild species to cultivated [11].

Chromosome elimination is established as a dynamic process of genome stabilization that occurs during hybridization and has been well explored and investigated in wheat with different kinds of *Poaceae*, among other intergeneric hybrid [12] [13]. Partial or total elimination chromosomes and exchange between parental chromosomes frequently occur during early development of the progeny [14], and present a barrier to the transfer of desirable traits from wild species to cultivated, but may act in favor of crop improvement programs [15] [16]. Chromosome elimination in hybrid is explained by asynchrony during synthesis of nucleoprotein and processes of cell division, multipolar spindles formation, chromosome degradation by specific nucleases, and inactivation of centromeres, incompatibility centromere and kinetochores proteins among parents, nondisjunction of chromosome uniparental during anaphase and micronuclei formation [13] [14] [17]. Other hypotheses pose the separation of the genomes in interphase and metaphase, and elimination nuclear by nuclear extrusion [16].

During meiosis the univalent chromosomes lagging at anaphase I and II, and micronuclei formation during telophase I and II cause meiotic disorders due to failure to enter in the newly nucleus and finally are eliminated [12] [14]. The chromosome elimination by micronuclei formation in intergeneric hybrids is given by the non-segregation of chromosomes or fragments during karyokinesis and the extrusion of chromatin during interphase [15]. The recognition and subsequent removal of DNA way micronuclei appears to be a function of topology specific chromatin that results in the activation of endonuclease and fragmentation of one of the genomes involved [16]. The chromosomes destined to elimination are located peripherally on the metaphase; these chromosomes present small centromere regions that no congregate in metaphase and lagging chromosome at anaphase [15] [16]. These observations are consistent with the classical mechanism of micronuclei formation. This work intended study the chromosomal elimination in intergeneric hybrid *O. sativa* \times *L. peruviana*, for known the stabilization process of hybrid gametes and plants.

2. Materials and Methods

The hybrid plants originated from simple crosses that used male sterile rice as the pollen grains receptor and wild parental *L. peruviana* as pollen donor (Figure 1). The embryo rescue was carried out by research assistant of the Laboratory of Anther Culture of CIAT, calli were regenerated of F1 hybrid plants *O. sativa* \times *L. peruviana* and were subjected to immersion in colchicine solution 0.05% and 0.1% by 48 hours at room temperature to induce to polyploidy, obtaining $2n = 48$ chromosomes. Crosses were performed in greenhouse of Rice Program of International Center Tropical Agriculture (CIAT) located in Palmira—Valle del Cauca $3^{\circ}16'N$, $76^{\circ}32'W$, at an altitude of 965 m.a.s.l. Cytogenetic evaluation was performed in the Laboratory of Light Microscopy of Virology Unit of CIAT.

Immature inflorescences of F1 hybrid *O. sativa* \times *L. peruviana*, with chromosome number $2n = 24$ y $2n = 48$,



Figure 1. Parental and hybrid plant. *L. peruviana* to left, *O. sativa* to right, Hybrid *O. sativa* × *L. peruviana* in the middle.

were fixed in Carnoy solution (absolute ethanol: glacial acetic acid, 3:1) at room temperature during 24 hours, last time the solution was renewed and stored in the refrigerator at 4°C. A flower of fixed material was spread over slides. Anthers were removed and realized a fast flattening over two drops of carmine acetic 1% during 5 minutes; subsequently was warmed quickly over alcohol burner, the artifacts are eliminated. The slide was again heated quickly, placed a small drop of 45% acetic acid over the sample for clarify the cytoplasm, finally was cover with coverslip and observe to microscope (Olympus BX60, Japan).

3. Results

F1 hybrids *O. sativa* × *L. peruviana* presented a drastic modification in the chromosome behavior (**Figures 2(A)-(H)**). Pairing and chromosome recombination between the two species was limited, *i.e.* not corresponding to the homeologous chromosomes due to genetic incompatibility and little evolutionary relationship between the both species. In the nucleus there were two different associations of DNA complexes, probably the two genomes were expressed independently during meiosis. The PMC behavior in the progeny $2n = 24$ chromosomes were varied; differences existing in the chromosome association degree (18-I, 3-II; 20-I, 2-II; 16-I, 4-II; 22-I, 1-II), multiples nucleoli, different spindles arrangements, cytoskeleton instability (**Figure 2(D)** and **Figure 2(E)**), loss of cytoplasm (**Figures 2(A)-(H)**), apoptosis process and pycnotic masses (**Figure 2(G)** and **Figure 2(H)**), micronuclei formation (**Figures 2(C)-(E)**), unbalanced gametes and sterile pollen grains of different size were observed. Despite to all irregularities suffered by PMC, microsporogenesis occurred until pollen grains formation.

During meiosis, laggards chromosomes and micronuclei formation caused meiotic disorders due to failure to enter in the newly nucleus formed, and that finally were eliminated. The chromosomes destined for elimination like micronuclei were located peripherally on the metaphase plate (**Figures 2(C)-(E)**). These chromosomes presented small centromere regions which not properly congregate in metaphase, leading to laggards chromosomes in anaphase [15] [16]; these observations were consistent with the classical mechanism of micronucleus formation.

The chromosome doubling by using colchicine in hybrids $2n = 48$, is a possible mechanism of exchange of genetic material between two species, allowing the production of polyploids karyotypically stable, expression of homology, recombination and synapse of chromosomes, In addition, increases cytoskeletal stability during chromosome segregation and reduces pycnosis and apoptosis processes, so their fertility can be restored with respect to hybrid $2n = 24$. The cell division was abnormal, asynchronous, due to different stage of meiotic division.

Micronuclei formation is a common phenomenon among intergeneric crosses and corresponds to cytoplasmic y nuclear bodies that reflect chromosomal aberrations resulting from the combination of two genomes with high

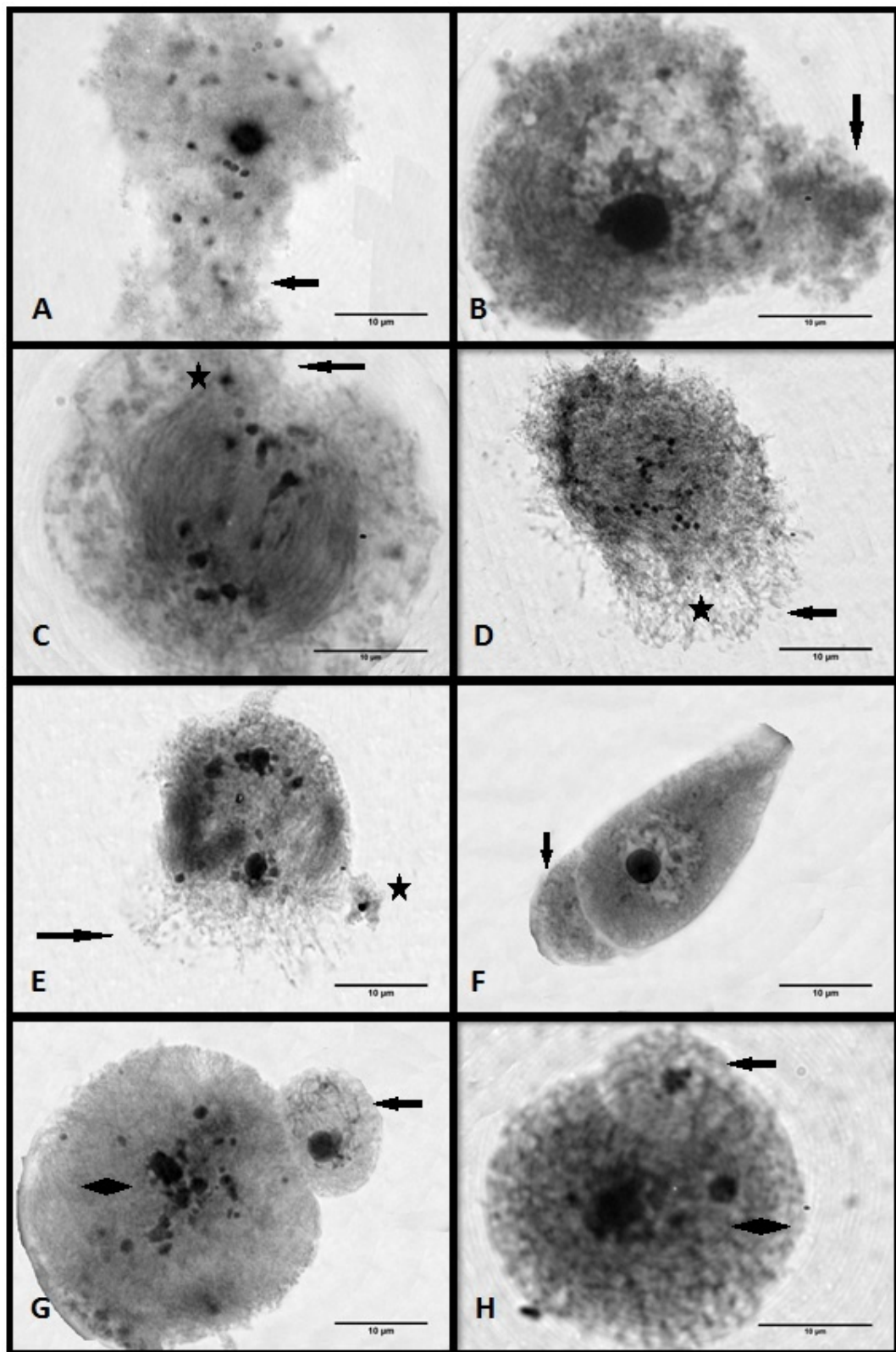


Figure 2. Chromosome elimination; (A), (B) Cytoplasm loss at prophase I; (C)-(E) Cytoskeletal instability at anaphase/telo-phase I; (F)-(H) Nuclear extrusions. ★ Indicate micronuclei. → Indicate cytoplasm loss. ◄ Indicate pycnotic masses.

genetic distance. In hybrids *O. sativa* × *L. peruviana* micronuclei formation was evident; these have different DNA contents and unbalanced microspores formation. The loss of cytoplasm and chromatin repeated at various stages of meiosis, from the beginning of the PMC to the formation of tetrad of microspores, like shown (**Figures 2(A)-(E)**). The chromosome elimination preferentially affects the chromosomes of one of the two species involved; the way and speed of this process, as well as transmission of recombinant chromosomes in following meiotic processes are crucial for the karyotype stabilization of the progeny, and conclude with the stable incorporation of genetic material.

The chromosome deletion is a dynamic process generating genome stabilization in intergeneric crosses by the recognition and subsequent removal of DNA, resulting from the activation of endonucleases and the fragmentation of one of the genomes. F1 hybrids between *O. sativa* × *L. peruviana* presented different mechanisms of chromosome elimination during all cell division. The elimination is explained by the spatial separation of genomes and chromosome elimination by nuclear extrusions like shown (**Figures 2(F)-(H)**), asynchrony during synthesis of nucleoprotein and cell division processes, formation of multipolar spindles, chromosome degradation by specific nucleases, centromere specific inactivation, incompatibility of centromere and kinetochore proteins between parental, uniparental non-disjunction of chromosomes, micronucleus formation by non-segregation of chromosomes or fragments during karyokinesis and extrusion of chromatin.

4. Discussion

Different interspecific crosses between *Oryza* species have abnormal meiotic behavior, a high degree of incompatibility, absence of chromosomal homology, incorrect segregation of chromosomes, cytoskeletal instability, abnormal spindle formation, microcytes formation, chromosome elimination, apoptosis processes, unbalanced microspores, PMC degeneration and sterility in pollen grains [8] [9]. In hybrids *O. sativa* × *L. peruviana*, aberrations during meiosis and complete sterility of pollen grains, cytoskeleton instability, multiple and multipolar spindles, irregular cytokinesis forming unbalanced microspores of different sizes, micronuclei formation, different mechanisms of chromosomal elimination and severe apoptosis processes due to condensation of chromatin that ends forming masses corresponding to pycnotic bodies and subsequently, cell death.

Incompatibility genomic and chromosome elimination is evidenced in many intergeneric hybrids and of equal way was observed in hybrid *O. sativa* × *L. peruviana*, due to some kind of genomic conflict that occurs after hybridization. It is possible that some factors may trigger the deletion for stabilization of the newly formed genome, however, the base of the cellular mechanisms involved in the process of chromosome elimination are poorly understood [13]. Genomic conflict occurs in meiosis, possibly by asynchronism and cell cycle length of the genomes involved, time differences in replication between parental species that leads to strand breaks and genomic rearrangements and that may be of unknown signals of protein synthesis or post-translational changes in existing proteins [12]. The complexity of the events involved in the cell cycle difficult to distinguish between different hypotheses.

The elimination of genetic material occurs in all meiotic stages by different mechanisms, including: absence of chromosomal homology; irregular chromosome segregation, oriented toward different regions of the poles; nuclear extrusions, multiple and multipolar spindles leading to micronuclei formation of different size and subsequent deletion [8] [9]. The elimination in hybrid *O. sativa* × *L. peruviana* was presented by chromosomes lagging which not congregate in metaphase plate and end forming micronuclei, cytoplasm loss, cytoskeletal instability during chromosomes segregation, multiple and multipolar spindles, apoptosis and nuclear extrusions.

Processes of chromosome elimination by problem in pairing and segregation associated with chromatin degradation or fragmentation of chromosomes, may be the reason for the changes in the chromosome number [12], including uniparental non-disjunction of univalent chromosomes in metaphase; if the force to one of the poles is more stronger than other, the chromosomes move toward to pole without separation of sister chromatids, causing nondisjunction [15] [17]. Lagging chromosomes due to spindle fibers fail to join the centromere, the chromatids were immobilized on the periphery of the cell and did not enter the polar regions where new nuclei were formed. The immobilization of chromosomes in the cell periphery may be related to malfunction of the sister kinetochores that leading to anchorage failures of spindle [14], if the force in both poles is equal, the chromosomes do not move from the equatorial plate and are excluded from the nuclei of the daughter cells [15]. Chromosomal bridges in anaphase, if the tension to both poles is strong and if cohesion in the centromere region is broken, the centromeres move to each pole without complete separation of sister chromatids, causing chromo-

some bridges, chromosome arm break or acentric fragments [12]-[15].

Studies show that part of chromatin may be removed directly from the cells before and during the course of meiosis by nuclear extrusion, as in the hybrid *O. sativa* × *L. peruviana*, possibly due to the spatial separation of parental chromatin, heterochromatinization and DNA fragmentation, chromosomal elimination is also explained by differences in time during the phases of the cell cycle, asynchrony in the synthesis of nucleoproteins and inactivation of chromosomes by nucleases and suppression of the function of centromere in deleted chromosomal [12] [13]. Another mechanism of elimination is given by forming chromatin mass of little condensed respect to other chromosomes and corresponds to pycnotic bodies of chromatin that is mainly characterized by the removal of one of the poles of the cell, always accompanied by sticky chromosomes fragmentation [13]; the hybrid *O. sativa* × *L. peruviana* also exhibited apoptosis processes and pycnotic bodies characterized by the elimination of one of the cell nuclei. It was proposed that the factors involved in chromosome deletion are originate from the nuclei and is not influenced by cytoplasmic factors [12], however in hybrid *O. sativa* × *L. peruviana* is evidenced loss of genetic material by instability in cytoskeleton during chromosome segregation.

5. Conclusions

Pollen grains fertility in the intergeneric hybrids was not restored using colchicine. However, doubling chromosomes reduced pycnosis and apoptosis, and allowed increase chromosome synapsis during meiotic prophase.

Chromosome elimination in the *O. sativa* × *L. peruviana* occurred as a process of stabilization of newly formed genome, under different nuclear and cytoplasmic elimination mechanisms, mainly for failure of pairing and chromosome segregation, cytoskeleton instability, abnormal spindles and nuclear extrusions.

Pollen from the hybrid plants is fully sterile due to lack of homology between the parental chromosomes and abnormal behavior throughout meiosis. This can be inferred that there is a low evolutionary relationships and a high genetic incompatibility between species involved. However, possibility of crossover indicates that genetic barriers can be overcome.

Acknowledgements

To Dr. Cesar Martinez, Dr. Wilmer Cuellar, Ms. Consuelo Martinez and all the people who gave me their support in International Center for Tropical Agriculture—CIAT.

Specially to Martha Escandón and all members of the Research Group of Neotropical Plant Genetic Resources of the National University of Colombia-Palmira.

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