

## Short Communication

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**Maternal genetic inheritance of red pericarp in the grain of maize****José Ron-Parra<sup>1\*</sup>, Moisés M Morales-Rivera<sup>1</sup>, Javier Jiménez-López<sup>1</sup>, Ángel A Jiménez-Cordero<sup>1</sup>, Lino De La Cruz-Larios<sup>1</sup>, José J Sánchez-Gonzalez<sup>1</sup>**

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**Abstract**

The diversity of colors in the grain of corn is wide, from whites to blacks and including a continuum of various shades of yellows, pinks, reds, purples and blues. The most abundant commercial colors are yellow and white, however other colors have become more important because of the presence of pigments to which are attributed favorable effects as a food. The pigments are also considered natural barriers of the grain against the invasion of pests and diseases in the production fields. The colors of the grain of corn occur in three different parts of the seed: the cover of the grain or pericarp, derived from the maternal tissue, with a diploid genetic content; the endosperm, including the aleurone layers that are cells in the grain immediately below the pericarp with a chromosome content of  $3n$ ; and the embryo, with a genetic content of  $2n$ . The red color considered in this study is present in the pericarp ignoring possible effects in other tissues of grain and other organs of the plant. In this study, we used materials with colorless or red pericarp, and white or yellow endosperm; with the purpose of describing the type of inheritance of this character in the grain of corn. The results indicated a maternal genetic inheritance with classical complete dominance of the red color of pericarp over the clear or transparent phenotype, where the red color of the grains on ear is determined by the genotype of the mother grain but not by the seed embryo genotypes, which is characterized by uniformity of grain color of the ear. This type of inheritance could be useful in the development of pigmented varieties of higher food quality for humans.

**Keywords:** pigments, human health, non segregation, ear color

**Introduction**

The grain of maize consists of three main parts, the pericarp, endosperm (including the aleurone) and embryo. Each one of these tissues is derived from a different set of progenitor cells. The pericarp is adult maternal tissue, the endosperm carries maternal (two doses) and parental chromosomes (one dose), while the embryo or germ is derived from the zygote with an equal contribution of female and male parents. The grain of corn contains genotypes of two generations, the female parent (pericarp) and offspring (aleurone and embryo), in differing combinations among these three tissue types. A grain of corn can be red by the presence of pigments in the pericarp and/or in the aleurone. The pigment in the aleurone is water soluble, but the pericarp pigment is not, which implies the existence of two routes of synthesis in the production of these pigments, as well as two independent systems of inheritance. The red color of the pericarp can occur in all racial groups, but is lost in the process of «nixtamalización» of grain for the development of the «masa» (wet flour) and in the washing of the grain for the preparation of «posole». In contrast, the red color in the aleurone is retained after these processes, resulting in pigmented «masa» for all their derivatives and grain for the pigmented «pozole». In this study,

the inheritance of grain color was followed across four planting generations, under open and hand pollinations in maize materials with red and colorless pericarp, to determine the mode of inheritance of this character in a cross between an improved and a native variety.

Normally improved maize varieties are of uniform grain colors; the most common in the market are yellow and white grains, distinguished by the presence or absence of yellow pigments in the endosperm (but without pigment in aleurone and pericarp). Occasionally these improved commercial white or yellow endosperm maize types are «painted» by native pigmented maize, planted near the improved varieties, with dominant alleles expressed immediately in the aleurone of the fertilized seeds (by «xenia» effects), or in the pericarp in the next generation progeny. When the pericarp expresses pigments, all the grains in the cob are pigmented, providing an example of maternal effect. In the case of grain or seed phenotypes, one must take into account that the tissue of the pericarp and the rest of the parts of the plant, e.g., stems, roots, leaves, etc, have the same genotype, whereas the endosperm and embryo could be different, depending on the type of the pollination from where they were originated, selfing or crossing. Self-

ing increases the proportion of genes in homozygous condition and a uniform appearance; in contrast, with the crossing, different genotypes can emerge.

In the University Center for Biological and Agricultural Sciences (CUCBA) at the University of Guadalajara, we have been collecting native varieties of maize in recent years (Ron et al, 2006), through the Institute of Management and Use of Plant Genetic Resources (IMAREFI). The purpose of this project has been the conservation, rescue, and utilization of native maize in all states of the national territory (Ortega et al, 2011). In the state of Jalisco, several collections of native maize have been made in different towns and cities, by acquiring seed samples and/or ears directly from the producers or in local or regional markets. The diversity in morphological characteristics and agronomic traits has been evident among the collections in this state; some of these characteristics have been of interest for study and possible exploitation, as is the case for corn of different colors (pigmented) and textures (hard, soft, wrinkled, etc) of the grain. The collection M0630, registered in the IMAREFI germplasm bank, is an accession of wrinkled grains, presumably with the recessive homozygous genotype *su1su1*, (Neuffer et al, 1997) and red pericarp color with several intensities under the control of *p1* gene located on the short arm of chromosome 1 (Neuffer et al, 1997). The objective of this study is to ascertain the mode of inheritance of the red color of the pericarp of this collection (M0630).

**Materials and Methods**

The materials involved in this study were the collection of maize M0630 with red-wrinkled grain, and a commercial corn hybrid with white grain and unknown origin.

In 2010, at the Experimental Field of CUCBA, six commercial hybrids of white grain adapted to Jalisco were planted to do all possible crosses among them for yield trial evaluation; also, in this hand pollination area, the red wrinkled grain type M0630 was planted as a border. The crosses among the commercial hybrids were evaluated in 2011 and 2012 at the Experimental Field of CUCBA and Las Garzas, Mpio. of Guachinango, Jal. At harvest time, in one of crosses plants appeared with red ears, even when all the grain planted was white. At harvest of the yield trials in Las Garzas, Mpio. of Guachinango, Jal, in 2012, two ears with red grain and cobs were selected. Each of these two ears was planted (100 seeds) in the CUCBA in 2013, under plastic greenhouse conditions. Some plants were self-pollinated within each family and others crossed by a line of white grain (male). In 2014, in CUCBA experiment station, selfed and crossed seed from the previous generation were planted and plants were self-pollinated. Also, some plants, from families, were crossed (as males) to a yellow wrinkled stock.

**Results**

The results of this study are presented below for growth cycle (Figures 1 and 2). Note that the Figures 1 and 2 are identical to the second generation, but are different for the third and fourth generations.

**CUCBA 10T**

In one of the crosses among the commercial hybrids of white grain, some plants, supposedly with the genotype *p1p1* (ignoring the color of the cob and other tissues of the plant), accidentally received pollen from border plants planted with seed of the wrinkled red collection M0630, supposedly with the genotype *P1P1* (like the previous case, ignoring the color of cob and other tissues of the plant). It is estimated that 2 or 3% of the white grain harvested from such crosses were the heterozygous genotypes *P1p1*, but no color is expressed because the pericarp of the harvested grains belonged to the genotype of the parents of the hybrid female white-grained (*p1p1*). Supposedly, the genotypes of the grain were: Pericarp (*p1p1*), embryo (*P1p1*) and endosperm (*p1p1P1*) (Figures 1 and 2).

**LAS GARZAS 12T**

In this locality, at harvest of the experiments with the crosses of white grain, we selected two ears (half-sib families) with uniformly red grains (red pericarp and white endosperm). The two plants were harvested from where the two ears of red grain (*P1p1*), surely had received most of their pollen from plants with white grain (*p1p1*) around them. The two ears

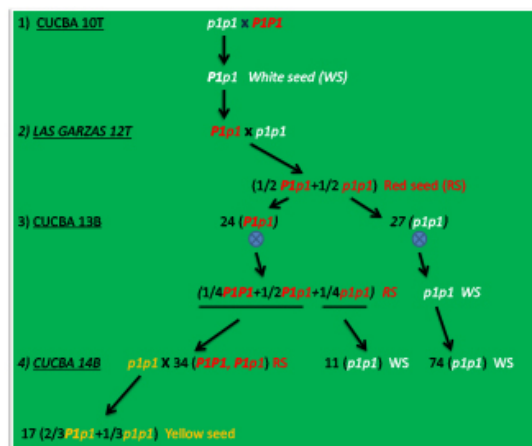
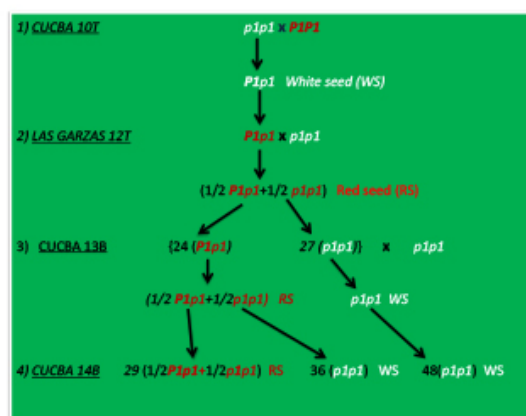


Figure 1 - Representation of the genotypes (alleles P1 and p1) and phenotypes (grain color of red, white, and yellow) of the locus *p1* for the color of the pericarp in the grain of corn in four generations of planting (1, 2, 3, 4). On the crosses shown (x), the first genotype is female, and the circle symbol represents self-pollination. The first arrow down after the harvested seed, and the second arrow down, to the genotypes of plants. Note, in the first growing station (CUCBA10T), the heterozygous seed (*P1p1*) is white but next generation (LAS GARZAS 12T) all progeny is red. Also, in growing stations 3 and 4, the recessive homozygous seeds are red, but next generation all progeny is white.



**Figure 2** - Representation of the genotypes (alleles  $P1$  and  $p1$ ) and phenotypes (grain color of red, white and yellow) of the locus  $p1$  for the color of the pericarp in the grain of corn in four generations of planting (1, 2, 3, 4). On the crosses shown (x), the first genotype is female. The first arrow down after the crosses indicates the genotypes and phenotypes of the harvested seed, and the second arrow down, to the genotypes of plants. Note, in the first growing station (CUCBA10T), the heterozygous seed ( $P1p1$ ) is white but next generation (LAS GARZAS 12t) all progeny is red. Also, in growing stations 3 and 4, the recessive homozygous seeds are red, but next generation all progeny is white, under self pollination or when crossed to a white.

were shelled and the seed was kept in separate envelopes. The supposed genotypes of the red grains from families were: Pericarp ( $P1p1$ ), and embryos ( $1/2 P1p1$  and  $1/2 p1p1$ ).

#### CUCBA13B

The two families of red grain were planted in the greenhouse and self-pollinated. In addition, some plants ( $P1p1$  and  $p1p1$ ) within each family were crossed as females with a line of white grain ( $p1p1$ ) as male. At harvest, 24 ears had all red grains and 27 had all white kernels; no ears segregated for white and red grains, which is a typical maternal genetic inheritance, where the maternal genotype determines the phenotype of the offspring. The assumed genotypes of the red kernels of the self-pollinated plants were (Figure 1): pericarp ( $P1p1$ ) and embryos ( $1/4 P1P1$ ,  $1/2 P1p1$ ,  $1/4 p1p1$ ), and for the plants crossed as females (Figure 2): pericarp ( $P1p1$ ) and embryos ( $1/2 P1p1$ ,  $1/2 p1p1$ ). The only and unique genotype of the seeds from the self-pollinated and crossed plants with ears of white grain was  $p1p1$ , for both pericarps and embryos (Figure 1 and 2).

#### CUCBA14B

The red and white kernels, from the selfed pollinations were planted and self-pollinated, again. Among the resulting plants, we found 34 plants with ears containing all red grains and 11 plants with ears containing all white grains. This suggests a 3:1 Mendelian inheritance, where the red color of the pericarp is dominant to the colorless pericarp. All seeds from the

74 self-pollinated plants with ears containing all white grains, produced ears with all white kernels (Figure 1). Plants from red seeds were crossed, as males, by a yellow sugary grain hybrid as female; at maturity, there were no grains of red color, only yellow grains, confirming the maternal genetic inheritance of the red color of the pericarp (Figure 1). The crosses of plants of ears with red kernels by the line of white grain, from previous generation, gave 29 plants with red grain and 36 with white grain, while 48 plants with ears of white grain crossed by the line of white grain, only gave ears of white grain. The one-to-one ratio of ears with red grain and ears with white grain, demonstrates, once again, the complete dominance of the red color of the pericarp over the transparent pericarp (Figure 2).

The statistical tests with the  $\chi^2$  (Chi-squared) distribution were carried out; proving all in favor of the segregations 1:1 and 3:1 expected, typical of the inheritance of a single Mendelian gene with complete dominance for the red color over the colorless pericarp.

## Discussion

The red color of the pericarp in this study could be considered as a type of maternal genetic inheritance, where the red color is dominant to the translucent. This character was stable and consistent across the generations of study, but there were variants in the intensity of the red color between ears, but no change in color of grain within ears. These variants of colors between ears could be explained by effects of epistasis, interaction of the dominant allele with alleles of other genes in the genome; in addition to that,  $p1$  is a locus with a high predisposition to changes in its sequence of bases that have an impact on expression of the color red due to changes in the metabolic pathways in the synthesis of the pigments present in the pericarp (Peterson 1990; Athma et al, 1992; Athma and Peterson, 1991; Lee and Harper, 2002; Chopra et al, 2003; Cocciolone et al, 2005; Zhanga and Peterson, 2005). In this study, the two ears (half-sib families) from which the controlled crosses were initiated (CUCBA13B), produced red color for both grain and cob, but no follow-up was given to cob color.

Another aspect to consider in this study, is the presence of three genotypes in the grain represented by two generations; the genotypes of endosperm and germ represent the current (maternal) generation and the genotype of the pericarp represents the prior maternal generation. In this study, inheritance was tracked using crosses and self-pollinations, taking into account the direction or sense of the crosses (male and female) what was allowed to have clarity in the influence of each sex on the color of the grain in the pericarp. Espinosa-rujillo et al (2009) made crosses among six pigmented native varieties and found an important influence of the maternal effects

for pigments in the pericarp, but the maternal genetic effects for red pericarp were confused by the presence of other colors in the parents, and by the type of analysis and interpretation of quantitative inheritance made.

The locus *p1* for the red color of the pericarp has been studied for many years (Anderson and Emerson, 1923) in terms of genetic transmission and degree of dominance. Since then, it has been pointed out that the red color dominates over colorless or translucent, but it has not been emphasized nor even pointed out that it is a classic case of maternal genetic inheritance, not to be confused with the cases of maternal inheritance related to the cytoplasmic or extra-nuclear inheritance.

In recent years, there has been renewed interest in organic foods and foods grown without synthetic chemicals, especially in those that benefit the health, by their natural chemicals (as is the case of the pigments). The red color of the pericarp in the grain of corn has been associated with the production or synthesis of compounds (Grotewold et al, 1994; Winkel-Shirley, 2001; Sharma et al, 2012) that could benefit the health of humans, and also benefit the health of grain free of pesticides (Dowd and White, 1995; Wilson et al, 1995; Cooke, 1997; Pilu et al, 2011). For this reason, the information from this study, could be implemented in projects or programs to develop improved varieties with red pericarp; in fact, some breeding methods have been published (Lago et al, 2014), and also, there are now some products of sweet corn and popcorn with red pericarp in the market.

### Conclusion

It is concluded that the red color of the pericarp in the collection of maize M06030 has a type of maternal genetic inheritance, where the red color dominates the transparent. This type of inheritance might be of interest in the improvement of red-colored maize of different types in terms of their textures (sweet, flour, dents, and popcorn) and pigments in the endosperm (red-purple, yellow, and white). This could contribute to less deterioration of grain in the field lowering damage of insect pests and toxic fungi, when application of synthetic pesticides is not desirable.

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