Presence or absence of each single fragment was coded as one or zero, respectively, in a binary data matrix. Jaccard's coefficient was selected to construct the similarity matrix. Cluster analysis was performed by the unweighted pair-group method analysis (UPGMA) method and the Infostat/P computer program v.1.6 (Grupo Infostat, FCA Universidad Nacional de Cordoba). The dendrogram in Figure 1 shows the genetic relationships based on SSR analysis.

In general, the grouping agreed with the pedigree information of the lines, although some discrepancies were observed. However, the cophenetic value of 0.547 indicates a low-moderate fit of the dendrogram with the similarity matrix generated with SSR data.

Results confirm microsatellites as an excellent complement to morphological and other conventional markers that are currently used to obtain plant variety protection for new maize lines (and eventually hybrids). However, their utility to assign new inbreds to heterotic groups remains to be explored.

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Evidence of natural occurrence of paramutation at *p1* locus

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The maize *P-rr* gene encodes a Myb transcription factor that activates the phlobaphene pigment pathway, regulating the flavonoid biosynthetic genes in kernel pericarp, cob, husk and skills (Chandler and Stam, Nature Rev. Genet. 5:532–544, 2004; Sidorenko et al., Plant Mol. Biol. 39:11–19, 1999). To understand the transcriptional regulation of the *P* gene, promoter regulatory sequences of the allele *P-rr*, identified by *Ac* transposition, were tested in transgenic plants (Sidorenko et al., Plant J. 22:471–482, 2000). Transgenic plant lines (*P-ww*) containing a *GUS* reporter gene and an enhancer fragment, called P1.2, produced F1 plants with reduced pigmentation on the pericarp when crossed with *P-rr* plants. The reduced pigmentation was transmitted to the next generations, being the original altered allele denominated as *P-rr'* (Sidorenko et al., 1999, 2000; Sidorenko and Peterson, Plant Cell 13:319–335, 2001). The paramutation was attributed to the P1.2 fragment, which probably altered the DNA methylation pattern and reduced the transcript levels of the *P-rr* allele (reviewed in Chandler and Stam, 2004). Natural occurrence of *P* paramutagenic alleles was recently described by Das and Messing (Genetics 136:1121–1141, 1994). However, the literature for description of P paramutagenic alleles is very poor.

In a program to study the inheritance of paramutagenic alleles and their effects during the inbreeding cycles, we have isolated a reduced pigmentation phenotype in the pericarp. Crosses between the Cusco, Piranão and ITA varieties activate an *Ac* transposon, producing a F1 generation expressing *P-vv* phenotype (Fig. 1). The F2 generation segregates and produces every possible phenotype related to the *P* locus as *P-rr, P-ww*, *P-wr* and several intermediate patterns of reduced pigmentation, as well as variegated pericarp. A series of cobs is presented in Figure 2. The variegated cobs always produce kernels with reduced pigmentation in the pericarp, and a gradient of color expression level can be seen. Some cobs presented a cream color that could not be related to any event or known allele, and was probably produced by new allele. On the other hand, some cobs presented a very intense full color in comparison to the original *P-rr* or *P-wr*. The increase on the level of color could be attributed to intensifier genes. We do not know about the occurrence of these genes in our material, but this possibility should be considered.

The kernel analysis was very difficult because the varieties involved present the alleles required to the anthocyanin pathway.

Figure 1. F1 *P-vv* phenotype produced after the crosses among the three varieties.

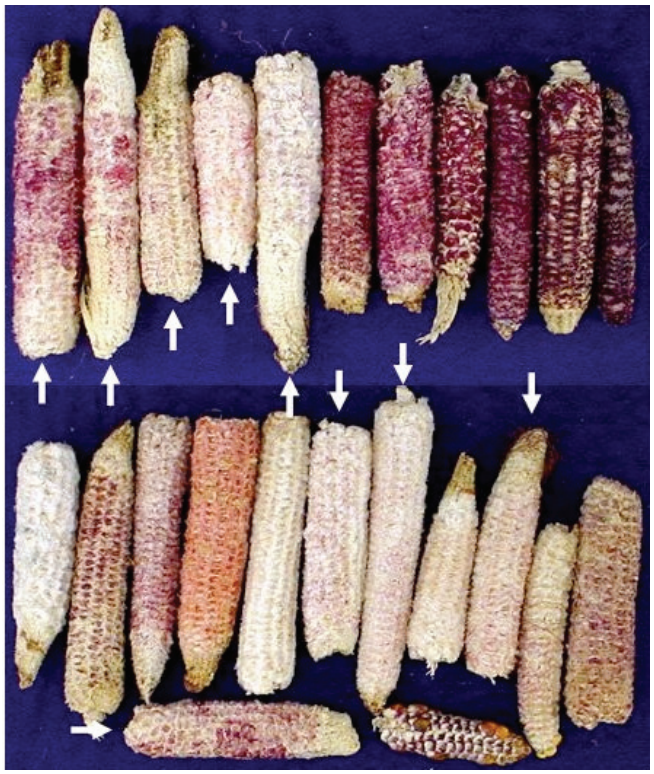

Figure 2. A series of ears where the arrows indicate the reduced color expression, possibly a *P'* (paramutable) allele. Note a variation in the expression level; the two first (top row, from the left to right) ears show variegation and strong colored sectors, while the other ears show sectors with a reduced color expression.

Paramutation in the *r* locus could be seen in some ears, deduced from the occurrence of white pericarp with color only at the top of the kernel (Fig. 3). Some kernels presented both paramutation events, and it should be interesting to investigate the interaction between these events in the same line.

Figure 3. Segregating ears showing paramutation phenotypes in the pericarp. The arrows indicate kernels that contain paramutation events in the locus *p* and *r*. These kernels have been isolated to study the interaction of different events of paramutation in the same plant. The other three ears (at right) show the difficulty in screening the pericarp paramutants with other loci segregating for kernel color.

As cited in the review above, the level of color is strongly correlated to the increase of methylation and decreased transcript levels, and the paramutation events have been attributed to the 1.2P promoter region. Our idea is that the *Ac* transposable element could be inserted near the 1.2P promoter region (described earlier by Sidorenko et al.), altering the pattern of methylation and promoting the paramutation in our material. Probably, an insertion in the promoter region could generate a *P-ww*-like phenotype derived from a full-imprinting, whereas insertion near the promoter region could affect the transcription levels by alteration in the methylation pattern as a result of the transposable element inactivation. The effect of the paramutation would be the more intense the shorter the distance between the promoter region and the insertion (Fig. 4). However, our main objective is isolating the alleles and studying their behavior in crosses with other stable alleles. Thus, inbred lines of each derived paramutant phenotype have been produced.

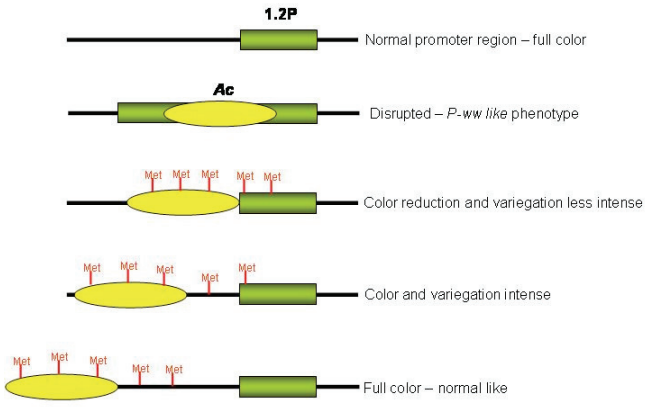

Figure 4. Hypothetical model showing how the *Ac* insertion, its inactivation by methylation, and the distance of promoter region in the *p* locus would affect the paramutation.

Testing crosses have been conducted among those paramutant phenotypes and stable ones, well as among the *P-vv* and stable ones, to reproduce the phenomenon. We believe our hypothesis could be tested by mapping the position of the insertions in this way.

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Development of the first fertile plants with an addition of the whole maize chromosome 10 to the oat genome

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The genome of maize, *Zea mays* L., has been dissected into 10 entities, reflecting the addition of individual maize chromosomes to hexaploid oat, *Avena sativa* L. A complete set of oat-maize chromosome additions is available and can be used to map markers to chromosome and analyze the expression of individual genes and their specific interaction with an alien background (Kynast et al., Funct. Integr. Genomics 2:60–69, 2002).

More than 50 fertile lines are available with monosomic and/or disomic addition of individual chromosomes of different maize lines in different oat cultivars serving as chromosome recipients; however, maize chromosome 10 has been available as a self-fertile line only as a telocentric derivative for the short arm. Whole chromosome 10 additions were previously sterile (Kynast et al., Proc. Natl. Acad. Sci. USA 101:9921–9926, 2004). The added maize chromosomes were identified by cytological and molecular means, as described elsewhere (Kynast et al., Plant Physiol. 125:1228–1235, 2001).

In a recently undertaken series of crosses with maize inbred lines B73 and Mo17 as chromosome donors, one inter-species hybrid (F1-5751-1) tested as a juvenile plantlet (F1-5751-1*) appeared to have retained the whole maize chromosome 10 in addition to the haploid oat complement, based on the positive test with maize chromosome arm 10S-specific SSR marker p-umc1293 and maize chromosome arm 10L-specific SSR marker p-bnlg1839 (Figure 1). Tests of genomic DNAs from flag leaves of the first four tillers (F1-5751-1a, F1-5751-1b, F1-5751-1c, and F1-5751-1d) verified the presence of maize chromosome 10 in the plant. The plant was a descendent of the cross Starter x Mo17 and showed limited fertility after self-pollination (Table 1). The panicle (231 spikelets) from the a-tiller had 2 seeds, of which one seed did not germinate. The panicle (190 spikelets) from the b-tiller had one seed. The panicle (201 spikelets) of the c-tiller had six seeds, of which one did not germinate. The panicle (194 spikelets) of the d-tiller was sterile.

We observed transmission of the maize chromosome in the seven F2-offspring. Eight markers were selected which were distributed across the length of the chromosome: *umc1380*, *umc1576*, *umc2034*, *gcsh1*, *umc1272*, *umc1084*, *umc2021*, and *csu48*. PCR assays detected these sequences in the F2-offspring, indicating that the maize chromosome is intact (Figure 1). We will assess stability and transmission in subsequent generations.