mechanism, transcription efficiency is enhanced by the increased stability of Z-DNA due to an enhancement in the stabilizing action on Z-DNA of the Z-DNA binding *Bg*-encoded proteins when the *Bg* dose increases from 1 to 2 or from 1 to 3.

Several explanations are possible concerning the role of Z-DNA located downstream of transcription start sites in the *Bg* sequence. The presence of these Z-DNA regions may increase fidelity of RNA splicing by a mechanism proposed by Wittig et al. (1992). Another explanation can be prompted by the aforementioned feature of an insignificant change in reversion frequency of the *o2-m(r)* alleles when *Bg* dosage increases from 2 to 3. For example, binding of *Bg*-encoded proteins at their enhancing concentration (when the dose of *Bg* elements increases from 2 to 3) to the indicated Z-DNA regions (especially if these regions show lower affinity to *Bg*-encoded proteins and are bound by such proteins at their high concentration) may hinder the movement of the next RNA polymerase molecule on these regions, thus lowering the efficiency of transcription. That is, the stabilizing action of *Bg*-encoded proteins on Z-DNA situated downstream of transcriptional start sites would affect gene transcription in a negative autoregulation mode.

In any case, the presence of Z-DNA binding domains in *Bg*-encoded products, and the ability of certain regions of the *Bg* transposon to form Z-DNA, may indicate the existence of mechanisms of *Bg* activity autoregulation through the interaction of Z-DNA forming regions of this transposon with its encoded proteins.

Z-DNA forming regions in other maize transposons sequences. Using the ZHunt program a search for Z-DNA forming regions in sequences of other maize transposons (*Ac*, *PIF*, *En*, *MuDR*) was carried out. Such regions were found in sequences of all transposons analyzed except *PIF* (GenBank accession number AF412282.1) (Table 1).

Table 1. Probable Z-DNA forming regions in sequences of different maize transposons revealed by the ZHunt program (Ho et al., 1986; Ho, 1994; Champ et al., 2004).

Transposon	Starting position	Length, bp	Z-score	Sequence
Bg	120	21	$2.2 \cdot 10^4$	ACCAGACGCGCGCACGAGAGC
	402	13	$9.3 \cdot 10^2$	CACGGACGCGCAG
Ac	381	16	$3.3 \cdot 10^3$	CCACGCGCCCACGCCG
	1261	28	$1.4 \cdot 10^5$	ATGTACGTGCACGTGCGCGTGGGCATGG
En	397	15	$1.8 \cdot 10^3$	GAGCGCGCACCTCCA
	5892	13	$4.7 \cdot 10^3$	TTCGCGTGTGCGA
	8035	17	$2.4 \cdot 10^3$	TGATGTGCGCGCAGTAA
MuDR	169	19	$1.8 \cdot 10^4$	TTCGCCCGCGCACACGCCG
	4756	20	$1.8 \cdot 10^4$	CGGCGTGTGCGCGGGCGAAC

Sequences used (according to the GenBank accession numbers) are as follows: X56877.1 (*Bg*); X05424.1 (*Ac*); M25427.1 (*En*); M76978.1 (*MuDR*). The Z-Score cutoff (minimum) is equal to 700.

Interesting results are observed for potential Z-DNA forming regions of the *MuDR* transposon. A characteristic feature of this transposon is the convergent transcription of its two major transcripts, *mudrA* and *mudrB*, initiated in terminal inverted repeats from opposite strands (Hershberger et al., Genetics 140:1087-1098, 1995). Transcription start sites of these transcripts (Hershberger et al., 1995) are located near revealed Z-DNA forming regions: the beginning of the first Z-DNA forming region (starting from position 169 of the *MuDR* element sequence, see table 1) coincides with the starting bp nucleotides of the first start site of the *mudrA* transcript; the second Z-DNA forming region (positions 4756-4775 of the *MuDR* element sequence, see Table 1) is located 5 bases downstream of the transcription start site of *mudrB* (position 4780; Hershberger et al., 1995). These results confirm one more time the predisposition of Z-DNA for transcription start sites and indicate the involvement of Z-DNA in the regulation of the transcription of the *MuDR* transposon genes.

Corrigendum. In the MNL 79 note on *Bg*-encoded proteins a misprint was made in the legend of Figure 1: the correct numbers for the first exon of PPBg3 are 813-1546.

PIRACICABA, SP, BRAZIL
ESALQ – Universidade de São Paulo
PONTA GROSSA, PR, BRAZIL
Universidade Estadual de Ponta Grossa

A seed-by-seed strategy to study the paramutation at *r1* locus

--Mondin, M; Gardingo, JR

The paramutation at the *r1* locus was largely studied by classical and molecular approaches, and several aspects of its behavior and origin were elucidated. Classical experiments provided information about the locus structure, genetic distance and phenotypical instability, and the molecular genetics revealed the sequence and the elements that comprise the locus, including transposable elements, genes and methylation. Many advances have been made towards the understanding of the control of the paramutation at the *r1* locus, however many questions remain unanswered. One question that has interested us is related to the instability of the phenotypes after the paramutagenic allele altered the paramutable one in the heterozygous state. Seven classes of kernel pigmentation are known, varying from colorful with a maximum deposition of anthocyanin to colorless without pigmentation, in the F2 progeny. The literature is categorical in descriptions about reversions to the original state of the allele after several cycles of self-pollination, it being well established that the paramutation is an unstable event. The knowledge about stable paramutant alleles is incipient and the selection of a stable phenotype of each class of pigmentation could represent material important for molecular investigation. In this work, the main aim is the development of a strategy that permits us to understand the instability of the alleles and the selection of possible stable alleles to produce inbred lines that could be used in the molecular investigation.

We had previously described the introgression of a paramutable *r* allele in traditional varieties of maize from Brazil (Gardingo and Mondin, MNL 77:60-61, 2003). Inbred lines have been derived from the varieties that express the paramutation. The phenotypical classes have been scored from 1 (colorless) to 7 (colorful) and every classes was observed in the S1. To obtain the S2, a bulk of the classes 3, 4 and 5 was selected. In the S3 generation each class was followed seed-by-seed and evaluated as to the seven seed color pigmentation classes. Here, we present some results in one inbred line derived from the Carioca variety (Ca).

The S2 generation was scored considering the ear as a whole. From all patterns of segregation expected, only six were observed (Table 1). In one case, a pattern did not present colorless seeds, and the seeds in the ears segregated from class 2 to class 6. A high number of colorless ears were recovered, which could be a

Table 1. S2 segregation pattern, derived from a bulk of seed of pigmentation color classes 3, 4 and 5.

Inbred Line	Segregation Mode*					
	Colorless	Colorless/2-4	Colorless/2-5	Colorless/6	2-6	All Classes (1 to 6)
Ca	81	47	71	13	5	19

* Number of ears scored.

result of the homozygous recessives. A complete imprint was not discarded, but new experiments should be conducted to consider this hypothesis. No colorful seed was recovered in any ear. Class 6 was scored in a low frequency, and the seeds were self-fertilized to observe the pattern of segregation in the S3 generation. Even in the ears segregating to different classes, the highly pigmented seeds were recovered in a low frequency. To exemplify this, the ears segregating colorless/2-5 were scored seed-by-seed. Table 2 presents the absolute numbers, and Figure 1 shows the average of each class from the ears. It is clear that the frequency of the class 5 was significantly lower than the other classes. We have considered this case as a pattern of segregation, since several ears have shown similar frequencies. We expected a higher frequency of reverting seeds, expressing color classes 6 and 7. We have postulated that the effect of the paramutable allele is very strong, and several generations of self-fertilization were needed to recover the colorful class.

Table 2. Total of seeds scored on the colorless/2-5* ears.

Inbred line	Seed Color Classes			
	Colorless	2-4	5	Total
Ca	7221	7230	674	15125

*Presented in the Table 1

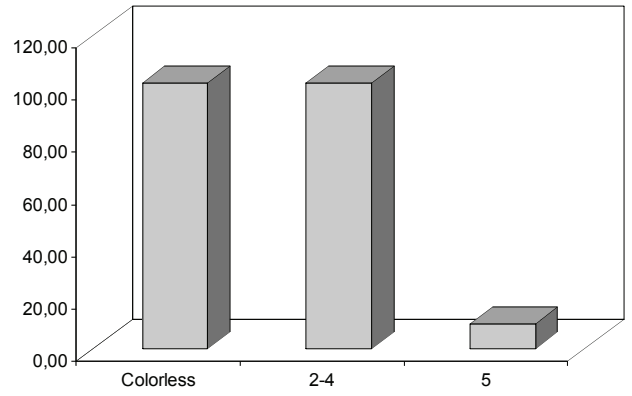

Figure 1. Average frequency of seeds from different classes of pigmentation scored in colorless/2-5 ears.

In the S3, colorful seeds were not recovered, however classes 5 and 6 were more frequent. The number of non-segregating ears was higher (Table 3). Seed class 1 resulted only in colorless ears. This case has been interpreted to be recessive homozygous, but this class has been analyzed carefully to identify possible reversions to color classes. Every seed class scored segregated colorless. We were expecting a higher frequency of classes 5, 6 and 7, but as they were not observed, the postulate described above seems to be true.

Analyzing seed-by-seed the non-segregating S3 ears derived from class 3 seed, a lower frequency of seeds in class 5 and a

Table 3. S3 segregation pattern derived from selected S2 seed color classes.

Seed Color Classes	Ears Scored	Segregating to colorless	Non-segregating	Colorless
1	40	0	0	40
2	16	14	1	1
3	50	39	7	4
4	30	24	5	1
5	11	7	4	0
6	4	4	0	0

higher frequency in class 4 was observed, while ears derived from class 5 seed presented a lower frequency of seed in the less pigmented classes (Table 4). Class 4 ears showed segregation for classes 2 to 6. Some ears showed a unique class of seed color, mainly when derived from class 5, and these ears have been selected for evaluation. The most important observation was the absence of colorless seeds. The classes observed in a non-segregating ear should be analyzed seed-by-seed, trying to minimize the segregation to different color classes. These results indicate that the selection of some seed color classes in non-segregating ears, followed by seed-by-seed analysis in the next generation, could be a good strategy to stabilize the paramutation. Some crosses between plants of the same class have generated seeds of the same class (data not shown), for example, crosses of class 4 produced ears fully class 4.

Table 4. Frequency of seed color classes on S3 non-segregating ears.

S2 seed color classes	Seed Color Classes				
	2	3	4	5	6
3	30	322	264	23	0
4	15	121	497	305	10
5	0	25	75	305	88

We believe that as a stabilized paramutation is obtained, some important aspects of the *r1* paramutation will be explained, such as the role of transposable elements in paramutation events. Moreover, the stabilization of a phenotype class could be a result of a chromatin conformation that is transmitted generation to generation without alteration, or a suppression of the recombination among the repeats of *r1*. All these interesting questions could be investigated utilizing these stable lines with molecular approaches.

PRESIDENTE PRUDENTE, BRAZIL
University of Western Sao Paulo

Changes in chromosomes in highly embryogenic cultured cells and in germinating stored seeds of maize

--Scandolieri, RF; Koyanagui, AP; Takahashi, FT; Fluminhan, A

In higher plants, an increased frequency of genetic and chromosomal changes is usually observed during the germination and development of plants derived from aged seeds and regenerated from in vitro cultured cells. Much evidence supports a close relationship between the age of stored seeds or cell cultures and the loss of vigour and germinability of seeds, and the regeneration ability of cultures and number of chromosome aberrations that are observed at the first mitoses of root meristems in surviving plants.

In vitro culture of plant cells has led to several useful approaches for biotechnology in the agricultural sciences, such as: the selection and clonal propagation of promising genotypes and