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### **Spontaneous chromosome doubling in maize haploids: the cases of high frequency**

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During creation of the double haploid lines we found that some genotypes have increased frequency of spontaneous chromosome doubling. These are the Rf7 line (frequency 6-7%) and Rf7 x Ku123 hybrid (frequency 12-16%). We decided to check whether this phenomenon is widespread and tested the lines Rf7, Ku123, MK109, MK01 and hybrids between them. The results in the table. The frequency of doubling is defined as the number of self-pollinated ears with at least one kernel relative to the number of plants that survived to the tassel stage.

Based on the results, the high frequency of spontaneous doubling in the variant n(Rf7 x Ku123) is a phenomenon not typical, but exceptional, due to a successful combination of genes. It seems that the Rf7 line carries genes responsible for the ability to spontaneous doubling, while the Ku123 line carries enhancers that do not manifest themselves, but in combination with the genes of Rf7.

Even higher frequencies were observed in the forms derived from Rf7 x Ku123 (see bottom of the table). Probably, in the lines rk-6 and rk-148 the favorable gene combination was fixed. This suggests that the number of genes affecting is not too large, no more than 5-6, otherwise it would be very unlikely to collect them in a set of 40-50 DH lines. The minimal model, explaining the experimental data, is digenic (one effector from Rf7 and one enhancer from Ku123).

In the hybrid Rf7 x Ku123 and derived forms, the efficiency of spontaneous doubling is high, but lower than with colchicine. It, however, is comparable to the efficiency of colchicine doubling in other genotypes of maize (10-30% depending on genotype), which allows to replace the labor-intensive colchicine doubling by a less laborious spontaneous one.

Table. Frequency of spontaneous doubling in haploids of different genotypes

Genitype	Frequency of spontaneous doubling	Frequency of colchicine doubling
n(Rf7)	12/189=6.3% (2011) 4/54=7.4% (2012)	
n(Ku123)	1/83=1/2%(2012)	
n(MK109)	1/77=1.3% (2012)	
n(MK01)	0/38=0 (2012)	
n(Rf7 x Ku123)	4/34=11.8% (2009) 9/71=12.7% (2010) 7/45=15.6% (2011)	10/50=20.0% (2010) 18/35=51.4% (2011)
n(Rf7 x MK109)	2/31=6.5% (2009) 2/22=9.1% (2010) 3/40=7.5% (2011) 2/103=1.9% (2012)	
n(Ku123 x MK109)	3/57=5.3% (2011) 1/49=2.0% (2012)	
n(MK01 x Rf7)	3/132=2.3% (2012)	
n(MK01 x Ku123)	0/112=0 (2012)	4/66=6.1% (2012)
n(MK01 x MK109)	2/61=3.3% (2012)	
n(rk-6 x Rf7)	29/134=21.6% (2011)	68/120=56.7% (2011)
n(rk-148 x Rf7)	75/242=31.0%(2015)	
n(rk-138 x Ku123)	26/213=12.2% (2015)	
n(Rf7 x Ku123) x Rf7	11/144=7.6% (2015)	
n(Rf7 x Ku123) x Ku123	8/161=5.0% (2015)	

Comment. rk-6 is DH line derived from the cross Rf7 x Ku123;  
rk-138 and rk-148 are DH lines derived from the cross Rf7 x rk-6