Maize Genetics Cooperation Newsletter vol 84 2010

MAIZE AND SORGHUM RESEARCH INSTITUTE
PASCANI, REPUBLIC OF MOLDOVA

FREQUENCY OF REVERSION OF *o2::rbg* ALLELES AS A CHARACTERISTIC OF SPECIFICITY OF THEIR INTERACTION WITH REGULATORY *Bg* ELEMENTS [1]

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[1] This note is a shortened version of a full size article which had been submitted for publication, however, due to termination of my work in Maize and Sorghum Research Institute its publication has not been finalized. However I would like to share main result and conclusions of this article with the maize community.

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Earlier (Maydica 48: 275-281, 2003), the specificity in interaction has been established between *Bg* elements and mutable *o2::rbg* alleles (both the regulatory *Bg-hf* and *Bg-lf* elements, and the responsive *o2-hf* and *o2-lf* alleles have been obtained under disruptive selection for whole endosperm revertants (WER) content, MNL 73: 76-79, 1999). Thus, in two or three doses the *Bg-lf* element determined low reversion frequency of the *o2-lf* allele but high reversion frequency of the *o2-hf* allele, whereas the presence in the same doses of *Bg-hf* determined high reversion frequency of both the *o2-hf* and *o2-lf* alleles (Maydica 48: 275-281, 2003).

Rather unexpected therefore was finding of very high reversion frequency of the *o2-hf* allele in presence of standard *Bg-Ref* element (this paper). Percentage of WER (i.e. phenotypically normal kernels) on homozygous *o2-hf*, *Bg-Ref* ears was higher than that of the *o2-hf*, *Bg-hf* ears obtained under selection for high WER content and often did not differ significantly from the content of normal kernels observed on heterozygous *O2* ears. Most variegated kernels on

homozygous *o2-hf, Bg-Ref* ears were characterized by small and very small opaque sectors surrounded by vitreous tissue (Figure 1).

***Possible causes of high frequency of reversion of* o2-hf *in presence of* Bg-Ref:**

Several causes could be connected with high content of phenotypically wild-type kernels in *o2-hf, Bg-Ref* genotypes: (i) action of modifiers; (ii) earlier (i. e. before meiosis) developmental stage of *o2-hf* reversion in presence of *Bg-Ref* (in this case significant amount of embryo revertants and their clustering on the ear should be observed); (iii) specific interaction between *o2-hf* allele and *Bg-Ref* element.

An assumption about action of modifiers is unlikely since sharply different on revertant content genotypes *o2-hf*, *Bg-Ref* and *o2-lf*, *Bg-Ref* have been obtained in the crosses of the same *o2-R*, *Bg-Ref* line with closely related strains *o2-hf*, $+^{Bg}$ and *o2-lf*, $+^{Bg}$ and differences in reversion frequencies of responsive alleles of these strains were not caused by modifiers unlinked to the *o2* locus (Maydica 44: 195-203, 1999; MNL 73: 76-79, 1999).

Most WER kernels on* o2-hf, Bg-Ref *ears are not embryo revertants and* Bg-Ref *does not condition earlier in development reversion of the* o2-hf *allele:

Out of 67 tested progenies of one of the two studied ears of the *o2-hf/o2-R, Bg-Ref/*$+^{Bg}$ genotype (ear 02-4574×4742p152) three were heterozygous *O2* plants. No heterozygous *O2* plants were found in 59 progenies of the second *o2-hf/o2-R, Bg-Ref/*$+^{Bg}$ ear (ear 02-4573×4742p131). In the progeny of selfed homozygous *o2-hf*, *Bg-Ref* ear (ear 02-4568p131) 18 plants can be considered as embryo revertants (Table 1).

Two plants homozygous for the wild-type *O2* allele (originated from WER kernels) were found in the progeny of selfed *o2-hf*, *Bg-Ref* ear (Table 1). Two heterozygous *O2* plants, descendent of *o2-hf/o2-R, Bg-Ref/*$+^{Bg}$ ear and 12 of homozygous *o2-hf*, *Bg-Ref* ear were found in progenies of 37 and 108 WER kernels, respectively, giving frequency of their formation in progeny of WER kernels equal to 5.41 (2/37×100) and 5.56% (12/(2×108)×100)) respectively.

It is necessary to mention that presence of variegated kernels in two out of three embryo revertant ears (i.e. in ears heterozygous for wild-type *O2* allele and non-mutable *o2-R* allele) found in the progeny of the *o2-hf/o2-R, Bg-Ref/*$+^{Bg}$ ear (Table 1A) is unexpected and needs to be explained (see below).

At 3 out of 16 plants (03-4225p4, 03-4411p4, 03-4432p4 in Table 1B) considered as *O2* heterozygotes (i.e. carrying wild-type *O2* and *o2-hf* alleles) in the progeny of selfed *o2-hf, Bg-Ref* ear, the content of variegated kernels in crosses with the *o2-R*, $+^{Bg}$, tester (used as female parent), was significantly higher than expected. This excess of variegated kernels is apparently conditioned by the same causes as the described above phenomenon of apparition of variegated kernels on heterozygous *O2/o2-R, Bg-Ref*/$+^{Bg}$ ears (see below). Significant deviations from expected ratios due to excess of normal kernels which are mostly observed in the crosses of the *o2-hf*, *Bg-Ref* strains with the *o2-R*, $+^{Bg}$ tester used as male parent and on selfed ears (Table 1B) are conditioned by high frequency of WER formation especially in presence of two or three doses of regulatory element.

Revealed embryo revertants did not belong to the same pairs of spikelets, however five embryo revertants on homozygous *o2-hf, Bg-Ref* ear (Table 1) were in two clusters consisting from two and three contiguous kernels presented in adjacent pairs of spikelets (data not shown).

Observed frequency of embryo revertants (5.41 and 5.56%) is approximately on the same level with this trait of the *o2-hf, Bg-hf* strains (1.82-8.18%) (Genetika (Moscow) 39: 709-712; 2003) indicating that reversion of the *o2-hf* allele in presence of *Bg-Ref* element (as well as in that of the *Bg-hf*) occurs mostly at the period from fertilization to the first division of the primary endosperm nucleus. The size of embryo revertant clusters formed from the kernels (of two and three kernels) was also equal to that of observed in homozygous *o2-hf*, *Bg-hf* genotypes (Genetika (Moscow) 39: 709-712; 2003). Proceeding from the frequency of formation of heterozygous *O2* ears in the progeny of WER kernels obtained from analyzed selfed homozygous *o2-hf*, *Bg-Ref* ear (the ear 02-4568p131) and the number of WER kernels on this ear (5.56% and 253, respectively) the apparition of two plants homozygous for wild-type *O2* allele found in the progeny of mentioned ear and the apparition of two kernel embryo revertant cluster, as a result of *o2-hf* reversion in gametes at postmeiotic stages of development is not excluded. However frequency of formation of three kernels embryo revertant cluster ($1.7 \cdot 10^{-4}$ or 0.0556^3) on selfed *o2-hf*, *Bg-Ref* ear indicates that premeiotic reversion of *o2-hf* is more probable. Small size of this cluster indicates on both the late stages of premeiotic development at which the reversion of *o2-hf* can rarely occur and confirms that the early reversion of this allele is not the main cause of high WER content in *o2-hf*, *Bg-Ref* genotypes.

Three observed features of *o2-hf, Bg-Ref* strains derivatives: 1) kernel phenotype characterized by small and very small opaque sectors in vitreous background (Figure 1); 2) presence of variegated kernels on some embryo revertant *O2/o2-R, Bg-Ref/+*^{Bg} ears (containing non-mutable *o2-R* allele, see Table 1); 3) excess of variegated kernels in 3 out of 16 heterozygous *O2/o2-hf, Bg-Ref/+*^{Bg} ears (see Table 1); can be explained by insertion of *Bg* or *rbg* elements in wild-type *O2* allele leading to inactivation of this allele and to apparition of opaque tissue in vitreous background. In case of *rbg* insertion it be classified as reinsertion since the wild type *O2* alleles had previously been originated due to excision of *rbg* element from the *o2-hf* allele.

***High frequency of reversion of the* 2-hf *allele in presence of* Bg-Ref *as a particular case of specificity of interaction between* Bg *elements and* o2::rbg *alleles and a possible mechanism of this interaction*:**

Generalizing obtained data it is possible to conclude than the most probable cause of high frequency of reversion of the *2-hf* in presence of *Bg-Ref* is the specificity of their interaction, an another example of this earlier described phenomenon observed for *o2-hf* and *o2-lf* alleles and *Bg-hf* and *Bg-lf* elements (Maydica 48: 275-281, 2003).

As a mechanism of this specificity of interaction could be participation of *rbg* product, together with *Bg* encoded transposase in transposition complexes. The possibility of such participation is indicated by sequence similarity between the *Bg* and *rbg* elements: *rbg* differs from *Bg* by small deletion and insertion events and the two elements share more than 75% homology based on sequence data (Hartings et al., Molecular and General Genetics 227: 91-96, 1991).

In this case the apparition of sharply different *o2-hf* and *o2-lf* alleles as a result of change in state of the initial *o2-m(r):3449* allele under disruptive selection for WER content (Maydica 44: 195-203, 1999) is conditioned by changes in the *rbg* elements affecting in opposite directions the ability of *rbg* products for interaction with transposition complexes responsible for *rbg* excision. Accordingly, changes in the initial *Bg-3449* element, which conditioned apparition of *Bg-hf* and *Bg-lf* elements under the same disruptive selection (Maydica 44: 195-203, 1999), could lead to the changes in their encoded transposases that affected affinity of these transposases toward *rbg* element products.

Another indication of effect of selection on *Bg* elements leading to their ability to control *rbg* excision is the higher frequency of reversion of *o2-hf* in presence of *Bg-Ref* element than in presence of *Bg-hf*. The changes in the *Bg-hf* conditioning certain upper level of the *rbg* excisions could be determined by used method of disruptive selection for high WER content (in which the *Bg-hf* element was obtained): the ears containing significantly more than 50% of WER were not selected for next cycle of selection (Maydica 44: 195-203, 1999) since in this case it were more difficult to distinguish the ears with high WER content from the ears heterozygous for normal *O2* allele.

TABLE 1 - *Kernel segregation in embryo revertants found in progeny of two ears of* o2-hf/o2-R, Bg-Ref/+^{Bg} *and homozygous* o2-hf, Bg-Ref *genotypes*

Plant number	Crossing with *o2-R*, +^{Bg} or with *o2-m(r)*, +^{Bg} ([b]) used as female parent				Selfing ([a]) or crossing with *o2-R*, +^{Bg} or with *o2-m(r)*, +^{Bg} ([b]) used as male parent				
	Number of kernels			$\chi^2_{1:1}$ [c]	Number of kernels			$\chi^2_{1:1}$	$\chi^2_{3:1}$
	n[d]	v	o		n	v	o		
1	2	3	4	5	6	7	8	9	10
A. Progeny of the ear 02-4574×4742p152 of *o2-hf/o2-R, Bg-Ref/+^{Bg}* genotype									
03-4210p1[e]	-	-	-	-	148	0	148	0.00	-
03-4210p13	-	-	-	-	166	8	175	0.00	-
03-4446p7	-	-	-	-	84	33	118	0.00	-
B. Progeny of the ear 02-4568p131 of homozygous *o2-hf, Bg-Ref* genotype									
03-4211p19	74	59	0	1.69	197	83	0	46.41***	-
03-4212p7[f]	26	12	0	5.16*	220[b]	77[b]	0[b]	68.85***	-
03-4212p19	78	61	0	2.08	210	50	0	98.46***	-
03-4219p3	88	75	0	1.04	174	70	0	44.33***	-
03-4225p4[e]	70	221	0	78.35***	147	40	0	61.22***	-
03-4226p16	71	0	0	-	158	0	0	-	-
03-4404p13[g]	97	97	0	0.00	147	67	0	29.91***	-

03-4404p16[g]	28	20	0	1.33	177	104	0	18.96***	-

Table 1 (continued)

1	2	3	4	5	6	7	8	9	10
03-4406p7[e]	66	62	0	0.13	122	45	0	35.50***	-
03-4411p4	80	107	0	3.90*	171	69	0	43.35***	-
03-4415p19	103	109	0	0.17	326[a]	39[a]	0[a]	-	39.89***
03-4418p13	28	41	0	2.45	171	95	0	21.71***	-
03-4420p4[e]	82	23	0	33.15***	219	181	0	3.61	-
03-4421p16	-	-	-	-	310	0	0	-	-
03-4428p3	70	76	0	0.25	112	59	0	16.43***	-
03-4432p3[ef]	128[b]	153[b]	0[b]	2.22	228[a]	32[a]	0[a]	-	22.33***
03-4432p4[f]	65	120	0	16.35***	137[a]	35[a]	0[a]	-	1.98
03-4433p13	59	50	0	0.74	117	67	0	12.78***	-

[c] For progenies of the ear 02-4574x4742p152 (part A of the table) the 1:1 ratio was the ratio of the sum of normal and variegated kernels to opaque ones; for the progenies of the ear 02-4568p131 (part B of the table) the ratios 1:1 and 3:1 were the ratios of phenotypically normal kernels to variegated.

[d] Here and in Table 3, n, v, o indicate phenotypically normal (normal or WER), variegated and opaque kernels respectively.

[e] Plant originated from variegated kernel (not marked are plants from phenotypically normal kernels).

[f], [g] Plants from clusters of three ([f]) and two ([g]) embryo revertants consisting from contiguous kernels presented in adjacent pairs of spikelets (data not shown).

*, *** Significance of deviation from expected at P=0.05 and P=0.001, respectively.

- Indicates not applicable (for the χ^2 test) or data not available

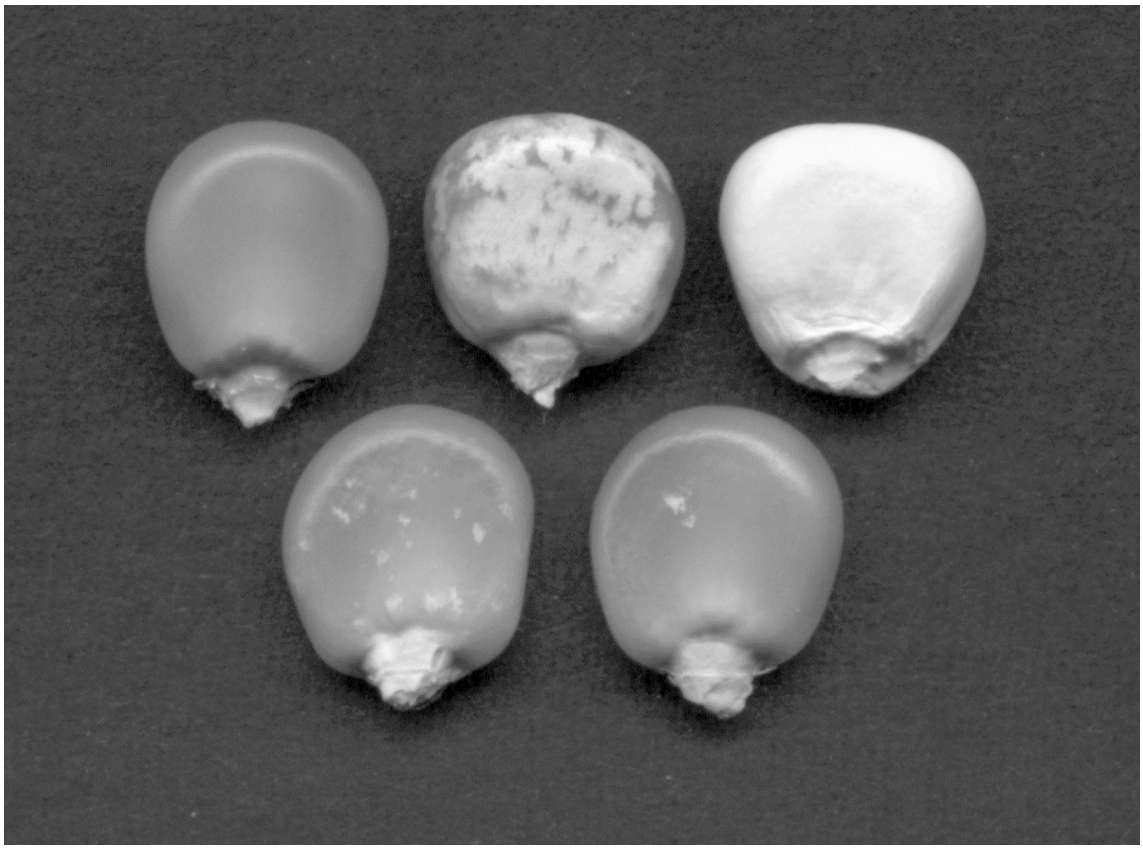

FIGURE 1 - Kernel phenotypes observed at *o2::rbg* alleles. Upper row (from left to right): phenotypically wild-type (whole endosperm revertant, WER) kernel formed as a result of *rbg* excision from *o2::rbg* before the first division of primary endosperm nucleus; variegated kernel (vitreous sectors in opaque background) arisen due to *rbg* excision during endosperm development, commonly observed at *o2::rbg* alleles; opaque kernel phenotype conditioned by the *o2::rbg* allele in absence of *Bg* element. Two variegated kernels in the lower row which are characterized by small and very small opaque sectors in vitreous background are usually observed in *o2-hf*, *Bg-Ref* genotypes.