On the mechanism of haploid production by RWS

Haploids have become one of the most effective tools in modern genetics and breeding. When a haploid inducer line is crossed as a male parent onto a diploid female, most kernels produced contain a diploid embryo and a triploid endosperm; however, a portion of the kernels has a haploid embryo and triploid endosperm. Such kernels germinate normally and grow into haploid plants. The haploid plants are maternal haploids because the female parent contributed the chromosomes.

In most cases, kernels with haploid embryos are selected using the *R1-nj* allele of the *R1* locus on chromosome 10. The kernels with haploid embryos are germinated, treated with a chromosome-doubling agent (such as colchicine), and pollen from doubled (diploid) sectors is used to self-pollinate the haploid plant. The kernels produced are doubled haploids and completely homozygous (instant inbreds).

RWS, a line developed at the University of Höenheim, Stütgart, Germany (Röber *et al.*, 2005, Maydica 50:275-283) is a widely used line for producing maize haploids. RWS and lines derived from it are used extensively by the corn breeding industry. However, the mechanism that haploid inducing lines produce kernels with haploid embryos is not well understood.

Two major mechanisms have been proposed to explain how these haploid inducers might produce kernels with haploid embryos. First, an abnormal fertilization event might take place in which one sperm fertilizes the two polar nuclei and the other sperm fails to fertilize the egg of an embryo sac producing a kernel that has a

triploid endosperm and a haploid embryo. Second, the normal double fertilization events would take place, and then the chromosomes contributed by the female parent are eliminated from the embryo after fertilization.

The following experiment was performed to distinguish between these two hypotheses. RWS (which is *Bm2, Lg1, Gl1, J1,* and *Gl*) was crossed as a male parent onto a female parent (Mangelsdorf's multiple chromosome tester) that is homozygous for recessive plant-expressed mutations on five of its chromosomes (*bm2, lg1, gl1, j1,* and *g1* on chromosomes 1, 2, 7, 8, and 10 respectively). 1200 kernels were field-planted. Of the 1108 plants that grew, 121 expressed all five of the recessive mutations (were maternal haploids). In addition, four exceptional plants were identified that expressed only one of the mutations (one was *bm2*, one was *lg1*, one was *gl1,* and one was *g1*). Each of these four plants had a morphology that was typical of a monosomic plant. Kernels of this same cross were also grown in a sand bench planting (it is only possible to classify *gl1* and *lg1* in sandbench plantings). Of the 535 seedlings, 35 of the seedlings were *gl1* and *lg1* and one exceptional plant was *gl1* and *Lg1*. Cytological analysis indicated that each of the five exceptional plants were monosomics (2n=19). These five exceptional plants could only be produced if chromosome loss occurs after fertilization. However, this experiment does not preclude the possibility that some of the haploids were produced by an event in which a sperm failed to fertilize the egg of an embryo sac.