Letter

Is rf2 a restorer gene of CMS-T in maize?

Recently Patrick Schnable's team reported that they had cloned *rf2* and determined its function [1,2]. For two decades [3], male fertility restoration of CMS-T (cytoplasmic male sterility-Texas) has been described as being under the control of two restorer loci, *rf1* and *rf2*. *Rf1* and *Rf2* (the restorer alleles) are dominant and are both required for male fertility restoration of CMS-T. But is *rf2* a restorer gene?

Cytoplasmic male sterility is a maternally inherited trait that results in the inability of plants to produce viable pollen grains. This phenomenon is often observed in nature in many plant species, and has been used extensively in hybrid production. Every time the cause of CMS has been identified, it has been mitochondrial [4].

Intra-genomic conflict [5] arises when genomes do not share the same pattern of inheritance, each genome maximizing its own transmission at the expense of the other. This is the case of cytoplasmic genes that are maternally transmitted, whereas nuclear genes are biparentally transmitted. Thus, mitochondrial genes that improve their transmission by blocking male gametogenesis will appear and spread (and are therefore mitochondrial male sterile genes) and generate a subsequent selective pressure that favors the emergence of nuclear loci restoring male fertility. These 'restorer' loci are believed to act specifically against the expression or the effect of sterilizing factors encoded by the mitochondrial genome. This nucleo–mitochondrial interaction can therefore be seen as the fruit of a host–parasite-like co-evolution [6]. In summary: (1) CMS requires the cytoplasmic inheritance of the genes that encode for sterilizing factors and not just the localization of these factors; (2) nuclear restoration is supposed to be specific and more-or-less exclusive (at least at the allelic level) to a given CMS.

Puzzlingly, Patrick Schnable's team have shown that the RF2 protein, which exhibits an aldehyde dehydrogenase (ALDH) activity, is necessary for pollen production, not only in plants bearing T-cytoplasm but also in plants bearing a 'normal' non-sterilizing cytoplasm (N). There is no difference in the accumulation of the RF2 protein, or in its ALDH activity, between the T- and N-cytoplasm plants.

Therefore, *rf2* does not appear to work as a restorer gene acting specifically against the expression of T-cytoplasm because it is expressed and necessary for male gametogenesis of N-cytoplasm plants as well. Hypothetically, *rf2* could appear as a restorer gene for any other CMS in maize.

I therefore suggest that *rf2* is not a restorer gene of T-CMS but rather a nuclear male fertility gene that is necessary for normal pollen development, regardless of cytoplasm. Consequently, it is not surprising to find *Rf2* (the functional allele) in most maize inbreds, even though they have never been exposed to T-cytoplasm. By contrast, and as expected, *Rf1*, the 'other' restorer gene is rare.

Even though *rf2* does not provide clues to the restoration mechanism in CMS-T (as *rf1* would if it were cloned), it does open up exciting avenues towards understanding the link between mitochondrial metabolism and male gametogenesis.

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Response from Patrick Schnable

Pascal Touzet asserts that the *rf2a* gene (previously designated *rf2* [1] is not a nuclear restorer of T (Texas) cytoplasm maize. He supports this assertion with the statement that *rf2a* is 'necessary for male gametogenesis of N [normal] cytoplasm plants'. He goes on to state that *rf2a* appears to be 'a nuclear male fertility gene that is necessary for normal pollen development, regardless of cytoplasm.'

The data we presented [2] do not support Touzet's conclusions. Plants that are homozygous for mutant alleles of nuclear male fertility genes are by definition male sterile regardless of their cytoplasmic background. We have previously stated that N-cytoplasm plants that lack a functional copy of the *rf2a* gene are not male sterile [1]. The point made in Figure 9 [1] is that the anthers from the lower florets of these plants fail to develop normally. Even so, because the anthers from the upper florets of these plants develop normally, these plants are male fertile. Indeed, the N-cytoplasm version of the inbred line R213, which is fixed for an *rf2a* mutant allele, produces copious amounts of viable pollen and is easily maintained by self-pollination. These data are not consistent with *rf2a* being a genic male fertility gene. In contrast to the male fertility conditioned by *rf2a* mutants in N-cytoplasm plants, R213 plants that carry T-cytoplasm exhibit *rf2a*-dependent male sterility. Hence, our data support the long-standing view that *rf2a* is indeed a nuclear restorer of T-cytoplasm maize.

Acknowledgements
Research on cytoplasmic male sterility in my laboratory is supported by grants from the USDA NRI Plant Genetic Mechanisms program (most recently, awards 0001478 and 0201414). I thank Chris Chase, Susan Gabay-Laughnan and Charlotte Bronson for discussions helpful in formulating this response.

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