chromatin. There are many possible reasons: heterochromatin has low levels of accessible methylcytosine residues, so that the antibody is unable to distinguish differences; or, the denaturation process was not adequate for the antibody to access all the methylcytosine residues

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α-tubulin-YFP labeled sperm cells for live cell imaging of the fertilization process in maize and relatives such as *Tripsacum dactyloides*

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In vivo imaging of double fertilization processes is a prerequisite to studying and understanding the underlying molecular mechanisms involved in cross-talk between the male (pollen tube) and female gametophyte (embryo sac), as well as sperm cell release, migration and fusion with the two female gametes (egg and central cell). Tremendous progress is currently being made in understanding these fundamental biological processes using *Arabidopsis* as a model system because a large number of tools have been developed over the last couple of years (Berger et al., Trends Plant Sci. 13:437-443, 2008). Comparable tools are missing for maize. In order to visualize these fertilization processes in maize also, we have screened a number of fluorescent protein tagged maize lines generated via the NSF project #0501862 (for review see http://maize.jcvi.org/cellgenomics/) to identify sperm cell marker lines.

One line could be identified displaying YFP-tagged α-tubulin in many cell types of roots, leaves, inflorescences, etc., but exclusive labeling of sperm cells inside mature pollen grains. During microsporogenesis, the YFP signal could be detected earliest after the first mitotic division in the generative nucleus. The generative nucleus then divided to form two sperm cells with similar YFP signal intensity. In the mature pollen grain, only the two crescent-shaped sperm cells displayed YFP signals. This marker line is superior even to *Arabidopsis* marker lines, as the entire sperm cell boundaries are labeled by cortical tubulin strands. Furthermore, the accumulation of thick tubulin bundles at both sides surrounding the sperm cell nucleus allows simultaneous visualization of the nuclei (Fig. 1A). There is no detectable signal in the vegetative cell of the developing pollen grain at any stage of microsporogenesis nor at a later stage during pollen tube growth. Due to its specific expression pattern during microgametogenesis, the endogenous promoter of this *α-tubulin* gene family member, which drives expression of the fusion protein, provides a tool to specifically deposit other proteins or RNAs inside maize sperm cells. Moreover, this marker line enables studies on sperm migration dynamics during pollen tube growth and fertilization. In pollen grains of maize and many other plant species, the two sperm cells lie in immediate proximity to each other and are connected by an unknown mechanism. During tube growth, the two sperm cells leave the pollen grain through the germ pore shortly after germination and migrate back and forth inside the growing pollen tube

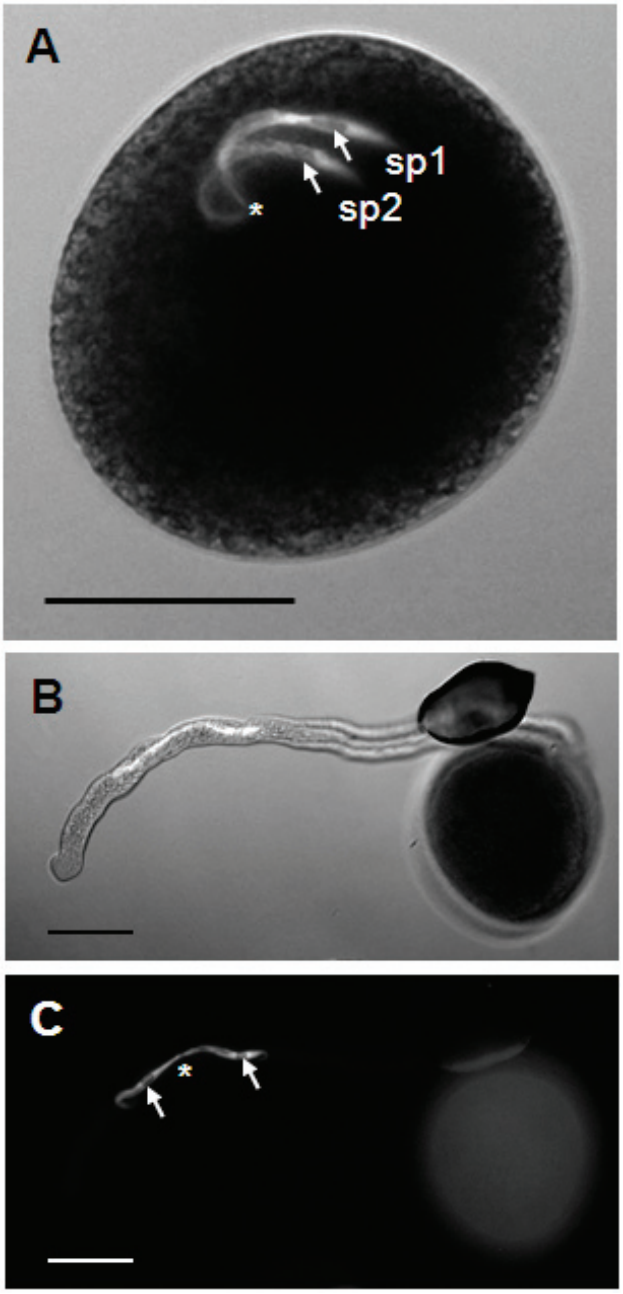

Figure 1. α-tubulin-YFP labeled sperm cells. **A**: Mature pollen grain containing two sickle-shaped sperm cells (sp1 and sp2). Arrows point at sperm nuclei and an asterisk marks the connection between both cells. **B**: Sperm cells migrating towards the tip of a growing pollen tube (merged bright field and fluorescence image). **C**: Same as B but only YFP signal is visible. Arrows and asterisk as in A. Scale bars 50µm.

while still attached to each other (Fig. 1B and C). As soon as the pollen tube reaches the embryo sac, it discharges its contents though interaction with the synergids. These processes occur inside the ovary, which is deeply embedded in maternal tissues, and have never been observed in maize and other grasses. However, a number of labs have already developed methodologies for in vitro fertilization and separation of the female gametophyte from maternal tissues, which will enable study of these fundamental processes using the marker line described above.

Maize sperm cells are also able to fuse with female gametes of related species, which will permit investigation of a number of additional fertilization related questions. In vivo studies using maize pollen and female flowers of maize relatives such as *Tripsacum dactyloides*, for example, showed a very high rate of fertilization events. In the apomict *T. dactyloides*, the unreduced egg cell develops autonomously into an embryo without fertilization, but the unreduced central cell requires fertilization by one of the two male gametes. What is the fate of the second sperm cell? Does it often fertilize a synergid, as a high number of twin embryos are observed in *T. dactyloides* (Bantin et al., Sex. Plant Reprod. 14:219-226, 2001), or does it degenerate? Moreover, this line will allow studies of the fate of sperm-deposited protein/RNA after fertilization, as the α-*tubulin* promoter used to generate this line is switched off after fertilization (Kliwer and Dresselhaus, unpublished). Seeds and promoter sequences of this transgenic line are available from the Jackson lab.

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Selection strategies for tolerance to Mal de Río Cuarto disease in different evaluation environments

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Mal de Río Cuarto (MRC) is the most important viral disease of maize in Argentina The production of maize for grain is greatly affected by MRC disease, which is caused by a virus of the family *Reoviridae*, genus *Fijivirus*, and transmitted by the planthopper, *Delphacodes kuscheli* Fennah (Homoptera: *Delphacidae*) (Nome et al., 1981; Ornaghi et al., 1993).

Characterizing genotype testing sites and identifying environments with negligible genotype x environment crossover interaction is important for plant breeders wishing to identify superior germplasm and (or) genotypes for a wide range of environments. Genotype x environment (G x E) interactions can be an impediment to genetic progress in maize (*Zea mays* L) breeding for Argentina. Therefore, identifying appropriate environments where the selection should be carried out is an important aspect of a plant breeding program in order to maximize the efficiency of selection. Indirect selection efficiency is related to the heritability of the trait and to the genetic correlation between environments.

The objective of this work was to study the effect of different environments on the relative efficiency (*E*) of direct selection in relation to indirect selection for MRC. The trials were conducted with 111 recombinant inbred lines (RILs) derived from a cross between a susceptible inbred line, Mo17, and a tolerant inbred line, BLS14. The trials were conducted during 2004, 2005 and 2006 at Río Cuarto (64º 20'W, 33º 8'S, 334 masl), and during 2004 and 2005 at Sampacho (64º 42'W; 33º 19'S, 510 masl). The year-location combinations were regarded as different environments (Río Cuarto 2004, Río Cuarto 2005, Río Cuarto 2006, Sampacho 2004 and Sampacho 2005). The experimental design at each environment was a randomized complete block design with two replications.

At the beginning of male flowering, 60–70 days after planting, the RILs were evaluated for several traits related to symptoms of MRC disease, which allowed us to estimate the grade of severity of the disease. A four-grade disease severity scale proposed by Ornaghi et al. (1999) (0: no symptoms; 1: enations; 2: enations and "hokey pole" ears; 3: enations, shortened superior internodes and small ears with few or no kernels) was used for each plant and data averaged for a plot.

Indirect selection efficiency is derived from the selection response model using the formula for the ratio of correlation to direct response:

$$E = r_g\, h_Y / h_X$$

where r_g is the genetic correlation for the trait measured in the environment of selection Y and in the environment of interest X, and *h*y and *h*x are square roots of the broad sense heritability of the grade of severity of the disease in Y and X environments, respectively. The efficiency model *E* is based on the assumption that selection intensities *i* of direct and indirect selection are the same. The equation of efficiency of indirect selection states that if $E>1$, indirect selection is more efficient than direct selection, and if $E<1$, direct selection is more efficient.

The genetic correlation between the environment of selection Y and in the environment of interest X to the grade of severity of the MRC, can be estimated from the equation:

$$r_g = \sigma_{g\,(XY)} / \sqrt{(\sigma^2_{g\,(X)}\, \sigma^2_{g\,(Y)})}$$

where $\sigma_{g\,(XY)}$ is the genetic covariance between X and Y, and $\sigma^2_{g\,(X)}$ and $\sigma^2_{g\,(Y)}$ is the genotypic variance component of the X and Y, respectively.

Heritability estimates of the grade of severity of the disease evaluated in each environment were estimated as:

$$h^2 = (\sigma^2_g)/[(\sigma^2_g)+(\sigma^2_e/r)]$$

where *r* denotes the number of replicates and the subscript g indicates variance components associated with genotypes and e, experimental error.

Table 1. Genetic correlation (r_g) for the grade of severity of MRC between environment of selection and environment of interest, and predicted efficiency (*E*) of indirect selection relative to direct selection.

Environment of selection[a]	Environment of interest	r_g	S.E.[b]	*E*
R4	S4	0.30	0.25	0.24
	R5	0.17	0.21	0.15
	S5	0.03	0.19	0.03
	R6	-0.03	0.23	-0.03
S4	R4	0.30	0.25	0.37
	R5	-0.21	0.16	-0.24
	S5	0.24	0.20	0.28
	R6	0.44	0.27	0.53
R5	R4	0.17	0.21	0.19
	S4	-0.21	0.16	-0.19
	S5	-0.02	0.15	-0.02
	R6	0.01	0.19	0.01
S5	R4	0.03	0.19	0.03
	S4	0.24	0.20	0.21
	R5	-0.02	0.15	-0.02
	R6	0.09	0.18	0.10
R6	R4	-0.03	0.23	-0.03
	S4	0.44	0.27	0.36
	R5	0.01	0.19	0.01
	S5	0.09	0.18	0.09

[a]R4 = Río Cuarto 2004, S4 = Sampacho 2004, R5 = Río Cuarto 2005, S5 = Sampacho 2005 and R6 = Río Cuarto 2006
[b]Standard errors of the genetic correlations between environments