



Restoration of c-type cytoplasmic male sterility in maize: Fine-mapping of *Rf4*

Susanne Kohls, Rainer Messmer, Peter Stamp

Institute of Plant, Animal and Agroecosystem Sciences, ETH Zurich, 8092 Zurich, Switzerland

Introduction

1.1 What is cytoplasmic male sterility (CMS)?

- Failure of pollen production induced by mitochondrial mutations
- Male fertility can be restored by nuclear “restorer-of-fertility” genes
- Restorer genes cloned so far mainly code for proteins of the **pentatricopeptide-repeat protein family (PPR)**
- **c-CMS** is one of three major CMS types in maize

1.2 Applications in plant breeding

- **Now:** cost-efficient hybrid production
- **Potential:** GMO pollen containment and yield increase through exploitation of xenia by allopollination



1.3 Limitations of c-type CMS

- Complex, largely unknown fertility restoration system
- Restorer properties of an inbred line has to be determined by time-consuming test-crosses

1.4 Objective

- Fine mapping of the major c-CMS restorer gene *Rf4*, located on the short arm of chromosome 8 (bin 8.00)

Material & Methods

2.1 Field trials and phenotyping of male fertility

Phenotyping of male fertility in F_2 : visual anther scoring in two F_2 populations (**B37c x K55**, $n_1=1669$ in 2007, $n_2=1317$ in 2008)

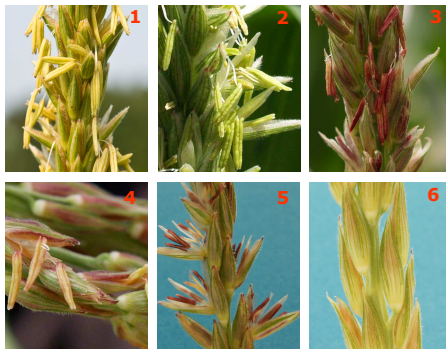


Fig. 1: Anther quality scale 1 (functional anthers) to 6 (no exertion of anthers)

Male fertility scoring follows 3 criteria:

1. Anther quality (Fig. 1): scale 1 - 6
2. Anther quantity: scale 0 - 5
3. Anthesis - silking interval

These 3 criteria are combined to assign plants to the male fertile, semi-fertile and sterile class:

- Male fertile: anther quality 1 - 2, anther quantity 5, ASI > - 8 days
- Semi-fertile: anther quality 3 - 5, anther quantity < 5, ASI < - 8 days
- Sterile: no anther exertion

2.2 Molecular analyses

1. Marker development in bin 8.00
 - by SSR motif detection (SSRIT)
 - by comparative sequencing of the parental lines
2. Genotyping & Mapping
 - **selective genotyping** in the phenotypic extremes of F_2 -2008 ($n=500$)
 - Mapping software RECORD + RQTL
3. *In silico* candidate gene search
 - RepeatMasking
 - Gramene pipeline
 - *ab initio* Fgenesh & GenScan gene prediction

Results

3.1 Phenotypic results: segregation of male fertility

- Occurrence of ~75 % fully restored plants suggests the presence of **one dominant restorer gene** (Tab. 1).
- Occurrence of semi-fertile plants complicates phenotypic assignment

		2007	2008
Male fertile (mf)	<i>(Rf4)</i>	74.7	77.3
Semi-fertile (sf)	<i>(rf4)</i>	24.3	24.3
Sterile (st)	<i>(rf4)</i>	1.0	1.1
		25.3	22.7

Tested ratio 3:1 $\chi^2 = 0.16$ $\chi^2 = 3.73$
(mf : sf+st)

Tab. 1: Segregation of male fertility in F_2 -2007 and F_2 -2008 [%]

3.2 Molecular mapping results

- *Rf4* was delimited to a 0.8 cM region (Fig. 2). This corresponds to a physical map region of ~ 500.000 bp
- Marker types: ETH1 indel, ETH 2-4 SSR
- No predicted genes were found in this regions which resemble previously cloned restorer genes (PPR proteins).

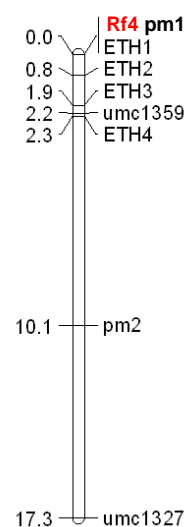


Fig. 2: Genetic map of *Rf4* (chr. 8) and close markers based on phenotypic extremes of F_2 -2008 ($n=500$).

Conclusions

- *Rf4* is inherited like a single dominant gene in this population.
 - The achieved map distance is sufficient for **marker assisted selection**: markers will be available after publishing.
 - Important: *Rf4* is not the only restorer gene of c-CMS! The restorer property of an inbred line can therefore not be predicted based on the *Rf4* genotype alone.
 - No PPR motifs were found in the 0.8 cM interval comprising *Rf4*
- *Rf4* might therefore be a novel type of restorer protein!