

# Silencing of the Aflatoxin Cluster in a Diploid Strain of *Aspergillus flavus*

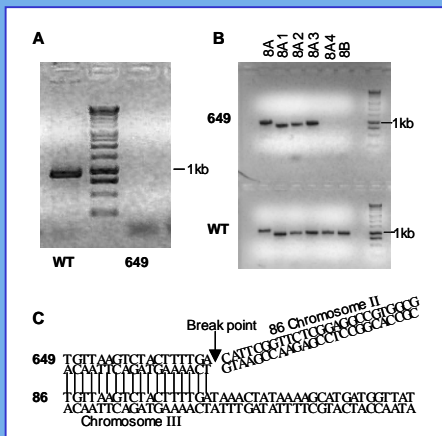


Carrie A. Jacobus<sup>1†</sup>, Dominique Robertson<sup>1,2†</sup>, Charles P. Woloshuk<sup>3\*</sup>, Gary A. Payne<sup>4†</sup>  
 Department of Genetics<sup>1</sup>, Department of Plant Pathology<sup>2</sup>, Department of Botany<sup>3</sup>  
 \*Purdue University, West Lafayette, IN 47907, USA  
 †North Carolina State University, Raleigh, NC 27606, USA

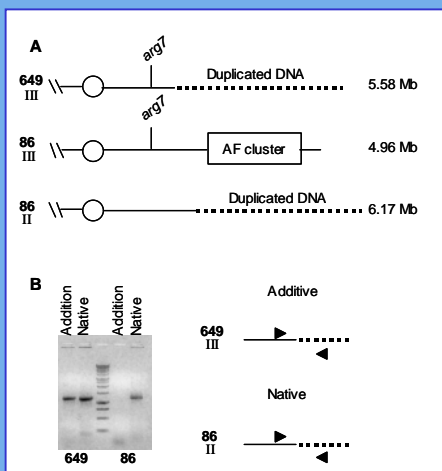


## ABSTRACT

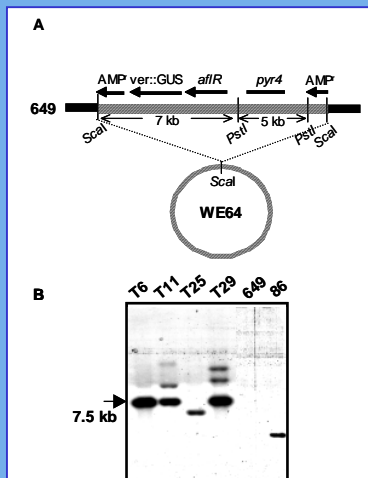
*Aspergillus flavus* is an asexual filamentous fungus that produces the toxic and carcinogenic compound aflatoxin. The parasexual cycle can be induced in this fungus for genetic analyses, including assessment of gene dominance in stable diploids. All known mutations in genes for aflatoxin biosynthesis are recessive in diploids except for *afl-1* in strain 649. Diploids between 649 and 86 (wild type) lack transcripts for the aflatoxin biosynthetic genes and fail to produce aflatoxin. We are characterizing this mutant to better understand the mechanism of this inhibition of aflatoxin biosynthesis. Loss of aflatoxin production in 649 is due to a 317 kb deletion that includes the aflatoxin gene cluster. In addition, this strain contains a 939 kb duplication. Failure to produce aflatoxin in 649 x 86 diploids does not appear to be due to a repressor of the transcriptional regulator AfIR as diploids between 86 and a strain of 649 carrying ectopic copies of *aflR* produce aflatoxin. These data suggest that the location of *aflR* in the genome dictates whether it is functional in the 649 x 86 diploid. One explanation for this is that some form of silencing is preventing aflatoxin production in the diploid strains. Investigations are currently underway to characterize the possible *trans*-sensing phenomenon.



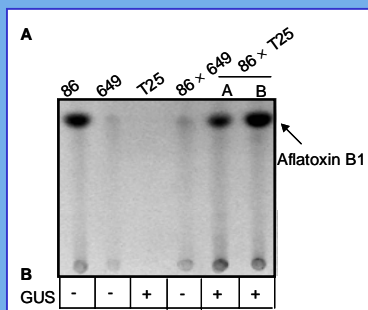
**Figure 1 - Identification and characterization of the deletion in 649.** (A) The telomere adjacent to the aflatoxin gene cluster has been deleted. (B) The break point region was narrowed down by a series of PCR reactions. (C) The exact break point was determined and the nature of an addition in 649 was characterized.



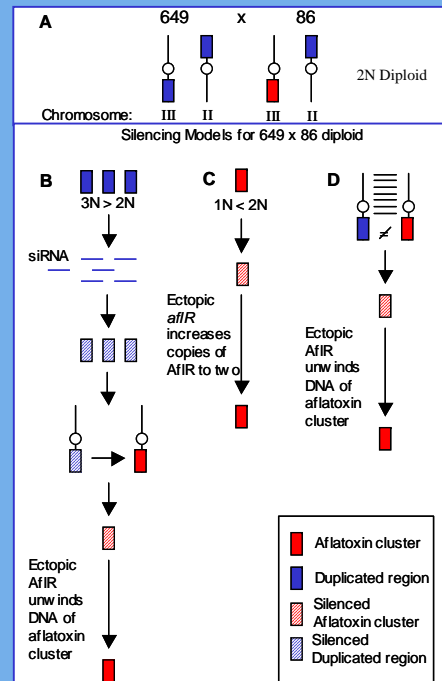
**Figure 2 - Identification and characterization of the addition in 649.** (A) 649 contains a 317 kb deletion as well as a 939 kb addition from chromosome II. (B) The addition in 649 is the result of a duplication and not a translocation.



**Figure 3 - Transformation of 649 pyr with WE64 carrying a copy of *aflR*.** (A) Construct WE64. (B) Southern blot probed with *aflR*. A size 7.5kb band indicates tandem integration. T25 contains a single integration of WE64.



**Figure 4 - Aflatoxin production is restored when a strain of 649 carrying an ectopic copy of *aflR* is crossed with a wild type strain.** (A) TLC plate exposed to UV to visualize aflatoxin. (B) GUS activity is detected when a functional ectopic copy of *aflR* binds to the *ver-1* promoter. Because AfIR is functional in the 649 background, this argues against an AfIR specific repressor in 649.



**Figure 5 - Possible models to explain the silencing phenotype in 649 x 86 diploids.** (A) Chromosomes carrying the aflatoxin cluster or the duplication in the 649 x 86 diploid. (B) 3 copies of the duplication may trigger post-transcriptional gene silencing (PTGS) and because of chromosomal position, the aflatoxin cluster may also be silenced. The ectopic copy of AfIR may relieve the silencing by opening up the aflatoxin gene cluster. (C) The aflatoxin gene cluster is not present in two copies. This could trigger silencing of the aflatoxin gene cluster. (D) When T25 is paired with 649 to form a diploid, *aflR* is present in two copies and this may prevent silencing of the aflatoxin gene cluster. (b) Chromosome III from 649 and 86 will not completely align. This may cause silencing of the aflatoxin cluster. As in part A of this figure, the ectopic copy of AfIR may relieve the silencing by opening up the aflatoxin gene cluster