

TITLE: SELECTION OF INSERTION MUTANTS OF *Fusarium proliferatum* RELATED WITH ALTERED PATHOGENICITY IN SOYBEAN

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ABSTRACT:

Fusarium proliferatum is an important pathogen associated with plant diseases, affecting mainly the aerial parts, and producing different mycotoxins that are toxic to humans and animals. In the last decade, this fungus was also described as one of the causes of red root rot (RRR) in soybeans, which causes extensive damage to this crop, being Brazil the second largest producer of soybean in the world, behind only the USA and corresponding to the third most exported product of the country, which contribute very significantly to the national economy. Thereby this study aimed to carry out insertional mutation (AIM) in *F. proliferatum* by *Agrobacterium tumefaciens* mediated transformation (ATMT), select mutants with loss of pathogenicity and evaluate the colonization of soybean roots with mutants by the observation of the expression of GFP and DsRed fluorescent proteins. The genetic transformation was performed by ATMT, using the *GFP* and *DsRed* genes. Soybean roots were inoculated with oat grains colonized by insertional mutants of *F. proliferatum* after seven days of germination. The plants were kept under environmental conditions for a period of 21 days after inoculation. The roots were washed and observed for the development of RRR root symptoms. Histological sections of the roots were prepared to visualize the expression of GFP and DsRed proteins. Among the 50 transformants tested, 58% were responsible for reducing the symptoms of RRR in soybean roots, in relation to the control, indicating the high efficiency rate of the technique for the inactivation of genes related to pathogenicity. It was possible to observe clear expression of the red and green fluorescence of the mutants inside the plants, in contrast to plants inoculated with the wild strain. These results emphasize the effectiveness of the AIM technique, associated to ATMT for inactivation of pathogenicity genes, and can be used for the identification of RRR-related genes in soybean. In addition, the use of GFP and DsRed proteins can be used for interaction studies between pathogen and host plant.

Keywords: red root rot; sudden death syndrome; insertional mutagenesis; fluorescence

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