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Construction of Gateway Binary Vector for Selection with Bialaphos or Carboxin and GFP Expression in Fungi

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Genomic data has created a growing demand for tools and methodologies for studying the genes function, which can be realized through loss of function experiments (gene knockout) or by RNA silencing (knockdown). The development of binary vectors for *Agrobacterium tumefaciens* mediated transformation (ATMT) has the advantage of being independent of protoplast formation and can be used directly on a wide variety of fungal species and tissue types. The selection of transformants using bialaphos and carboxin has the advantages of low cost in the transformation and availability of different selectable markers, also allowing the analysis of several genes and combination of study by knockout or knockdown, using selectable markers in the same transformant. Thus, this study aimed to build two binary vectors containing reporter gene and selectable markers that confer resistance to carboxin and bialaphos. The cassettes were constructed using the Gateway system to two fragments. The gene encoding the GFP protein and *Ptox*A and *Ptrp*C promoters were cloned into pDONR P1-P5R plasmid. Genes that confer bialaphos and carboxin resistance, *bar* and *cbxr* respectively, were cloned into pDONR P5-P2 plasmid. The pPGW plasmid was used as destination vector. The *gfp* gene transcription is controlled by *Ptox*A promoter and the *bar* and *cbxr* genes transcriptions are controlled by *Ptrp*C promoter. These binary vectors were named pGWGFP-BAR and pGWGFP-CBXR. The assembly of cassettes was confirmed by sequencing, and the validation of vectors is being accomplished through transformation (ATMT) with the plant pathogens Mycosphaerella fijiensis and Fusarium oxysporum f. sp. cubense. Financial support: CNPq and FAPEAM