Quantitative real-time PCR assessment of cassava transgenic plants: copy number estimation and quantification of gene expression

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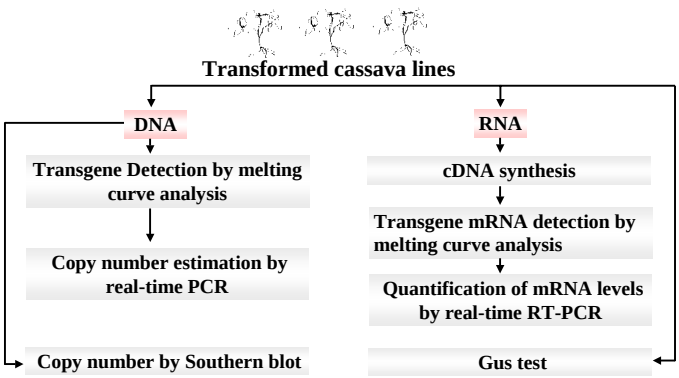
INTRODUCTION

To improve the conventional molecular analysis of cassava transgenic plants, we developed qPCR methods to estimate copy number and quantify mRNA levels of transgenes in cassava lines obtained through *Agrobacterium*-mediated transformation.

Six lines were also analyzed by Southern blot. The copy number was concordant in most cases with those estimated by qPCR. Most of the transgenic events (86.6%) had low copy number (1 or 2), thus corroborating that *Agrobacterium* generally inserts a low copy number of transgenes into plants.

Quantitative mRNA expression data grouped the transgenic lines into three expresser categories —high, medium, and low— for hygromycin phosphotransferase (*hpt*II) and ß-glucuronidase (*GUSPlus)* genes. *GUSPlus* mRNA data agreed with results from histochemical GUS staining.

METHODS



RESULTS AND DISCUSSION

• Copy number estimation by real-time PCR

Copy numbers for the genes *GUSPlus* and *hpt*II were estimated in 15 transgenic lines

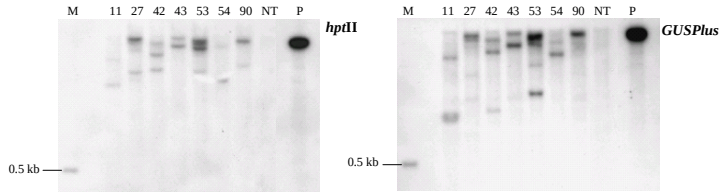
Table 1. Copy number for the *GUSPlus* and *hpt*II genes, estimated by qPCR for 15 transgenic cassava lines

Line	Copy number for gene: *GUSPlus*	*hpt*II
2	1	1
11	2	2
27	2	2
29	1	2
42	2	2
43	2	2
46	1-2	1-2
50	2	2
51	2	1
52	1	1
53	3	3
55	3	3
60	1	1
90	1	1
131	1	1

Most lines contained one or two copies of each gene; in some, the copy number was different for the two genes, suggesting rearrangements of the T-DNA

• Southern blot and qPCR, for copy number estimation

The analysis revealed different integration patterns, confirming that the lines arose from independent transformation events.



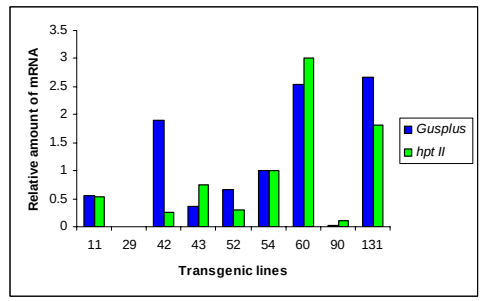
Of the six lines evaluated, five coincided exactly with the qPCR estimates indicating an 83.3% agreement between the two methodologies:

Table 2. Comparison of copy numbers for two transgenes, as estimated by Southern blot and qPCR for six transgenic cassava lines

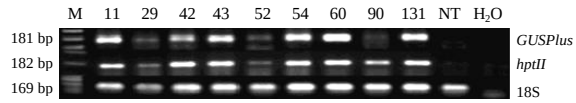
Line	Copy number for gene: *GUSPlus* qPCR	*GUSPlus* Southern blot	*hpt*II qPCR	*hpt*II Southern blot
11	2	2	2	2
27	2	2	2	2
42	2	3	2	3
43	2	2	2	2
53	3	3	3	3
90	1	1	1	1

• Quantifying transgene expression using qRT-PCR

By comparing the highest and lowest expresser lines, we calculated differences of up to 380 times for *GUSPlus* and 3000 times for *hpt*II:

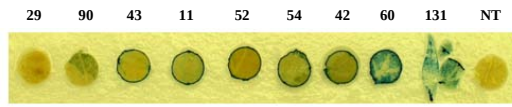


RT-PCR results agreed with the qRT-PCR data for most samples and for both transgenes:



• GUS test

A differential pattern of intensities for GUS can be seen. These intensities mirrored the data obtained with qRT-PCR:



• Summarizing, qPCR was more efficient than the laborious techniques conventionally used to detect transgene copy number and expression.

• Results suggest that, in 3-year-old transgenic cassava plants, high and stable expression of transgenes can be found.

PERSPECTIVES

•Use qPCR for future characterization of transgenic events in cassava, which could facilitate the selection of the most promising events.

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