

Two cassava promoters related to vascular expression and storage root formation

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Introduction:

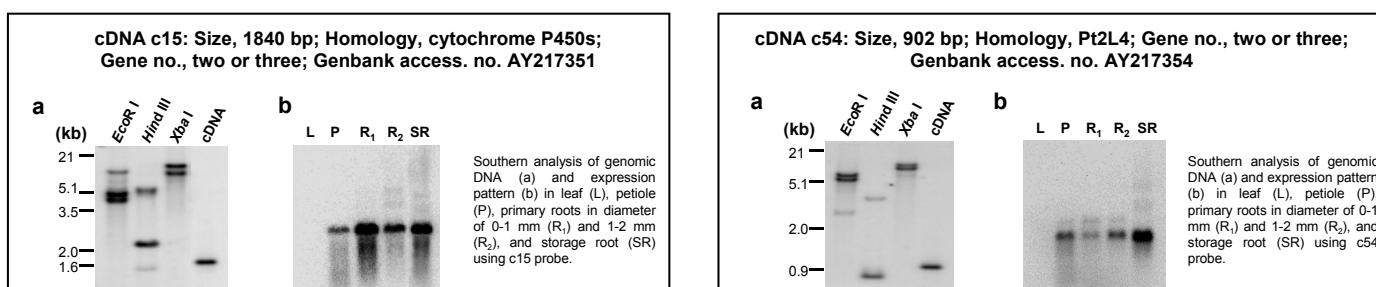
Cassava storage roots develop from primary roots by a peculiar secondary growth and accumulate starch up to 85% of their dry weight. To improve cassava root traits via genetic engineering, the availability of tissue specific promoters is a prerequisite. Here we describe the isolation of two root specific genes, *c15* and *c54*, and their corresponding promoter regions, both of which are related to vascular expression and storage root formation.



Results:

1. Identification and expression analysis of c15 and c54

Two cDNAs, c15 and c54, were identified and purified from a cassava storage root cDNA library in differential screening by hybridising to radioactively labelled storage root mRNA and leaf mRNA.



2. Cloning of the c15 and c54 promoters and stable plant transformation

Sequences of promoters *p*15/1.5 (a, AY217352) and *p*54/1.0 (b, AY217353)

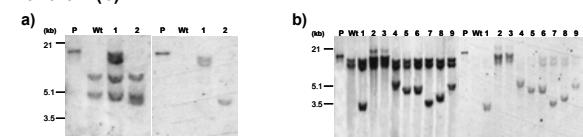
b)	<pre> 1) GGATCCAGG CTGGGGCA ACTTACACG CCAGAACAA ACCCTAAATC GAGAATTTCG 61) TCAAGCTT TTCTACTA CAGCATGC ATAGCTTCG GGGCAACAA AGAACAGCT 111) AGACAGGG CTTCGGGT GGGCAACAA ATCTGTTG CGGGCTTG TGCTGTTG 181) ATATTCTCG AAGCTTTGTT ATTITTTGTTT CTTTCTTG KTTTCTTG CAGGTTAAAT 241) AACGGCAAT CCTTAACTA TTGGGTTA CTCGGATG CTCGGATG GGGGTTTCTT 301) GGTGATCAAC TCACTGGT TGTAAATTG CTTGGTGGGTTT CAGGCTGGTGTGCTGGTGTG </pre> <p style="text-align: center;">B C G T T</p>
361)	TATTAAGA ATAGCTTA ACCATTAAC TAATTTAC ATTCAGCTT ATTTGATG
421)	ATATCTGC AAGCTGCTA GGATTTTGA CCTTCATCC TATGCTGTTT TCTCCGGGTT
481)	TTTTGGGT CCTCTGGT ACACATGGT TCAGCTTCG ATCTGGCTT AAATATTTTAA
541)	TCCTCTGG ATTAATTAAT TAATTAACG TTCTTAATG TTTTTTTTTT
601)	AAAATTAAGA GAAAGAAAGA AGCTGGGGT AGAAAGGAA AGAAGGCTT CTGAGCTTACG
661)	GCTGCTCA AGCGCTAG CAAACAAAGG CAAAGGAGG AGATGCTT AGGGTAAAGA
721)	GGGGCTGG TTTCTGGT TTCTGGCTT AAGCTGGTAT CAGGGCTTG TAAAGAAGA
781)	AAAGTCAGC TTCTTATTTT TTTTATTTCA NNCTTCTAA GCTTCTTCTT GGGGGGGG
841)	TATCTTCCTT GATTTGGT ACAGAGGG GGGGGGGGGG AATCCGGAA AGCTGGGGT TCTCTCTC

Notes: Bold letters underlined with dashed arrows indicate putative binding sites for transcription factors Athb-1, MYB, Ph, SBF1 and P. The translation start is indicated by a bold arrow below the ATG codon.

Expressing vectors: pCP15GUS

The diagram shows the pCP15GUS vector structure. It consists of a blue bar representing the vector backbone with restriction sites EcoRI and Ncol indicated. A red box labeled "p15/1.5" is positioned above the vector backbone, representing the p15/1.5 probe. Another red box labeled "uidA" is positioned below the vector backbone, representing the uidA probe.

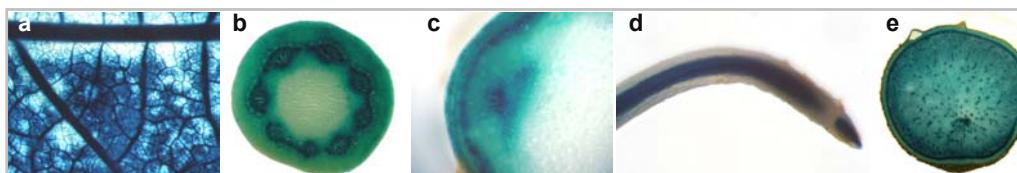
Southern analysis of transgenic cassava containing *p15/1.5::uidA* (a) and *p54/1.0::uidA* (b)



Notes: Total genomic DNA was digested with EcoRI. P, plasmid pCP15GUS in (a) and pCP54GUS in (b); Wt, wildtype control; Symbols 1 and 2 of (B) and 1 to 9 of (C) represent different transgenic escaper lines.

3. GUS expression driven by promoter p15/1.5 and p54/1.0

p15/1.5::uidA in cassava



p54/1.0::uidA in cassava

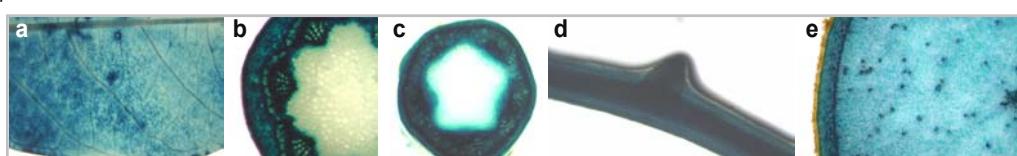
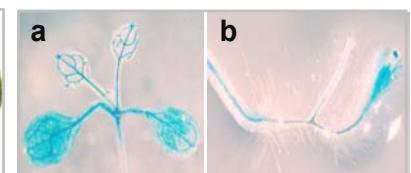
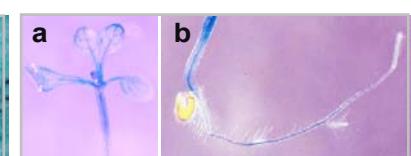


Figure 1.5.1.1. Bifurcation diagram of the logistic map.



p54/1.0::uidA in *Arabidopsis*



Not applicable

Conclusion:

Our results demonstrate that the two promoters are related to vascular expression and secondary growth of storage roots in cassava. The results presented here on histochemical localisation of GUS activity in mature cassava organs suggest that these two promoters are valuable candidates for targeted gene expression for genetic improvement of cassava, such as delayed post-harvest physiological deterioration and nutritional improvement of cassava storage roots.