Conserved expression of a root-hair specific promoter *LeExt1.1* from *Lycopersicon esculentum* in cassava

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Introduction:

Using root hair specific promoter to target gene expression in trichoblasts has great potential to improve nutrient uptake and transformation from soil in cassava (*Manihot esculenta* Crantz), a crop usually growing on acidic soils low in nutrients, particularly phosphorus. For example, when expressing a secretory phytase gene directed by a trichoblast-specific promoter *LeExt1.1* (Fig. 1) in potato, transgenic plants accumulated 40% more P in leaves than wildtype plants[1]. The promoter *LeExt1.1* is isolated from tomato (*Lycopersicon esculentum*) and directs the expression of an extensin-like protein LeExt1 in its root hair[2]. Here we demonstrate the conserved expression of the promoter *LeExt1.1* in transgenic cassava and suggest that it can be used to target transgene expression in trichoblasts of cassava.

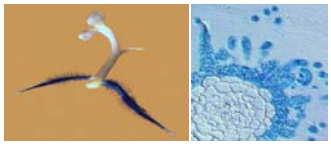


Fig.1 Histochemical localization of *LeEXt1.1::GUS* activity in transgenic potato. A, Primary roots of a seedling; B, Cross-section of a primary root. (After Bucher et al., 2002)

Materials and Methods:

1. Plasmid and *Agrobacterium* strain: The *A. tumefaciens* LBA4404 harboring the plasmid BinΔgen1.1GUS was used for cassava transformation.

Fig. 2 Schematic representation of T-DNA region of BinΔgen1.1GUS.

2. Cassava transformation: Plant transformation was conducted using the embryogenic suspension of TMS60444 via *Agrobacterium*-mediated gene delivery[3].
3. Molecular analysis of transgenic cassava plant lines: transgenic plants were confirmed by Southern analysis. The expression patterns of *LeExt1.1::GUS* were analyzed by Gus assay.

Results and Discussion:

1. Confirmation of transgenic cassava plant lines

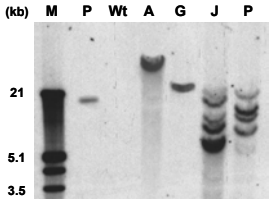
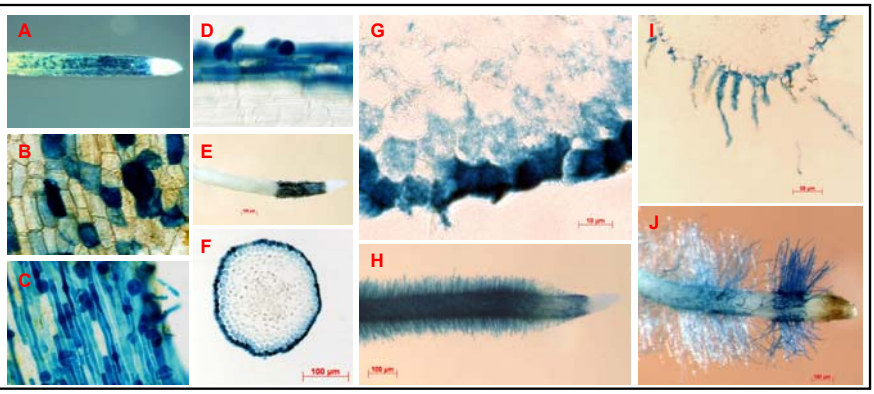


Fig. 3 Southern analysis of four transgenic lines by hybridizing with a GUS probe. Five ug of total genomic DNA was digested with *Sac*I to check the insertion number of transgene. M, molecular marker; P, plasmid Δgen1.1GUS; Wt, wildtype control; A, G, J, P, different transgenic lines.

2. Histochemical localization of GUS activity in cassava tissues

Fig. 4 Histochemical localization of *LeEXt1.1::GUS* activity in transgenic cassava. A, a primary root of an *in vitro* plant. B-C, rhizodermis of primary roots. D, a longitudinal root section. E-G, a primary root of a greenhouse-grown plant and cross sections. H-I, induced root hairs and a cross section of the root. J, a Gus stained primary root showing the expression of GUS in the hairs of the differentiation zone.



Conclusions:

The trichoplast-specific expression of *LeEXt1.1::GUS* was confirmed in transgenic cassava plants. The promoter *LeEXt1.1* can be used to target transgene expression in trichoblasts of cassava.

References:

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2. Bucher M, Brunner S, Zimmermann P, Zardi G, Amrhein N, Willmitzer L and Riesmeier JW (2002) The expression of an extensin-like protein correlates with cellular tip growth in tomato. Plant Physiol, 128: 911-923.
3. Zhang P, Potrykus I, Puonti-Kaerlas J (2000) Efficient production of transgenic cassava using negative and positive selection. Transgenic Res. 9: 405–415.