TRANSFORMATION OF CASSAVA WITH MODIFIED *E. coli* *glgC* GENE FOR INCREASED STARCH PRODUCTION

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INTRODUCTION.

•Cassava's starchy and tuberous roots are sources of cheap calories for the developing world people in. Cassava is also used industrially in the production of ethanol, mannitol, glucose, sorbitol, animal feed, and as a raw material for the starch industry (Cock, 1982).

•Cassava tolerates poor soils and drought and for this reason it is favored in places with infertile soils or adverse climates (Kock, *et al.*; 1994). The roots can be left in the ground without harvest for three years. This makes it important for security against famine.

•Also, cassava has a high efficiency in conversion of CO_2 into sucrose (Hunt, *et al.*; 1977). It's high rates of photosynthesis (43 μmol $CO_2/m^2/s$) is comparable to C4 plants (Angelov, *et al.*; 1993, Edwards *et al.;* 1990, Hunt *et al.*; 1977).

•For these reasons, cassava is potentially an excellent candidate for increased starch production .

•The rate limiting step in starch synthesis is ADP-glucose production catalyzed by the enzyme ADP-Glucose pyrophosphorylase (AGPase). We are using the bacterial AGPase gene (*glgC*) which has a single gene product unlike the plant heterodimeric enzyme (Preiss, 1988).

•The bacterial AGPase has two important allosteric regulation sites: Gly336 and Lys 296 (G336 and K296) for inhibition of allosteric feedback regulation by FBP .

• The allosteric regulation sites of the bacterial AGPase, has been modified, K296E/G336D, thereby introducing two mutations. This confers the enzyme with activity comparable to the wild-type AGPase but lacking the allosteric regulation (Kumart *et al.*, 1989).

•Our hypothesis is that we could increase starch production in transgenic cassava expressing a bacterial gene encoding a modified ADP-glucose pyrophosphorylase (AGPase) which has a higher Vmax than the plant enzyme.

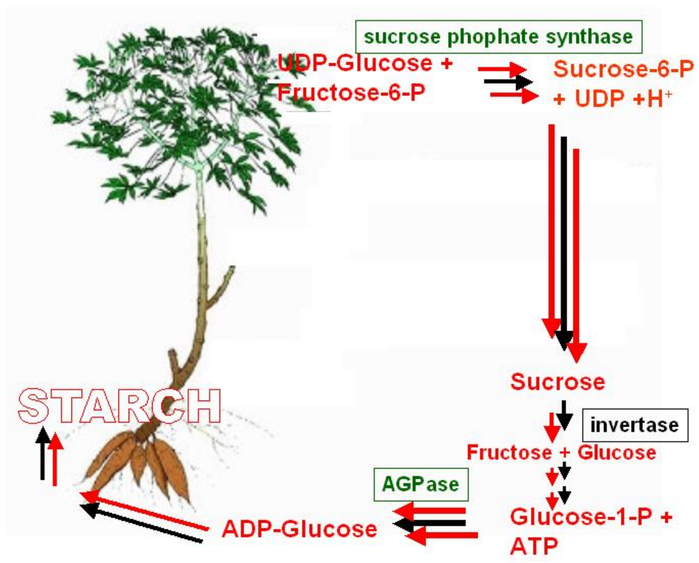


Fig.1. Sketch of biochemical events.

MATERIALS AND METHODS

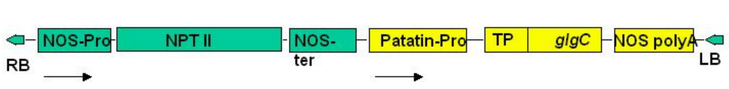
Bacterial Strain and Constructs: Cassava was transformed via *Agrobacterium*-mediated transformation using strain LBA4404 with the plasmid construct (3D: patatin/TPglgC). The 3D plasmid contains DNA sequences encoding a chloroplast transit peptide (TP) sequence at the 5' end of the structural gene **(Fig. 2).**

Transformation: Transformation was carried out with cassava embryo cotyledons. The explants were grown on somatic embryo induction medium supplemented with 75 mg/l paromomycin and 500 mg/l carbenicillin after cocultivation with *Agrobacterium*. Embryos are transferred to embryo maturation medium supplemented with 75 mg/l paromomycin and on germination will be transferred to the growth medium supplemented with 75 mg/l paromomycin.

Reverse Transcriptase-PCR (RT-PCR) Analysis: Total RNA was extracted from roots of *in vitro* grown putative transformed cassava plants using Qiagen's Rneasy Plant Mini Kit. RT-PCR and PCR amplifications were then performed with primers corresponding to the *glgC* 3' end and nos terminator 5' end (PCR) . Control reactions were carried out with cassava wild type total RNA.

Southern Blot: Genomic DNA was isolated from putative transformed plants according to the methods of Dellaporta *et al.* (1988). The DNA was used in Southern blot analysis using the same probe amplified by RT-PCR.

Enzyme Assay: AGPase enzyme assay was carried out according to the methods of Sowokinos (1976).



•Figure 2: T-DNA Region of the binary vector 3D Containing the muatted *glgC* gene with chloroplast transit peptide (TP) driven by the patatin promoter .

RESULTS AND DISCUSSION

•We have regenerated putative transformed cassava plants carrying *glgC* gene plus chloroplastic transit peptide and plants carrying *glgC* gene. The plants were transplanted to the soil in the greenhouse for tuberization and enzyme assays.

•Transformation experiments carried out with somatic embryo cotyledon explants were successful with TMS 71173 and have a transformation efficiency of 0.5 – 1% .

•We have analyzed the plants by Southern blot and RT-PCR using primers to screen for the promoter/gene border fusions and confirmed that the plants are transformed (**Fig. 3-4**,). RT-PCR analysis of the leaves of the transformed cassava plants was negative for expression of the *glgC* gene (**Figure 5**). Southern blot analysis confirmed the plants were transformed**.** The results of the AGPase assays show that some transformed plants have more than 85% increase in enzyme activity (**Fig. 6**)**.**

• Transformed plants had a better yield overall as indicated by increased number of leaves, increased stem and root/stem junction weight and increased root number and weight (**Table 1**). The fresh weight yield of the tuberous roots of the transformed cassava plants was 50% to 160% more than the wild type (**Table 1**). The average number of tuberous roots per transformed plant was also higher than that of the wild type, ranging from 8 – 12 compared to 7 of the wild type. The statistical analysis of the yield of transformed cassava and wild type show statistical differences in fresh weight of most of the categories analyzed. There was statistical difference between the fresh weight of leaves of the transformed plants 3D-1 and 3D-3 and the wild type but not between the wild type and 3D-2 which did not overexpress AGPase activity (**Table 1**). The dry weight measurements had the same trend as fresh weight (**Table 2**).

•We speculate that expression of the modified *glgC* gene in cassava roots has enhanced starch biosynthesis in cassava roots. An active AGPase by continuously utilizing hexoses (products of invertase hydrolysis of sucrose) for starch synthesis,presumably increase sink strength in roots. This in turn leads to more sucrose downloading and hydrolysis by invertases, leading to increased starch biosynthesis (Sonnewald *et al.*, 1997, Sturm, 1999).

	WT	3D-1	3D-2	3D-3
Leaf (g)	**221** +/-46^{c} (100)	**335** +/-27^{a} (156)	**284** +/-5^{ab} (127)	**299** +/-38^{a} (135)
Number of Leaves	**92**+/-9.^{b} (100)	**123** +/-10^{a} (134)	**109** +/-18^{ab} (116)	**114** +/-13^{ab} (124)
Stem (g)	**374**+/-75^{c} (100)	**584**+/-47^{a} (156)	**432**+/-85^{b} (116)	**529**+/-60^{ab} (144)
Stem-Root Junction (g)	**59**+/-7^{a} (100)	**86**+/-5^{a} (146)	**81**+/-13^{a} (137)	**64**+/-15^{a} (106)
Root (g)	**74**+/-19^{b} (100)	**199**+/-29^{a} (266)	**113**+/-31^{ab} (152)	**123**+/-23^{ab} (166)
Number of Roots/Plant	7+/-1^{b} (100)	**12**+/-3^{a} (174)	**8** +/-2^{b} (114)	**11**+/-1^{a} (157)

Table 1: Fresh weight comparison of transformed cassava and wild type plants.

	WT	3D-1	3D-2	3D-3
Leaf (g)	29+/-15 (100)	49.6+/-15 (166)	47.5+/-13 (162)	49+/-14.6 (166)
Stem (g)	50+/-12.9 (100)	98.2+/-55 (196)	99.4+/-46 (198)	86.8+/-36 (174)
Junction (g)	15.7+/-5.4 (100)	20.3+/-5.7 (133)	26+/-4.7 (173)	13.3+/-4.6 (86)
Root (g)	12.72+/-5 (100)	24.9+/-11 (200)	14.42+/-7 (116)	21.6+/-11 (175)

Table 2: Dry weight comparison of transformed and wild-type cassava

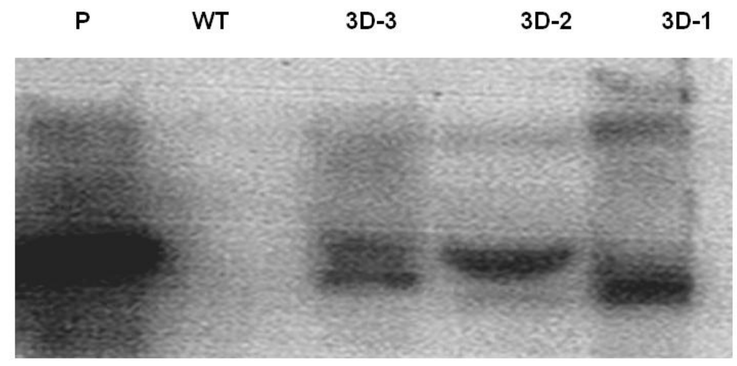


Fig 3. Southern blotting of TP-*glgC* transgenic cassava lines, the untransformed wild type and the plasmid control.

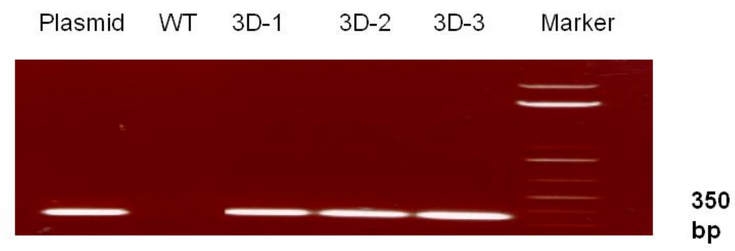


Fig. 4: RT-PCR Analysis of roots of TP-*glgC* Cassava Plants

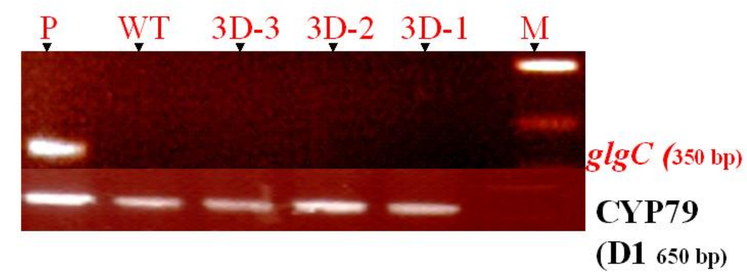
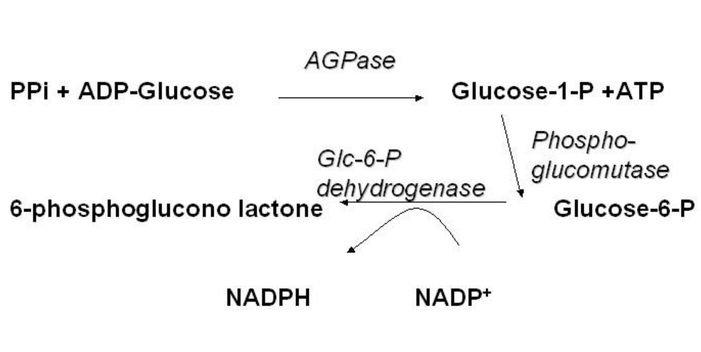


Fig. 5. Cassava leaf RT-PCR for *glgC* and CYP79 (D1).



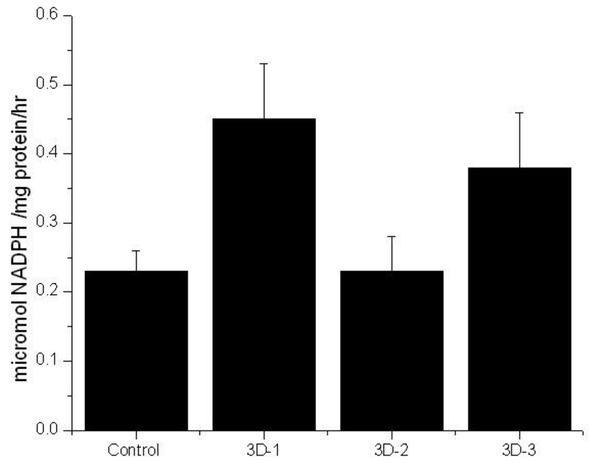


Figure 6. Root AGPase activity of cassava wild-type (71173) and transformed plants

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