SCREENING OF SOUTH AFRICAN CASSAVA (*MANIHOT ESCULENTA* CRANTZ) CULTIVARS FOR THE PRODUCTION OF EMBRYOGENIC TISSUES

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INTRODUCTION

Cassava ranks second to sugarcane and is better than both maize and sorghum as an efficient producer of carbohydrate under optimal growing conditions (Fregene and Puonti-Kaerlas, 2002). The main attention in Africa has been on the viruses causing cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Until recently, mainly due to selection problems, cassava was considered recalcitrant to genetic engineering. Optimized conditions for generating embryogenic structures in different cassava cultivars are required if effective transformation systems are to be established for the crop. The transferability of both the organogenesis-based regeneration and transformation capabilities to African cassava cultivars was already initiated at International Laboratory for Tropical Agricultural and Biotechnology (ILTAB), Missouri, USA by Taylor et al. (1996). These technologies must be expanded to regionally most important varieties where cassava has an important socioeconomic niche like in Southern Africa. This poster outlines the collaborative work undertaken between University of the Witwatersrand and ILTAB, which is aimed at transferring a genetic transformation capability for resistance to CMD caused by *South African cassava mosaic virus* (SACMV) to South Africa (SA).

MATERIALS AND METHODS

Plant material

Cassava cultivars T200, T400, P4/4 and P4/10 were brought to ILTAB from SA, whereas TMS60444 and a Zimbabwean cv. TMS303337 were obtained from ILTAB. All cassava cvs. were maintained as shoot cultures in Murashige and Skoog (1962) basal medium supplemented with 2% sucrose (MS2). Mother plants were prepared by transferring nodal cuttings from the stock plants to MS2 at ten per Petri dish and subcultured at 4 week intervals.

Induction of embryogenic tissues

The induction of primary embryogenic tissue took place on MS2 supplemented with 50 µM picloram. *In vitro* immature leaf lobes were excised from mother plants and placed on MS2 supplemented with either 50 µM picloram alone or NAA (1,10 and 25 µM). Further experiments with two SA-grown elite cvs. T200 and T400 were carried out on MS2 supplemented with 50 µM picloram supplemented with NAA (1, 10 and 25 µM) under three light regimes [dark; low light (0.44 µmol.m^{-2}.s^{-1}) and high light (7.1 µmol.m^{-2}.s^{-1})]. Ten Petri dishes per treatment were used with each Petri dish containing ten leaf explants. After 28 days in culture, production of embryogenic tissues from leaf lobe explants was scored. Embryos were regenerated into plantlets by sequential subcultures on medium consisting of MS basal salts supplemented with 5 µM NAA and 2 µM benzyl aminopurine (BAP). Regenerated plantlets were grown and rooted on MS medium devoid of growth regulators prior to transfer to the greenhouse.

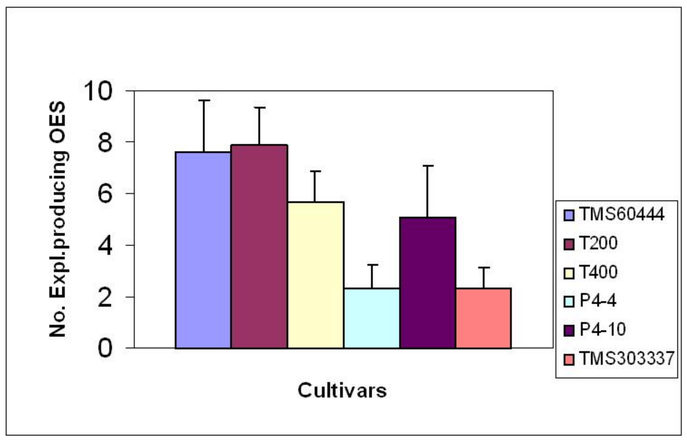

Figure 1. The effect of induction medium on the production of organised embryogenic structures from Southern African cassava cultivars. *Error bars represent the standard error of the mean*

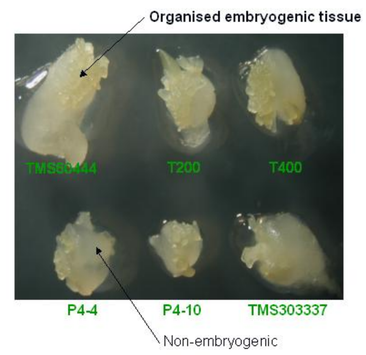

Figure 2. Organised embryogenic structures produced by immature leaf lobes after 28 days in MS2 medium supplemented with 50 µM picloram.

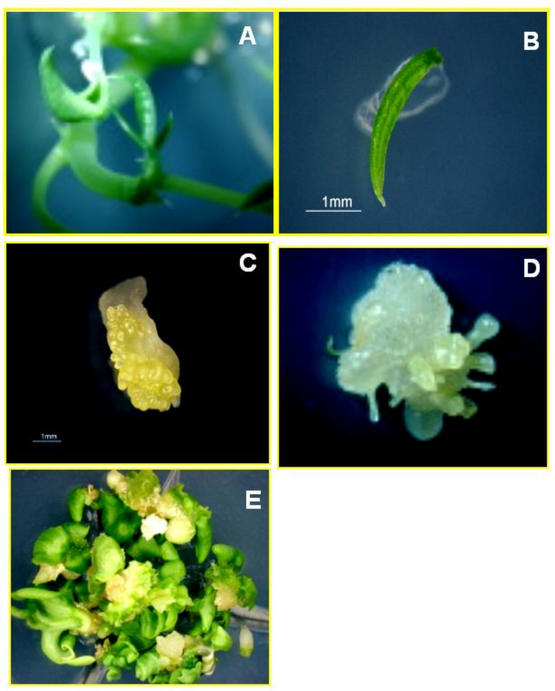

Table 1: Amount of organised embryogenic structures produced by *in vitro* leaf lobe explants on induction medium.

Cultivar	Score
TMS60444	++++
T200	++++
T400	+++
P4/4	+
P4/10	++
TMS303337	+

Figure 3 (A-E). Stages involved in OES production. (A) *In vitro* leaf lobes on the mother plant before excision, (B) a newly excised leaf lobe, (C) emergence of OES after two weeks surrounded by non-embryogenic callus (i), (D) mature embryos after four weeks of culture initiation, (E) multiple shoots four weeks on shooting medium

RESULTS AND DISCUSSION

A reproducible transformation procedure in cassava in developing countries necessitates the development of culture procedures that facilitate that production of embryogenic tissues across a significant proportion of regionally important cultivars. The step considered to be the most important during the production of target tissues in cassava gene insertion is the induction of primary embryogenic tissues from the explant. Failure to establish efficient procedures for this step in order to eventually produce transgenics, makes cassava genetic engineering impossible.

The frequency of somatic embryo production is summarized in Figure 1. South African cultivars T200 and T400 performed well, producing OES at frequencies (79 and 59%) approaching that of the model cv. TMS60444 (76%). Both cvs. T200 and T400 were shown to be significantly superior to other two SA cvs. P4/4 and P4/10 (23 and 51% respectively) and a Zimbabwean cv. TMS30333 (23%). Although cv. P4/10 scored a higher frequency of 51%, its embryo proliferation score was recorded to be 2, half to that of good cvs. TMS60444,T200 and T400 (Table 1) (Figure 2).

Further investigations on the combination of a weak and a strong auxin indicated that addition of 10 µM NAA increased OES production in all cvs. However, the addition of 25 µM NAA decreased OES production in cvs. TMS60444 and T200 but showed a slight increase in cv. T400 (Data not presented).

High light significantly improved OES production in all the three cvs. TMS60444, T200 and T400 ($p<0.05$). Further trials did not change the observed result (Data not presented).

Findings of fec tissue induction study conducted by Kokora et al. (2001) were extended to both S.African cvs. T200 and T400, which were found to be excellent for the generation of OES. Preliminary results indicated that both SA cvs. appeared to be responsive to this step with addition of 125 µM proving to be beneficial. In order to regenerate plantlets *in vitro*, embryos of cvs. TMS60444, T200 and T400 were regenerated by sequential subcultures on medium consisting of MS basal salts supplemented with 5 µM NAA and 2 µM BAP (Figure 3A-E). Regenerated plantlets were grown and rooted on MS medium devoid of growth regulators prior to transfer to the greenhouse.

CONCLUSIONS

- SA cv. T200 has proven to be the most efficient with T400 the least, in OES production.
- This study acknowledges the critical importance of all the above tissue culture stages and further studies are currently underway in our laboratory in order to eventually optimize each one of them.
- Further screening of more SA cvs. for their potential to generate OES remains a top priority.

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