Influence of Carbohydrate Concentration on Cassava Growth In Vitro

Thomas W. Zimmerman, University of the Virgin Islands
Agricultural Experiment Station, RR2 Box 10,000, Kingshill, VI 00850 E-mail tzimmer@uvi.edu

ABSTRACT

Cassava line TMS 60444, which has been used in cassava transformation, was grown under varying sucrose concentrations in vitro. Nodal segments from the cassava were grown on agar gelled Murashige and Skoog salts and vitamins and supplemented with 0, 1, 2, 4 or 6% sucrose. Shoot growth and development was monitored over a five week period. Sucrose levels of 2% and 4% produced the most active growth and tallest plants. Plant inter-nodal length and leaf size was reduced at the 6% level. This was attributed to the increased osmotic potential of the medium. The elevated sucrose concentration should allow more carbohydrates to be stored in the plant tissue for it to draw on as it becomes photoautotropic. Though the growth rate was reduced at the 6% sucrose level, the plants may be more adapted to acclimatization ex vitro.

INTRODUCTION

Cassava or manioc (*Manihot esculentum*) is a shrubby, tropical plant that has a starchy storage root used for food. Evidence of cassava use in the Virgin Islands goes back to the time of the Carib Indians. They cultivated the bitter manioc and used it as a source of starch in their diet. Cassava is third, after rice and corn, as a food source for people living throughout the tropical zone. Research is being conducted to improve the disease resistance, nutritional value and the starch quality of the fleshy storage roots. Cassava line TMS 60444 has been one of the first successfully used for genetic transformation. Though this line has been very successful in vitro, establishing it ex vitro has posed challenges.

Figure 1. Cassava growth after five weeks in vitro with varying sucrose concentrations.

MATERIALS & METHODS

Cassava plants from line TMS 60444 were grown and maintained in vitro through propagation of nodal segments. Nodal segments, from five week old cultures were cut and transferred to fresh Murashige and Skoog medium with vitamins, agar and varying levels of sucrose. The sucrose concentration range evaluated was 0, 10, 20, 40 or 60 g/L sucrose. The media were mixed and the pH adjusted to 5.8 prior to autoclaving for 25 minutes at 121°C and 1.2 kg·m^2. The medium was dispensed, 10 ml, into sterile 25 mm shell vials in a laminar flow clean bench. Nodal segments were placed in the center of the vials, capped and sealed with parafilm. Each treatment contained 36 replications. The cultures were grown in at 25°C in a 16 hour photoperiod. Observations were made daily and data collected twice a week. After five weeks, the developing plants were transferred to 7.5 cm square pots containing ProMix potting medium.

Figure 2. Cassava during acclimatization after one week ex vitro

RESULTS & DISCUSSION

Growth was evident from the cassava nodal segments by the third day in culture on the sucrose containing media. Root growth and development was initiated within one week. However, very little or no grown was observed in the 0 g/L sucrose treatment after five weeks. The 10 g/L sucrose treatment produces stunted and spindlier plants (Figure 1). Internodal length increased with increased sucrose concentrations up to 40 g/L (Figure 1). The 60 g/L sucrose level produced shorter plants with thicker stems than the 20 and 40 g/L sucrose levels. This may be due to the increased osmotic potential of the medium imposing a slight stress on growth.

Rooted plantlets were transferred to pots of potting mix and covered with a plastic dome (Figure 2). The clear plastic domes were used one week to provide a high humidity environment for the plants to become established. All plants survived for the first week ex vitro under domed protection (Table 1). However, after the dome was removed, the mortality rate increased. The plants originating from 10 g/L sucrose had a 90% mortality rate by week three (Table 1). The low level of sucrose in the culture medium may not have allowed the developing plants to store carbohydrates to survive on as it converts to becoming dependent on photosynthesis. The cassava plants from the higher sucrose levels acclimatized and survived better than those grown on lower sucrose media. Using a sucrose concentration of 40 or 60 g/L in vitro can have a beneficial impact the on survival rate ex vitro.

Table 1. Cassava survival during acclimatization following five weeks on MS medium with varying levels of sucrose

		% Survival		
		Under Lights		Greenhouse
Sucrose g/L	# of Reps	Covered Week 1	Uncovered Week 2	Week 3
10	30	100	20.0	10.0
20	31	100	61.9	59.1
40	32	100	94.7	80.9
60	29	100	95.5	87.9