



## Indigenous cassava clones as a new source of lycopene

Nagib Nassar<sup>1</sup>, Carla Simone Vizzotto<sup>2</sup>, Carlos Alberto Schwartz<sup>2</sup> and Osmindo Rodrigues Pires Júnior<sup>2</sup>

*1 –Departamento de Genética, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, 70910-900, Brazil*

*2 –Departamento de Ciências Fisiológicas, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, 70910-900, Brazil*

### ABSTRACT

Indigenous cassava clones acquired through their domestication a large diversity in relation to many economic traits such as high content of carotenoids and excellent palatability among other characters. One of these clones, which has been grown by indigenous Brazilian farmers and now being maintained in the Univ. of Brasilia gene bank, showed a high level of lycopene content (5 mgm per kilogram viz. a viz. zero in common cultivars , and 12-20 mgm kg<sup>-1</sup> in tomato, a lycopene- rich ). This is the first report of a cassava clone rich in lycopene.

Key words: domestication; indigenous cassava, lycopene, ritual, selection

### INTRODUCTION

Cassava is the most important crop in the tropics and a staple food for more than 800 poor people. Cassava is also the principal food for about 60 million people living in northeast Brazil. The majority of cassava clones grown and consumed in this region are known to be free of carotenoids, which leads to many health problems to inhabitants of this region. One of the approaches to develop cassava clones rich in carotenoids is to screen indigenous clones for this substance. This concept is based on the fact that indigenous clones of this crop accumulated desirable mutations that were further selected by indigenous farmers in their long history of cultivation.

The nutritive importance of carotenoids is attributed to its conversion to vitamin A as it is the case of  $\beta$ -carotene, and to its antioxidant property and ability to quench singlet oxygen as in the case of lycopene. Lycopene interacts with free radicals eliminating their poisonous effect (Palozza and Kirinsky, 1992). Early screening for carotenoid contents in these indigenous clones revealed one of the most striking features of this crop domestication: the clone UnB 400 showed 4 mgm kg<sup>-1</sup> of b-carotene (Nassar et. al., 2005). The clone UnB 401 also attracted our attention due to its red root flesh which may be regarded as an indication of lycopene.

### MATERIALS AND METHODS

The clone UnB 401, an indigenous cassava clone grown by in the Amazon, and maintained at Univ. of Brasilia living *Manihot* species collection was analyzed for lycopene content. This clone has a stem gray, 1.5 height, scars largely raised, 2-3 branches. Its leaves are 7-lobed, and the leaf lobe is linear, with the margins slightly sinuous, whereas the medium lobe shows 10 to 12 cm length. The leaf has a green petiole, and the young foliage is reddish. The inflorescence is a 3 to 6 cm glabrous panicle. Bracts and bracteoles are inconspicuous and caduceus. Flowers are monoecious; showing the pistillate flowers a basal opening, whereas the staminate apical opening occurs 3 weeks later. Fruits are green and winged and the roots are conic, with a rough, pink-brownish surface. The root flesh is slightly red and turns to dark red after cooking (Photo1).

*Extraction for lycopene analysis* 10 grams of mature roots were extracted three times with acetone (5 ml per gram). The filtered acetone extract was added in separation funnel containing petroleum ether, and distilled water. The aqueous fraction was discarded, and the organic fraction was submitted to saponification. Saponification was preferred since it removes accompanying lipids. In our work, the optimal conditions for mild saponification were achieved with 10% methanolic potassium hydroxide solution (100 ml) overnight at room temperature. After saponification the aqueous fraction was discarded and the organic fraction was dried with anhydrous sodium sulphate. The organic fraction was evaporated to dryness at 30 °C, re-suspended in 1000 µl of ethyl acetate and methanol (v,v;50,50) and submitted to a HPLC system.

*Equipment* Carotenoid analyses were performed by Shimadzu LC-10A HPLC equipped with a photodiode array detector SPD MXA-10 and a Rheodyne injection valve with 20 µL loop. The separation was carried out on a C18 Vydac 218TP54 column 250 x 4.6 mm i.d. (5 µm particle size) with 100% MeOH as mobile phase at a flow rate of 1 mL/min at temperature of 15°C. The chromatograms were processed at wavelengths of maximum absorption (450 nm). The identification of carotenoids was achieved by retention time (TR) comparisons with those of the standard compounds and using the wavelength of maximal absorption ( $\lambda_{max}$ ) and the shape of the spectrum between 300 to 600 nm compared with data available in the literature (Davies, 1976).

*Quantification* The calibration curves for lycopene were purchased from Sigma Inc., and purified from tomato, while for trans- $\beta$ -carotene (purchased from Sigma Inc.), and for  $\alpha$ -carotene (purified from "alfafa") were constructed with a minimum of the concentration levels thrice. All curves showed a good relation of area and concentration achieving a coefficient of determination ( $R^2$ ) of 0.96, 1.00 and 0.98 , for lycopene, trans- $\beta$ -carotene and trans- $\alpha$ -carotene, respectively. The cis isomer of lycopene was quantified using the calibration curve of lycopene.

## RESULTS

The chromatogram file of lycopene isolated from tomato and the cassava clone extract are shown in Fig. 1A and Fig. 1B, respective. Lycopene showed to be the major catotenoid, although  $\alpha$ -carotene and cis-lycopenes were also found. The identification and characterization of the peaks are given in Table 1. Others carotenoids were not able to be identified in the cassava clone, which shows a concentration of 5 mg of lycopene per gram of wet root weight.

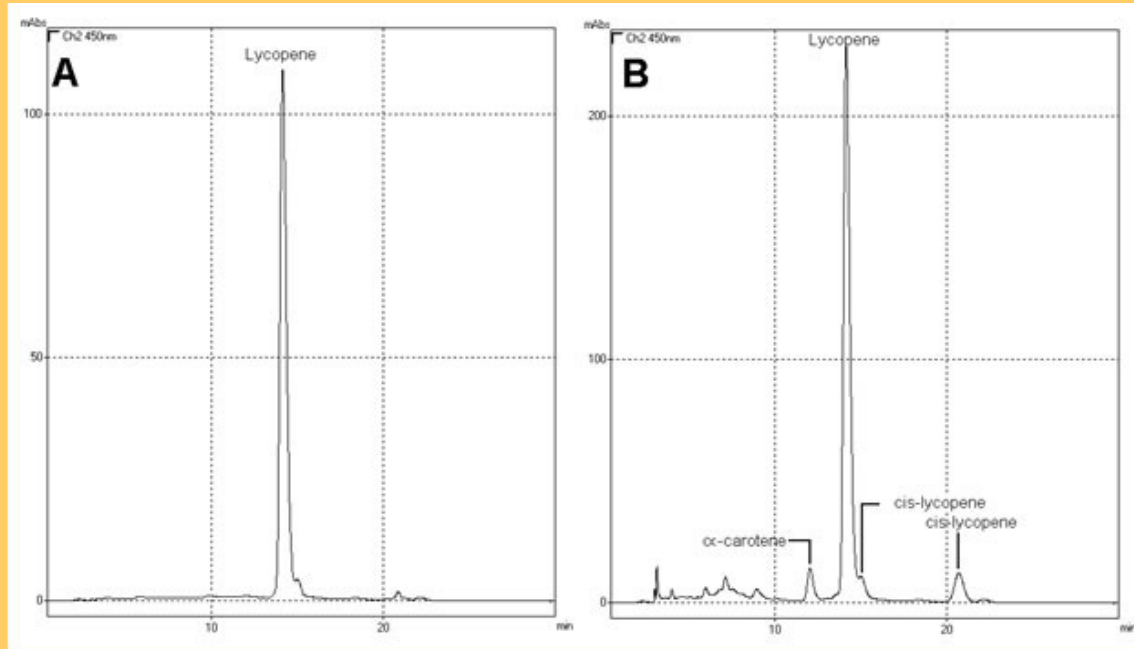


Figure 1. Chromatogram profile of (A) lycopene and (B) cassava clone, showing peaks of trans- $\alpha$ -carotene, lycopene and cis lycopene. HPLC analysis conditions: RP column C18 Vydac 218TP54 column 250x4.6 mm, mobile phase 100% MeOH, flow 1.0 ml/min.

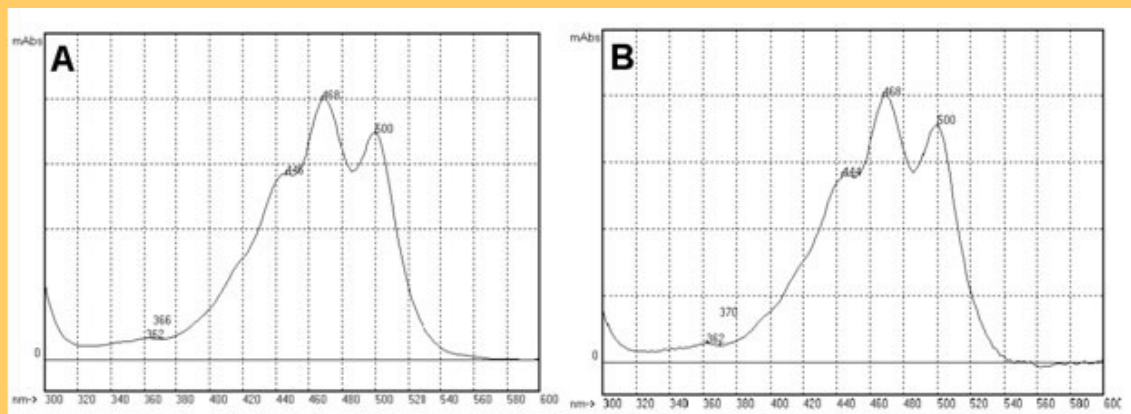


Fig. 2 . Spectrogram profile (range 300-600nm) of peak referent to lycopene (A) isolated from tomato and (B) of cassava clone roots, showing a similarity of 0.98. RP column C18 Vydac 218TP54 column 250 x 4.6 mm, mobile phase 100% MeOH, flow 1.0 ml/min.

The retention time in HPLC system, and the similarity of the spectrogram profile 300-600nm in photo-diode array of 0.98 (Fig. 2) confirms the presence of lycopene in this cassava clone.

## DISCUSSION

The most striking result of this research was the lycopene content in this cassava clone that was not previously reported in this crop species to the best of the authors' knowledge. This research provides means of better understanding cassava domestication and further breeding by indigenous Amazon farmers. The clone is grown in the Amazon, and from there it was brought to the State of Sao Paulo, where it was further grown by a few farmers. This clone could originate from a gene mutation that breaks the sequence of  $\beta$ -carotene formation, then adopted by Amazon's farmers, who used it probably for ritual or cultural ceremonies. This clone forms few roots compared to other improved cultivars. However increasing its root yield appears feasible by crossing with another clone possessing high combining ability for root yield.

Lycopene occur in tomato, guava, watermelon and pink grapefruit, and lycopene appears to be associated with reduced degenerative diseases. Other potential human health benefits include a possible role in the fight against digestive tract, breast and prostate cancer (Di Mascio et al., 1989; Handelman, 2001). Other researchers have also emphasized lycopene's protection against lung, stomach, and prostate cancer (Gerster, 1997; Sies and Stahl, 1998; Stahl and Sies, 1996). Epidemiological studies have shown that high intake of vegetables containing lycopene is inversely associated with the incidence of certain types of cancer. For example, habitual intake of tomato products has been inversely associated with the risk of cancer of the digestive tract. Lycopene is a precursor of  $\beta$ -carotene, whose synthesis includes an enzymatic cycle in the chain-end (Krinsky, 1994). The high lycopene level found in this cassava clone may indicate a dysfunction in the biosynthesis of  $\beta$ -carotene. The lycopene accumulation in this cultivar may therefore be the result of a deficiency in the  $\beta$ -carotene synthesis due to a mutation.

## ACKNOWLEDGEMENT

*We thank the Brazilian Conselho Nacional de Desenvolvimento Científico (CNPq) and the Fundação de Aperfeiçoamento de Pessoal Superior (CAPES) for their financial support. We are also grateful to Rodomiro Ortiz for his editing help.*

## REFERENCES

- Davies, B. H. 1976. Carotenoids. In: *Chemistry and Biochemistry of Plant Pigments* (T. W. Goodwin, Ed.), Vol. 2, pp. 38-165. Academic Press, London.
- Di Mascio, P., Kaiser, S., Sies, H. 1989, Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* 274:532-538.
- Gerster HY. 1997. The potential role of lycopene for human health. *J. Am. Coll. Nutr.* 16:109-126.
- Handelman, G. J. 2001. The evolving role of carotenoids in human biochemistry. *Nutrition* 17:818-822.
- Krinsky, N. I. 1994. The biological properties of carotenoids. *Pure Appl. Chem.* 66:1003-1010.
- Nassar, N.M.A, Vizzotto, C.S., Silva, H.L., Schwartz, C.A., Pires Junior, O.R. 2005. Potentiality of cassava cultivars as a source of carotenoids. *J. Food & Agric. Env.* 3:33-35.
- Palozza P, Krinsky, N.I. 1992. Antioxidant effects of carotenoids in vivo and in vitro: an overview. *Meth. Enzymol.* 213:403-420.
- Sies, H, Stahl, W. 1998. Lycopene: antioxidant and biological effects and its bioavailability in the human. *Proc. Soc. Exp. Biol. Med.* 218:121-124.
- Stahl, W., Sies, H. 1996. Lycopene: a biologically important carotenoid for humans? *Arch. Biochem. Biophys.* 336:1-9.



Photo 1. Cooked roots of clone UnB 400