AMADEPA

Association Martiniquaise pour le Développement des Plantes Alimentaires

29ème
CONGRES ANNUEL
ANNUAL MEETING
REUNION ANNUAL

Agriculture Intensive dans les Iles de la Caraibe : enjeux, contraintes et perspectives
Intensive Agriculture in the Caribbean Islands : stakes, constraints and prospects
Agricultura Intensiva en la Islas del Caribe : posturas, coacciones y perspectivas

INFLUENCE OF LIGHT EXPOSURE ON THE TUBER STORAGE PROTEIN PROFILES IN YAM BEAN (*PACHYRHIZUS EROSUS* (L) URBAN).

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ABSTRACT

Light-exposure of sprouting tubers of both sweet potato and yam bean caused "greening" in the periderm; however, the light-induced inhibition of tuber growth observed in sweet potato was not observed in yam bean tubers. Examination of the protein profiles and isoperoxidase banding patterns revealed no significant differences between light-exposed and dark-grown tuber tissue. While the basis of this light-mediated effect on starch accumulation may not be easily determined within the limits of biochemical assays, it has now become possible, with the emerging new molecular techniques, to determine whether this phenomenon is due to the
differential expression of the genes coding for the enzymes responsible for starch biosynthesis viz A(U)DP glucose pyrophosphorylase and starch synthase. The paper highlights the further advantages of using molecular approaches to the understanding of the tuberisation process.

INTRODUCTION

Tropical tuber crops comprise an important staple food for the Caribbean population, hence the Mexican yam bean (jicama) belonging to the genus, *Pachyrhizus*, family Fabaceae reported as having the highest tuber production capacity in the genus (SORENSEN, 1990) has the potential of being a leguminous root tuber crop of major economic importance in the region (SIRJU-CHARRAN *et al.* In Press). The plant grows vigorously, is tolerant to drought, requires little or no chemical

fertilisers or pesticides since it fixes nitrogen and is moderately resistant to a wide range of pests and diseases (SORENSEN, 1990). Marketable tubers weighing up to 1 kg. may be harvested 5-6 months after planting. The recent renewed interest in this crop has led to a spate of research being conducted, with the aim of realising its full potential. However, studies on the process of tuberisation have been limited to the effects of environmental factors (PAULL *et al* , 1988) and the anatomical changes which take place in tuberising roots (DABYDEEN and SIRJU-CHARRAN, 1990).

The light inhibition of tuber growth in sweet potato *(Ipomoea batatas (L) Lam)* (AKITA *et al*, 1966; SIRJU-CHARRAN, 1978, SIRJU-CHARRAN and Wickham, 1988) has been used as a model system in an attempt to explain the biochemical basis of tuberisation. Such studies were based on three parameters identified by WILSON 1970) as being important for tuberisation in sweet potato viz:

i) suppression of lignin biosynthesis
ii) formation of secondary cambia resulting in cell division and cell expansion
iii) enhanced storage of carbohydrates.Changes in activities of the housekeeping enzymes (e.g. IAA oxidase/peroxidase, invertase, phenylalanine) in accumulating and non-accumulating tissues were examined, however the possible significance of tuber storage proteins in the tuberisation process was not considered. This study attempts to address this deficiency by examining the effects of light exposure on the tuber protein profile in yam bean.

MATERIALS AND METHODS

Mature tuberous roots of yam bean were harvested from the field and planted with their proximal ends exposed to light in 4l. tins containing a mixture of sand, soil and clay as described for sweet potato by SIRJU-CHARRAN and WICKHAM,(1988). Tubers sprouted 7-10 days after planting and were harvested 15 weeks after planting.

PROTEIN EXTRACTION

Twenty grams (20g) of pith tissue was removed from both light-exposed and buried portions of the planted tuber and each sample was homogenized with 2 volumes of 50mM Tris-acetate buffer pH 7.5, containing 1.0mM EDTA, 1% w/v ascorbate and 0.5M sucrose. The supernatant obtained after centrifugation was fractionated with 35-50% solid ammonium sulphate and the proteins which precipitated was dissolved in 50mM Tris-acetate buffer, then dialaysed against 50mM Tris-acetate buffer pH 7.5 containing 1mM EDTA. The 45% dialysates were stored at -70 °C for use in further analyses. All extraction procedures were carried out at 4 °C.

ISOELECTRIC FOCUSSING

This was performed on native protein samples according to a modification of the method of ROBERTSON *et al*, 1987. A mini gel (0.75mm thick) of 5% polyacrylamide containing 4% carrier ampholyte (pH3 - 10) and 10% glycerol was pre-focussed for 10 minutes at 100V and 10 minutes at 200V.

Gels were fixed in 12.5% trichloroacetic acid containing 30% methanol for 15 minutes, rinsed with distilled water, stained with 1% solution of Coomasie Brilliant blue (R-250) for 20 minutes and destained in a solution of methanol/acetic acid/water (5:1:4).

POLYACRYLAMIDE GEL ELECTROPHORESIC (PAGE)

Electrophoresis under non-denaturing conditions was performed according to the method of DAVIS (1964) while PAGE in the presence of 0.1% sodium dodecyl sulphate (SDS) at pH 8.3 was performed according to the method of LAEMMLI (1970).

RESULTS AND DISCUSSION

Morphological examination of tubers harvested 15 weeks after planting revealed an inhibition of tuber expansion and greening of the periderm in light-exposed sections, whereas tuber portions kept in the dark continued to expand. A similar response has been reported for sweet potato (SIRJU-CHARRAN and WICKHAM, 1988).

The effects of light on gene expression are well-documented in the literature e.g. ALVAREZ and BOVERIS (1993) reported on the light induction of anti-oxidant enzymes and DT-Diaphorase in human blood nuclear cells. It was therefore envisaged that the light mediated effect on inhibition of tuber growth in yam bean might be reflected in changes in the tuber protein profile. Data (Fig. I) however revealed no significant differences in the protein profile of light-exposed and dark-grown tuber tissue.

GOMES *et al*, (1992) reported that the bulk of yam bean storage proteins was precipitated in the 45% ammonium sulphate fraction and that this protein fraction could be separated by SDS - PAGE under non-denaturing conditions, into two major bands (YBG1 and YBG2) and a minor band (YBG3). Further characterisation of these proteins by N-terminal sequencing revealed that YBG1 and YBG2 showed a high degree of sequence homology with cysteine proteases (e.g. papain) while YBG3 showed identity with soybean trypsin inhibitor.

Data (Fig. 2) indicated that separation of these proteins extracted from dark-grown (Lane 2) and light-exposed (Lane 3) tuber tissue, using SDS - PAGE and heated for 15 minutes at 70 °C showed no differences in protein profiles, however addition of DTT (Lanes 4 and 5) resulted in separation of a large number of low molecular weight polypeptides which were more abundant for the light-exposed sample (Lane 5). Further, the amount of YBG1 which separated was much less than that for dark sample indicating that YBG1 was being hydrolysed to a much greater extent under light conditions.

There seems to be no difference in the YBG2 fraction which separated suggesting that YBG2 was not being influenced by light. Like YBG1,

YBG3 also seemed to be in lower concentrations in light-exposed tissue. These results suggest that while light may not be responsible for regulating gene expression in yam bean tuber tissue, it could play an important role in either determining the conformation of the protein molecule or in the regulation of its hydrolysis. Light might therefore be exerting its regulatory effect at the post-translational level.

Tuber storage proteins may perform the dual role of acting as an inhibitor, thus serving as a deterrent to insect attack or as an important source of amino acids for developing sprouts. Based on the above observations, YBG1 seems to function in the latter role while YBG 3 in the former. The function and regulation of YBG2 is still to be determined.

REFERENCES

1 SIRJU-CHARRAN, G., N. LACKHAN, A. MAXWELL, A. EDWARDS HENRY and C.R. Mc DAVID (1992). Research Initiatives at the University of the West Indies in the identification of parameters for a *Pachyrhizus* Rich ex DC crop ideotype. In Proc. of the First International Symposium on tuber legumes. Guadeloupe F.W.I. 21-25 April (In Press).

2 SORENSEN, M. (1990) Observations on Distribution, Ecology and Cultivation of the tuber bearing legume genus *Pachyrhizus* rich ex DC Wageningen Agricultural University Papers 90:1-38.

3 PAULL, R.E., N.J. CHEN and S.K. FUKUDA (1988). Planting dates related to tuberous root yield, vine length and quality attributes of yam bean. Hor. Sci. 23:326-329.

4 DABYDEEN S. and G. SIRJU-CHARRAN (1990). The developmental anatomy of the root system in yam bean. (*Pachyrhizus erosus* (L) Urban) Ann. Bot. 66:313-320.

5 AKITA, S., M. YAMAMOTO, M. ONO, M. KUSUHARA, M. KOBAYASHI and S. IKEMOTO (1962). Studies on the small tuber-setting method in sweet potato cultivation. Bulletin Chugoku Agricultural Experimental Station 8:75-128.

6 ALVAREZ S and A. BOVERIS (1993) Induction of Anti-oxidant enzymes and DT-Diaphorase in human blood mononuclear cells by light stress. Arch. Biochem. Biophys. 305:247-251.

7. SIRJU-CHARRAN G. (1978) Enzyme Genes is in Sweet Potato tuber tissue. Ph.D. Thesis UWI Library, St. Augustine.

8. SIRJU-CHARRAN, G. and L. Wickham (1988). The development of alternative storage sink sites in sweet potato (*Ipomoea batatas Lam*). Ann Bot 61:99-102.

9. WILSON, L.A. (1970) The process of tuberisation in sweet potato (*Ipomoea batatas (L) Lam*). In Plucknett, D.L. (ed) Proceedings of the International Symposium in Tropical Root and Tuber Crops. University of Hawaii. I:24-26.

10. ROBERTSON, F.E., H.K. DANNELLY, P.J. MALLOY and H.C. REEVES (1987) ... Anal. Biochem 167, 290-294

11. DAVIS, B.J. (1984)... ANN N.Y. Acad. Sci. 121:404-427

12. LAEMMLI, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T. Nature 227:680-685.

13. MAESHIMA, M. T. SASAKI and T. ASAHI (1985) Characterization of major proteins in sweet potato tuberous roots. Phytochem. 22:1687-1693.