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Delimitation of Physiological Regions in Yam Tubers (*Dioscorea* sp.) and Distribution Pattern of Saccharide Degrading Enzymes, Cell Sap pH and Protein in These Regions

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Abstract. Physiological regions of yam tubers were morphologically defined in different specie into 'Head', 'Middle' and 'Tail', while the limits of these regions were studied using phosphorylase activity. Variation in enzyme activity, pH and protein concentration was found in different regions of the tubers. Old yam tubers had significantly higher activities of saccharide degrading enzymes, hexokinase, phosphorylase, glucose-6-phosphate dehydrogenase, phosphofructokinase and pyruvate kinase, than the new tubers. However, activity of phosphofructokinase in new *D. rotundata* was higher than that of old tuber. The high activity of phosphorylase in different regions of all the yam tubers examined indicates a very important role of this enzyme in starch degradation in *Dioscorea* species. The measured pH and protein concentration were also higher in old yam tubers. Except for phosphorylase, these enzymes had alkaline pH optima.

The yam tuber is a very important staple food in Nigeria but unfortunately it has a short storage life. In the absence of pathogens, the yam tuber tends to degrade itself by metabolic activity and loss of water by evaporation. There are three important species cultivated in Nigeria. These are *Dioscorea rotundata* (White yam), *Dioscorea cayenensis* (Yellow yam) and *Dioscorea alata* (Water yam). Morphologically yam tubers can be divided into three distinct regions, the 'head', 'middle' and the 'tail'. *D. rotundata* is generally cylindrical and often broader at the proximal end (head) and gradually tapers towards the distal end (tail). Sculpturally, the tuber consists of wavy slits vertically arranged and parallel to each other. At the head region the slits go deeper but are shorter ranging from 1 to 5 cm. The middle is marked by shallow slits ranging from 1 to 3 cm, while at the tail, where a certain amount of tapering is encountered, the slits are again shallow but short ranging from 1 to 2 cm. The head is also marked by numerous nodules which decrease towards the tail region. *D. alata* tuber is more or less oval in shape with large middle and narrow tail. The wavy slits are distributed as described for *D. rotundata*. *Dioscorea cayenensis* is generally irregular in shape with prominent lobes at the distal end (tail). The head is narrower than the tail while the middle is the largest in size. The wavy slits are both vertical

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and horizontal thus giving a scale-like appearance to the entire tuber. The scale-like appearance is more prominent at the tail region than the middle, while the lightest is the narrow head. The nodules prominent in *D. rotundata* are scanty at the head and decrease progressively towards the tail. In general, the heads of yam tubers are the most viable part used by farmers in yam propagation. The viability decreases towards the tail region.

BARKER and EL-SAIFI (1953) reported a fall in pH of potato tubers metabolizing under nitrogen as a result of lactic acid accumulation. In white yam tuber, changes in enzyme activity during prolonged storage have been reported (UGOCHUKWU *et al.* 1977), while the effect of anaerobiosis on metabolism of saccharides has been investigated (UGOCHUKWU and ANOSIKE 1979). It has been established that in mammals pH and enzyme compartmentation exist. It is probable that cell sap pH, enzyme activities and protein content may vary in different regions of long yam tubers. There has not been any report on delimitation of physiological regions in yam tubers nor any report on the distribution pattern of enzymes, cell sap pH and protein in these regions.

The aim of this work is to delimit the physiological regions in yam tubers using phosphorylase activity, study the variation of protein with age and to investigate the distribution pattern of saccharide degrading enzymes and pH in different regions of yam tubers.

MATERIALS AND METHODS

Materials

Yam tubers were obtained from the author's experimental farm in Benin City Nigeria, and were each about 30 cm long. They were planted in late March and harvested in October/November, 1984. 'New' yam tubers were those studied within two weeks after harvesting, 'old' yam tubers were those stored for about six months in a well-aerated place and at a temperature of between 28 and 29 °C before they were studied. All the reagents were of analytical grade and were obtained from Sigma Chemical Company.

Delimitation of Physiological Regions

The yam tubers were peeled and 10 g were removed at 2 cm intervals, washed in cold distilled water and homogenized in 40 cm^3 of cold extraction medium described by CRABTREE and NEWSHOLME (1972). The homogenate was squeezed through several layers of muslin and then filtered through cold No. 4 sintered glass filter. The filtrate was used as enzyme extract for determination of phosphorylase (EC 2.4.1.1)

pH Measurement

The head, middle and the tail regions of selected yam tubers were peeled and washed in distilled water. Samples (5,g) from each region were separately homogenized in 100 cm^3 deionized double distilled water and squeezed quickly through four layers of muslin and the filtrate centrifuged. The pH of the supernatant liquid was determined using a digital pH meter.

Protein Determination

The protein content of each region, prepared as detailed in pH measurement, was determined using the protein-dye binding method described by

BRADFORD (1976). Bovine serum albumin was used as protein standard. Protein concentration was expressed as mg per gramme of fresh mass of yam tuber.

Preparation of Crude Enzyme

A 25 g sample of yam tuber from each region was separately homogenized in 100 cm^3 of extraction medium (CRABTREE and NEWSHOLME 1972) and phosphorylase activity extracted as described under delimitation of physiological regions. Phosphofructokinase (EC 2.7.1.11) activity was determined in an extract made by homogenizing 25 g of yam tuber from each region in 100 cm^3 50 mM Tris/HCl buffer pH 8.2, containing 5 mM $MgSO_4$, 2 mM EDTA, and 10 mM β-mercaptoethanol (OPIE and NEWSHOLME 1967). The homogenate was filtered and extract centrifuged at 6000 *g* for 10 min at 4 °C. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and hexokinase (EC 2.7.1.1) activities were similarly extracted using extraction medium of UGOCHUKWU *et al.* (1977), and centrifuged at 25 000 *g* for 5 min. Pyruvate kinase (EC 2.7.1.40) was extracted in a similar manner using 0.1 M Tris/HCl buffer pH 7.5 containing 50 % glycerol and EDTA (1.0 mM).

Determination of Optimum pH

pH of maximum activity of phosphorylase was determined in 0.05 M collidine buffer in the pH range of 6 to 7.5, while pH optima of other enzymes were determined in pH range of 7 to 9.5 using Tris/HCl buffer and appropriate assay methods.

Enzyme Assay

Phosphorylase activity was assayed in the direction of starch synthesis (UGOCHUKWU *et al.* 1977) but using 0.5 cm^3 of extract and 1.0 cm^3 of trichloroacetic acid (10 % m/v). The inorganic phosphate liberated was determined in 0.5 cm^3 of the supernatant using molybdenum reduction method (WOOTTON 1974). Hexokinase activity was assayed by coupling ADP to pyruvate kinase/LDH system and measuring the rate of NADH oxidation at 340 nm. The assay mixture contained 60 mM Tris/HCl buffer pH 7.5, 0.5 mM ATP, 4 mM $MgSO_4$, 5.0 mM KCl, 0.4 mM PEP, 0.3 mM NADH, pyruvate kinase (100 units), 250 units LDH, 0.2 cm^3 enzyme extract and 0.4 mM glucose in a total assay volume of 3.0 cm^3. Glucose-6-phosphate dehydrogenase activity was determined by coupling the oxidation of glucose-6-phosphate to reduction of $NADP^+$ and measuring the rate of increase in NADPH at 340 nm (KACHMAR and DONALD 1976) except for the use of 3 mM $NADP^+$, 0.7 cm^3 distilled water and 0.3 cm^3 enzyme extract, in a total assay volume of 3.0 cm^3. Pyruvate kinase activity was assayed at 30 °C by measuring the rate of disappearance of phosphoenolpyruvate (PEP) at 230 nm (PON and BONDAR 1967). The standard assay mixture contained 40 mM Tris/HCl buffer pH 7.5, 80 mM KCl, 8.0 mM $MgSO_4$, 0.4 mM PEP, 0.2 cm^3 enzyme extract and 0.2 mM ADP in a total volume of 2.5 cm^3. The reaction was initiated by the addition of PEP. Phosphatase activity was similarly determined except the omission of ADP. Phosphofructokinase activity was assayed at 30 °C by coupling fructose-1,6-bisphosphate produced to aldolase/glyceraldehyde-3-phosphate dehydrogenase system and measuring the rate of oxidation of NADH at 340 nm (ASHIHARA *et al.* 1972). The method was slightly modified

by using 60 mM Tris/HCl buffer pH 8.3, and 0.2 cm^3 enzyme extract in a total assay volume of 3.0 cm^3. The temperature was stabilized by means of a constant temperature circulator.

RESULTS AND DISCUSSION

Fig. 1 shows the phosphorylase activity determined at 2 cm interval along the length of the tubers. In *D. rotundata* and *D. alata*, the activities of phosphorylase were constant from the vine end (head) up to 9 cm of the length of the tubers, rose gradually and remained virtually constant for a length of about 13 cm along the tubers. However, the activity dropped through the remaining length (6 cm) of the yam. Phosphorylase activity in *D. cayenensis* followed the same trend described for the other two species up to 24 cm but rose gradually towards the tail end of the tuber. These results show that yam tubers are clearly divided into three distinct physiological segments namely the head, middle and tail. The head region covered 30 % of the length of the tuber starting from the vine end, the next 50 % represented

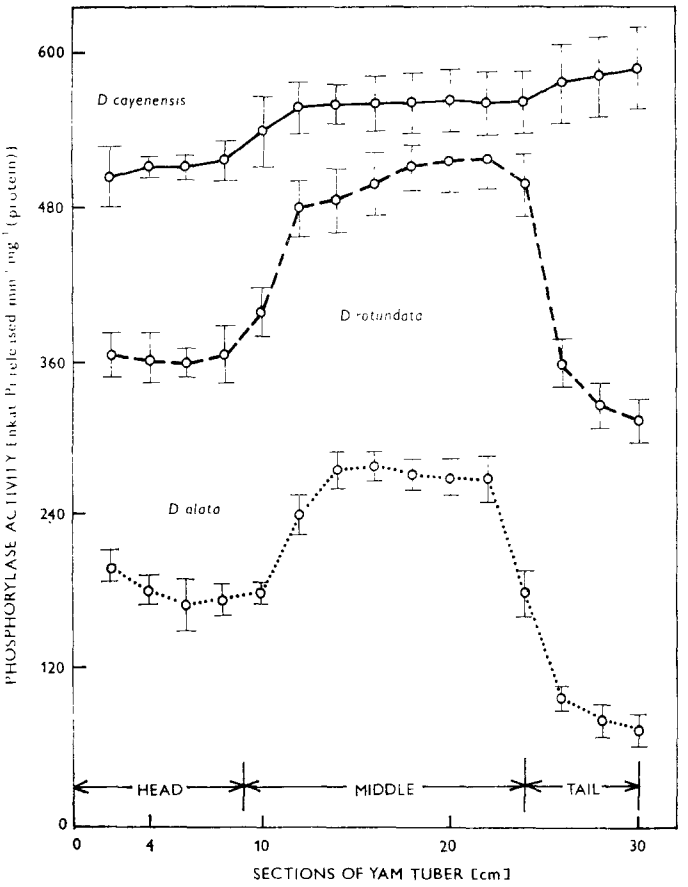

Fig. 1. Phosphorylase activity in head, middle and tail parts of yam tubers. Each point represents the mean of 8 samples. Standard error of mean is indicated by vertical bar.

TABLE 1

pH and protein variation and distribution of saccharides degrading enzymes in old yam tubers. Enzyme activity, protein content and pH were measured at three physiological regions of yam tuber as detailed under materials and methods. Each value represents the mean of 10 samples ± standard error of mean. Protein concentration and enzyme activity differences were computed ($P < 0.01$) while the significance of pH differences were evaluated ($P < 0.05$)

Enzymes	Enzyme activity (nkat g^{-1} of fresh mass of yam tuber)		
	Physiological Regions		
	Head	Middle	Tail
	White Yam (*Dioscorea rotundata*)		
Hexokinase	14.0 ± 0.90	20.30 ± 1.0	30.30 ± 1.50
Phosphorylase	386.7 ± 14.0	536.80 ± 20.60	353.40 ± 5.00
Phosphofructokinase	10.1 ± 0.20	15.0 ± 0.50	9.0 ± 0.20
Glucose-6-phosphate dehydrogenase	4.0 ± 0.13	5.70 ± 0.20	7.0 ± 0.33
Pyruvate kinase	11.0 ± 0.60	15.40 ± 0.30	14.0 ± 0.20
Protein [mg g^{-1} fr. m.]	40.0 ± 1.20	60.0 ± 3.0	31.20 ± 1.40
pH	7.84 ± 0.14	6.91 ± 0.21	6.20 ± 0.06
	Water Yam (*Dioscorea alata*)		
Hexokinase	8.50 ± 0.46	6.0 ± 0.21	4.30 ± 0.12
Phosphorylase	200.0 ± 10.0	260.0 ± 16.20	70.0 ± 2.50
Phosphofructokinase	7.0 ± 0.33	21.40 ± 0.50	18.90 ± 0.40
Glucose-6-phosphate dehydrogenase	0.60 ± 0.03	1.20 ± 0.07	4.20 ± 0.17
Pyruvate kinase	ND	ND	ND
Protein (mg g^{-1} fr. m.]	30.0 ± 1.40	42.0 ± 2.10	20.0 ± 0.12
pH	7.90 ± 0.21	6.90 ± 0.20	6.20 ± 0.11
	Yellow Yam (*Dioscorea cayenensis*)		
Protein [mg g^{-1} fr. m.]	31.0 ± 2.0	29.0 ± 1.0	34.0 ± 1.70

ND = not determined

the middle, while the tail region covered the remaining 20 %. This gives physiological credence to the distinction into head, middle, and tail regions very common in the cultural practice of yam growth.

Protein Determination

Table 1 shows that the protein content of different regions of old yam tubers is significantly different ($P < 0.01$). The highest protein concentration was recorded at the middle of both *D. alata* and *D. rotundata* tubers. The lowest value was found at the tail regions, while the value at the head was about the mean of the values found in the middle and the tail. In *D. cayenensis*, the protein content seemed to be evenly distributed. This even distribution in this species might have been influenced by injury, since part of the head region had been cut off during harvesting. This is likely to upset the actual distribution profile just as it affected enzymes in injured potato tubers (VERLEUR 1969, GERBRANDY and VERLEUR 1975). Preliminary work in this laboratory also showed no pH and enzyme activity differences along the length of this tuber. In new yam tubers (Table 2), the protein distribution

TABLE 2

Enzyme activity, protein concentration and pH distribution in new yam tubers. Enzyme activity, protein content and pH were measured at three physiological regions in yam tubers using the appropriate method as detailed under materials and methods section. Each value represents the mean of 10 samples ± standard error of mean. Protein was expressed in mg g^{-1} of fresh mass yam tuber. Protein concentration and enzyme activity differences were computed ($P < 0.01$), while the significance of pH differences were evaluated ($P < 0.05$)

Enzymes	Enzyme activity (nkat g^{-1} fresh mass of yam tuber)		
	Physiological Regions		
	Head	Middle	Tai
	White Yam (*Dioscorea rotundata*)		
Hexokinase	7.0 ± 0.40	5.50 ± 0.30	9.20 ± 0.65
Phosphorylase	90.0 ± 4.0	110.0 ± 6.20	76.0 ± 4.21
Phosphofructokinase	14.0 ± 0.70	18.0 ± 0.40	24.60 ± 1.40
Glucose-6-phosphate dehydrogenase	1.70 ± 0.03	1.90 ± 0.05	1.70 ± 0.03
Pyruvate kinase	8.0 ± 0.41	12.20 ± 0.46	11.80 ± 0.50
Protein [mg g^{-1}]	20.0 ± 1.0	25.0 ± 1.0	15.0 ± 0.11
pH	5.50 ± 0.16	5.30 ± 0.20	5.90 ± 0.06
	Water Yam (*Dioscorea alata*)		
Hexokinase	3.30 ± 0.19	3.0 ± 0.20	1.70 ± 0.10
Phosphorylase	45.30 ± 2.90	66.70 ± 3.30	13.30 ± 0.78
Phosphofructokinase	3.60 ± 0.16	9.90 ± 0.72	9.60 ± 0.80
Glucose-6-phosphate dehydrogenase	0.40 ± 0.02	0.80 ± 0.05	1.80 ± 0.13
Pyruvate kinase	ND	ND	ND
Protein [mg g^{-1}]	15.0 ± 0.08	20.0 ± 1.0	10.0 ± 0.49
pH	5.0 ± 0.10	5.90 ± 0.11	5.45 ± 0.15
	Yellow Yam (*Dioscorea cayenensis*)		
Protein [mg g^{-1}]	20.00 ± 2.0	19.50 ± 1.0	20.00 ± 1.50

ND = not determined

followed the same pattern as those found in old yams but the values were very significantly reduced ($P < 0.01$), being about two times lower than the values in the old tubers. Protein content appears to vary as the overall total enzyme activity. The region with the highest total enzyme activity had the highest protein concentration.

Table 1 also shows enzyme activity distribution in old yam tubers. Hexokinase in old *D. rotundata* increased from the head to the tail. In *D. alata*, the reverse is the case. The highest activity of phosphorylase was in the extract from the middle in both *D. rotundata* and *D. alata* tubers, the lowest was at the tail. The highest activity of phosphofructokinase was found in the middle of both white and water yam tubers. However, the lowest activity was measured in the extracts from the tail of *D. rotundata* and in the head of *D. alata*. Glucose-6-phosphate dehydrogenase activity in white yam and water yam tubers increased from the head to the tail. Pyruvate kinase activity differences in *D. rotundata* were similar to that of phosphorylase. With the exception of hexokinase and glucose-6-phosphate dehydrogenase, the

TABLE 3

pH of maximum activity of the enzymes examined in yam tubers. pH of maximum activity of enzymes was determined in the pH range of 6 to 9.5, using the appropriate buffers and assay methods, as described in the materials and methods section

Enzymes	pH Optima	
	Dioscorea rotundata	*Dioscorea alata*
Hexokinase	7.5	7.5
Phosphorylase	6.8	6.8
Phosphofructokinase	8.5	8.5
Glucose-6-phosphate dehydrogenase	8.3	8.3
Pyruvate kinase	7.5	7.5

highest activities of the saccharide degrading enzymes in old yam tubers were in the middle regions. There are statistically significant differences in the distribution of enzyme activity between various regions of the tubers ($P < 0.01$).

Table 2 also shows the enzyme distribution in new yam tubers. In white yam, all the enzymes exhibited their highest activities at the middle with the exception of hexokinase and phosphofructokinase whose highest activities were located at the tail. The activity of glucose-6-phosphate dehydrogenase at the tail and the head was the same. In water yam, the highest hexokinase activity was at the head while that of glucose-6-phosphate dehydrogenase was found at the tail of the tuber. The highest phosphorylase activity was found at the middle while the activity of phosphofructokinase at the tail and the middle was almost identical. There is also a significant difference in distribution of enzyme activity in various regions of new yam tubers ($P < 0.01$).

pH distributions in different regions of old and new yam tubers are shown in Tables 1 and 2, respectively. The pH of *D. rotundata* and *D. alata* increased from the tail to the head (Table 1). There is a significant difference in values obtained in various regions ($P < 0.05$). pH distribution in new yam tubers was very significantly different from that in the old tubers (Table 2). In new *D. rotundata*, the pH was highest at the tail, while the values at the head and the middle were identical. New water yam tubers had the highest pH at the middle and the lowest at the head. The value obtained at the tail was near the mean of the values at the head and the middle. The significance of the observed differences in pH in the new yam tubers when compared with the old yam tubers ($P < 0.01$) is not clear. In all the species examined, the pH of new yam tubers was less than 6.

There was less enzyme activity in new yam tubers than in the old ($P < 0.01$). This confirms the result of UGOCHUKWU *et al.* (1977). Similar increases in enzyme activity have been reported in aged potato tubers (SACHER *et al.* 1972) and in injured or infected potato tubers (VERLEUR 1969, GERBRANDY and VERLEUR 1975). However, phosphofructokinase activity in new *D. rotundata* tuber was higher than in the old tuber (Tables 1 and 2). This contrasts with the values obtained for other enzymes in all the species examined.

This high activity of the enzyme suggests increased rate of glycolysis (LEHNINGER 1975) in new white yam tubers. The high activity exhibited by phosphorylase in different regions of all the yam tubers examined, irrespective of age and species, demonstrates the important role of this enzyme in starch metabolism in *Dioscorea* species. The very high phosphorylase activity recorded in *D. rotundata* also strongly suggests a reason for the very short storage life associated with the tubers of this species. pH was also lower in the new yam tubers (Table 2) compared to the old tubers (Table 1). The high water content (about 65 %) of new yam tubers and the resultant enzyme dilution may be a means of controlling enzyme activity and slowing down saccharide degradation. PAETKAU and LARDY (1967), FRIEDEN (1969), and IDE *et al.* (1971) showed that enzymes from different sources were inactivated by dilution in mildly acid medium because of dissociation into subunit polypeptides. However, this inactivation is reversible. The pH of yam tuber rose as the yam got older. This rise in pH may be attributed mainly to loss of water by evaporation (COURSEY 1967).

Table 3 shows that pH optima of four of the five enzymes examined were alkaline. Phosphorylase had an acid optimum. The pH optima were independent of region of location. The determined pH optima confirmed the view expressed by DAVIES (1973), that the pH of maximum activity of virtually all enzymes are alkaline. Glucose-6-phosphate dehydrogenase was assayed at pH 7.5 to create a condition less favourable to 6-phosphogluconate dehydrogenase activity. Interference by phosphatase in the determination of pyruvate kinase activity in plant tissues has been reported (DUGGLEBY and DENNIS 1973). However, this is not a problem as the optimum pH for the phosphoenolpyruvate phosphatase activity (pH 5.5) in yam tuber is far removed from the pH used in the assay. In phosphofructokinase assay, NADH oxidase was removed by ammonium sulphate precipitation and phosphofructokinase activity was corrected for ATPase activity.

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BOOK REVIEW

TOKÁR, F.: BIOMASA VYBRANÝCH CUDZOKRAJNÝCH DREVÍN U LESNÝCH POROSTOCH JUHOZÁPADNÉHO SLOVENSKA. [Biomass of Selected Introduced Woody Plants in Forest Communities of South-West Slovakia]. — VEDA, Bratislava 1986. Kčs 13.—.

The publication presents the results of biomass measurements carried out on selected tree species in eleven permanent research plots, in the forest districts Partizánske, Palárikovo and Levice. In pure stands of *Castanea sativa*, *Quercus rubra* and *Juglans nigra* as well as in mixed stands with *Quercus petraea* L., *Tilia cordata* M. and *Pinus silvestris* L., the following parameters were measured: age and number of trees, stand basal area at breast height, dimensions of a mean stem, mean annual volume increment, fresh and dry matter of above-ground biomass, and the leaf area index. Among the relevant results of comparative measurements in stands aged 18—19 years, the author emphasizes a high volume increase of above-ground biomass in a mixed stand of *Castanea sativa* M. with *Quercus petraea* L. As concerns the species *Quercus rubra* L. and *Juglans nigra* L., maximum production was established in pure *Quercus rubra* L. stand and in mixed stand of these two trees with a predominance of *Quercus rubra* L.

It is a pity that the principal results obtained on all 11 plots have not been shown in a Table form. It would show that the growths examined are not homogeneous enough to accept the presented results unequivocally. Considerable differences in the number of stems on individual plots point to differences in tending or in spacing right at the planting of trees. In addition, the differences in heights of trees of nearly the same age would indicate a different site class, *i.e.* sites of unequal productivity.

From the methodical viewpoint the paper brings a number of interesting data, *e.g.* on the assessment of biomass amount, leaf area index and the distribution of biomass between stem, branches and shoots.

For the application of the results in the forestry practice it would be necessary to extent the investigation to all age classes and compare the production in mixed stands of trees near to the planned rotation age.

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