CHEMICAL ASSAY OF THE ANTHOCYAN PIGMENTS IN SWEETPOTATO

— *by* —

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Only a very limited amount of information is available on the chemical composition of the sweetpotato, *Ipomoea batatas*(1). Its anthocyanin pigments have only recently been identified as acylated glycosides of cyanidin and peonidin, (2) and some correlation studies have been carried out in connection with the syntheses of carotenoids and of anthocyanins in the stems and tuberous roots of the sweetpotato (3).

The breeding of sweetpotatoes at the University in Trinidad takes into account the need to eliminate a trait which produces purple mottles in the flesh of the tuberous roots. The pigments concerned are the sap soluble anthocyanins which, in comparison with the orange coloured carotenoids, have been little studied quantitatively in the sweetpotato. In quantitative work, any visual assessment of colour intensity suffers from the defect known as the "human element." So only major differences in purple colour intensity are likely to be detected visually in the flesh of any group of freshly-cut tuberous roots, as in the sweetpotato, where the background may vary from cream to yellowish orange. Other significant errors are likely to arise in a method which replaces the human eye with a colorimeter, like the Hunter colorimeter(4), to measure directly the intensity of the purple flecks at a surface.

In this communication, a more objective method is described for routinely assessing the purple pigment concentration in the flesh of sweetpotato tuberous roots. The method proposed should help the geneticist to follow the occurrence of the previously mentioned undesirable trait in the sweetpotato. The method requires a photoelectric colorimeter to determine the intensity of purple colour in a standard solution extract of the plant tissue. A suitable colorimeter using an Ilford spectrum filter is the EEL Portable Colorimeter (manufactured to give an accuracy of about two percent by Evans Electroselenium Ltd., Essex, England).

A whole sweetpotato tuberous root typical of the cultivar under test was sliced in the direction of its growth so as to provide a central section one-eight inch in thickness. This section was considered to be a reasonably good sample of the root with respect to its anthocyanin content. The periderm areas were removed from this section, and then it was finely chopped. A ten gram subsample of flesh material was thus collected and left to stand in the dark overnight, stoppered in a jar containing 100 ml. one per cent aqueous hydrochloric acid solution. This extraction process was found to be efficient, and the absorbance of the filtrate derived was easily measured directly against a blank of one percent aqueous hydrochloric acid, using a suitable filter on the EEL colorimeter (See Table 1). The filtrate gave a maximum near 525 nm (See Fig. 1), so the green filter No. 624 was found the most appropriate for this colorimetric method.

Absorbance readings higher than 0.10 units were found to be associated only with solutions which were visibly pink inside the one-centimetre diameter glass cell used in the colorimeter. Cultivar C9 provided an anthocyan-free extract (See Table 1) and consequently a more desirable colorimeter blank.

The method was extended for preliminary study of the variation of anthocyanin pigment along the stems of a cultivar subjected to a range of growing conditions.

Table I

Roots of Cultivar	*'EEL Absorbance (Units) + 0.05*
C9	0.10
13/56/5	2.30
13/56/12	4.70
049	0.10
C26	0.10

The tuberous roots contained 70 – 80% moisture.

In this case, the freshly harvested stems (minus leaves) were chopped into two-inch sections at a number of distances measured from their growing points. The average cross-sectional diameter of each two-inch stem section was measured with a micrometer screw-guage, and the length of each stem section was quickly reduced to give each section the same total outer (curved) surface area. Each section was then de-pithed, and the anthocyanin-free pith was discarded. The outer residual strips from similarly located sections of two stems from each agronomic treatment were combined, and each combined lot was left to stand in the dark overnight, stoppered in a jar containing 25 ml. of one percent hydrochloric acid in ethyl alcohol. This extraction process was found to be efficient and provided a filtrate which gave a maximum near 525 nm. (See Fig. 1). So the green filter No. 624 was used, with the acidic alcoholic solution as the blank, in the EEL colorimetric estimation of the colour intensity of the filtrate.

Some results are shown in Table 2 (and Figure 2) for two cultivars 049 and A138. The stems of 049 show no visible sign of pink anthocyanin-type pigmentation, but the stems of A138 are mainly purple coloured to the eye. The method described indicated that the anthocyanin content per unit surface area of the stem of cultivar A138 (at any location up to about three feet away from its growing point) depended upon the conditions of its growth. When growth was encouraged, by the application of nitrogenous fertilisers to A138, the rate of increase of anthocyanin content was suppressed along its stem.

It is likely that anthocyanin synthesis is genetically controlled (3), even though the actual formation (6) of anthocyanin pigments may be dependent on environmental and cultural factors. The production of anthocyanins and other flavonoid compounds (6) seems to depend on the availability of cinnamic acids and other phenylpropane compounds. (See Fig. 3). Studies correlating anthocyanin content with that of certain biogenetically related compounds in the sweetpotato might throw some light on the nature of the intermediate substances produced by plants during the formation of anthocyanins from phenylpropane compounds.

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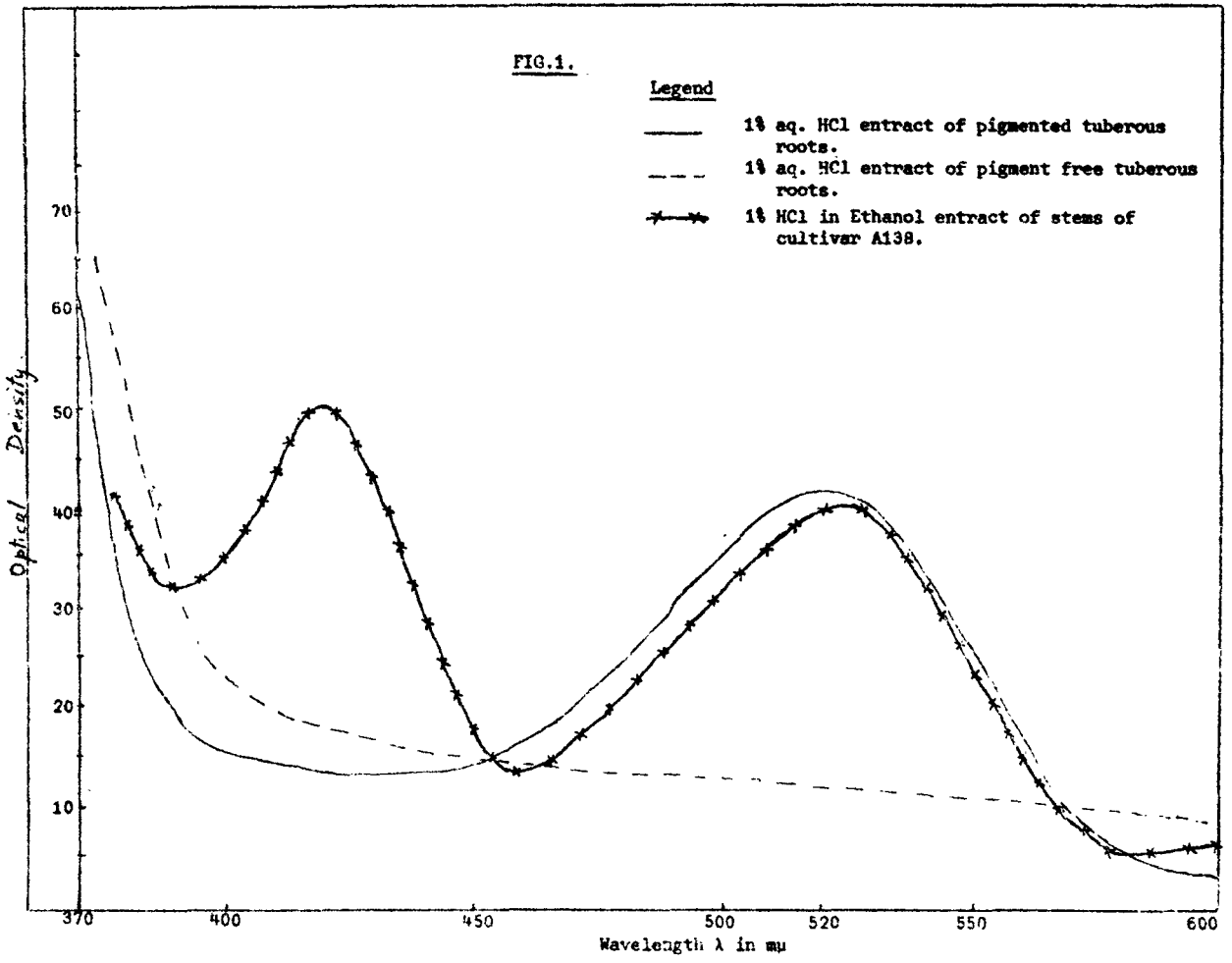


FIG.1.
Legend
1% aq. HCl entract of pigmented tuberous roots.
1% aq. HCl entract of pigment free tuberous roots.
1% HCl in Ethanol entract of stems of cultivar A138.
Optical Density
70
60
50
40
30
20
10
370
400
450
500
520
550
600
Wavelength λ in mμ

Table 2

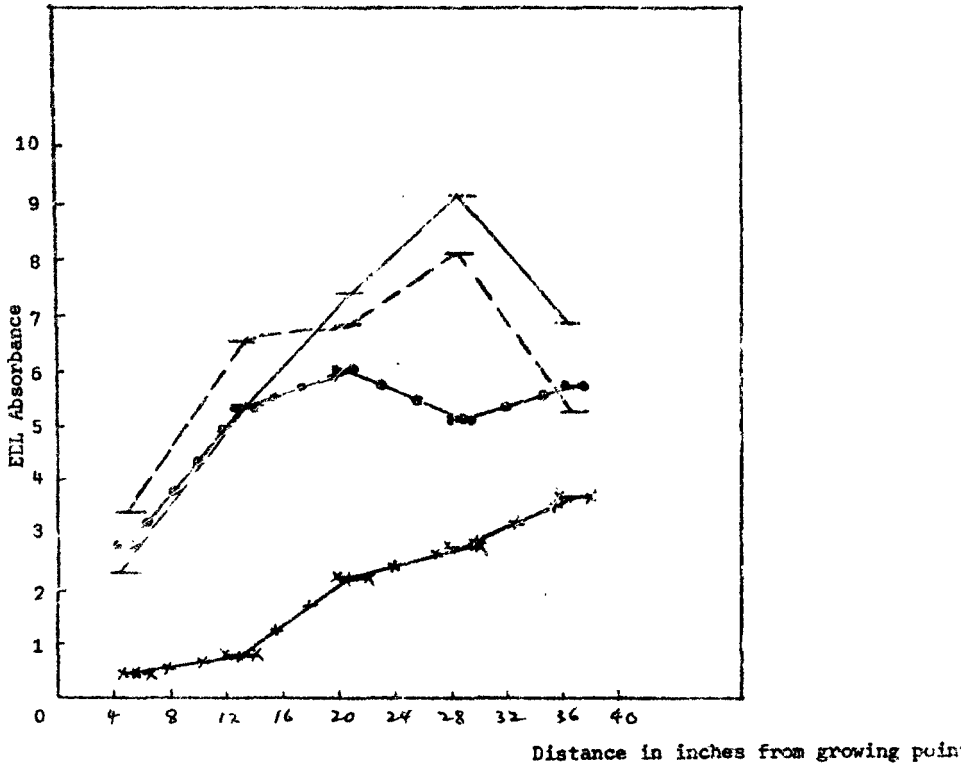
Cultivar	Treatment of Nitrogen and Staking	Diameter in mm. of stem sections		Absorbance units/unit area at locations shown measured from growing point in inches				
		Youngest	Oldest	4"-6"	12"-14"	20"-22"	28"-30"	36"-38"
A138	$N_0 S_0$	4.07	4.37	2.3	5.2	7.2	9.0	6.7
A138	$N_1 S_0$	3.37	4.01	3.5	6.4	6.8	8.0	5.0
A138	$N_0 S_1$	4.09	4.29	2.8	5.2	5.9	4.9	5.5
A138	$N_1 S_1$	3.15	3.32	0.4	0.7	2.0	2.6	3.4
049	$N_1 S_1$	4.35	5.50	1.3	1.0	1.1	1.1	—
049	$N_0 S_0$	4.35	4.20	0.9	1.0	1.1	1.1	—

N_0 – Control (No nitrogen applied)

S_0 – Control (No staking treatment)

N_1 – Nitrogen applied at fixed level.

S_1 – Staking treatment applied.



Legend: For Cultivar A138

N_0S_0

N_1S_0

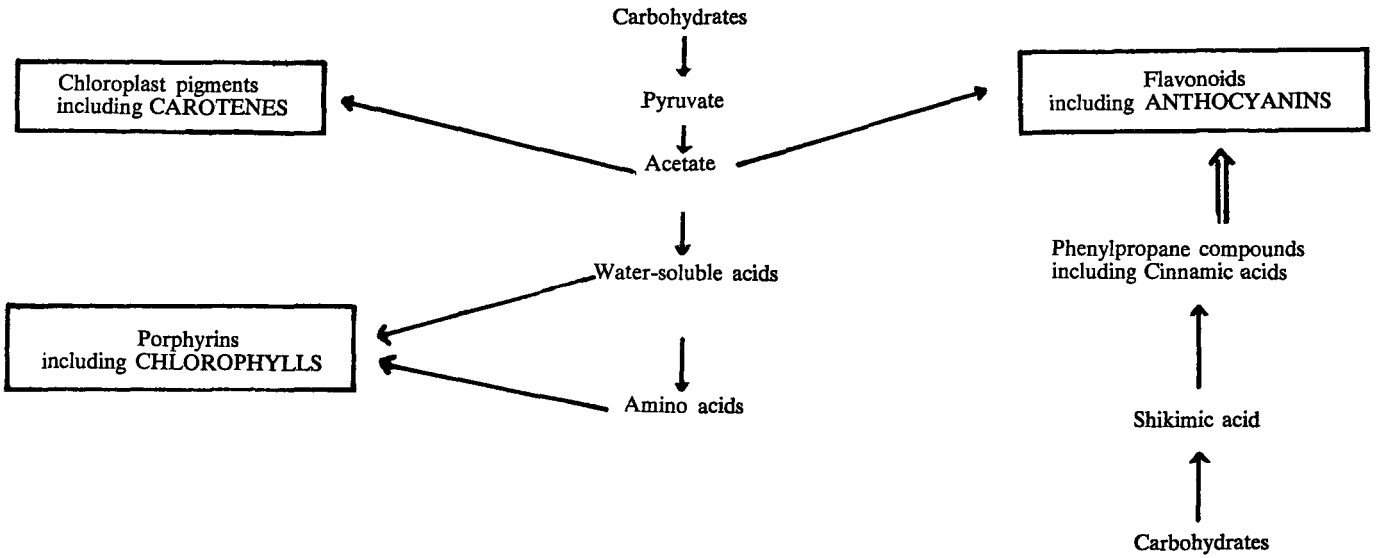
N_0S_1

N_1S_1

Fig. 3

Sugge

Suggested Biogenetic Schemes for Pigment formation in the Sweetpotato
Cf. Ref. 6. Goodwin (1965)



KEY:

Pathway controls biogenesis of flavonoid compounds

Pathway not specific to flavonoid biogenesis

Pathway may not be entirely specific for pigment synthesis.

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