Influence of Fertilizer Application on Browning and Polyphenol Oxidase Activity in Cut Tubers of Yam[1]

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ABSTRACT

The browning potential of surfaces of cut yam tubers has been observed to be significantly higher in fertilizer-grown yam tubers of *Dioscorea rotundata* when compared with non-fertilizer-grown yam tubers. Similarly polyphenol oxidase activity was significantly enhanced in fertilizer-grown yam tubers. No positive correlation was found between browning potential and polyphenol oxidase activity, indicating that the browning reactions in *D. rotundata* are likely to be non-enzymic. The enhancement effect of fertilizer on browning potential and polyphenol oxidase activity is discussed.

COMPENDIO

Se ha observado que el potencial obscurecimiento de la superficie de tubérculos cortados de ñame (*Dioscorea rotundata* Poir), es significativamente más alto en tubérculos crecidos con fertilizantes que en aquellos no fertilizados. De manera similar la actividad de la polifenol oxidasa fue significativamente aumentada en tubérculos fertilizados. No hubo una correlación positiva entre el obscurecimiento potencial de ñame y la actividad de la polifenol oxidasa, que indicara que estas reacciones podrían ser no enzimáticas. El efecto del fertilizante sobre el obscurecimiento y la actividad oxidante del polifenol, es discutido en este artículo.

INTRODUCTION

The white yam (*Dioscorea rotundata* Poir) is one of the most important *Dioscorea* species cultivated in the West African Yam Zone (4). With a record contribution of 78 per cent of the world production, Nigeria clearly exceeds all other countries in the cultivation of yams (14).

Following mechanical or physiological injury, the tuber is known to develop a brownish-black discoloration (13). The discoloration is known to vary in intensity among cultivars of a given yam species (15). These color changes, also referred to as browning reactions, have been attributed to two complicated reactions: an enzymic process (13), and a nonenzymic process (6, 8, 17). Discoloration by the former process involves the catalytic oxidation of phenols to quinones by the enzyme polyphenol oxidase (PPO) (EC 1.14.18.1). The quinone formed may polymerise or interact with other cell constitutents to form brown or dark-colored products. Whereas potato polyphenol oxidase has been characterised by Patil and Zucker (16), interest in the elucidation of yam PPO is recent (2, 3, 15). Mapson *et al.* (12) observed that the enzymic browning reaction could be affected by two sets of intrinsic factors, the concentration of phenolic substrates and concentration of PPO.

This particular investigation of the influence of nitrogen – phosphorus – potassium (NPK) fertilizer on the browning potential and PPO of *D. rotundata* was prompted by reports from Nigerian farmers and housewives that fertilizer-grown yam tended to show more browning on the cut furfaces than non-fertilizer grown yams.

In most cases browning is so intense that cut surfaces of tubers become totally blackened and are completely discarded for fear of poisoning. In Nigeria NPK mixtures of ratio 15:15:15 are clearly the most common inorganic fertilizers used to increase yam yield.

In the southern Guinea Savanna Zone of Nigeria, application of fertilizer at a rate of 30 kg/ha could yield 15-17 t/ha compared to 9-10 t/ha in unfertilized crop land (Ogunwale, personal communication). Thus increases of 5-7 t/ha could be obtained following the use of NPK fertilizer. The investigation has also attempted to find out if there is any relationship between PPO and browning reactions in *D. rotundata*. Such data may be useful in controlling browning in yams and yam products.

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MATERIALS AND METHODS

D. rotundata tubers harvested in the 1985 cropping season were used for the study. The tubers were separated into two lots of NPK fertilizer-grown and non-fertilizer-grown. The NPK fertilizer was applied at the rate of 30 kg/ha. The cultivars used in this study were Okun, Okunigba, Okunmodo and Borki, depending on the locality from where each was obtained. Of all these names, only Borki is registered by Lawton (11) as a cultivar of white yam.

Determination of browning potential

Browning potential (BP) was determined according to the method of Walter and Purcell (20). Thirty segments of tubers from both fertilizer and non-fertilizer-grown yams were exposed for 24 h at ambient temperature in a dark cupboard. Thereafter, a 1.5 cm diameter cork borer was used to obtain 3 g tissue randomly from each of five segments through the exposed surface. Five samples of 3 g tissue was then homogenized with 20 ml of distilled water in a Waring blendor. The homogenate was further diluted with 40 ml distilled water and centrifuged for 10 min at 2000 rev/min on a Gallenkamp cf-590 centrifuge. The absorbance of the filtrate was read in a Unicam Sp 1800 UV spectrophotometer. Control tissues were freshly cut and unexposed. BP was estimated as the ratio of absorbances at 450 nm, of homogenates of time exposed (B^t) and unexposed tissues (B^o) as shown below, since a linear relationship exists between color change and exposure time (7).

$$BP = \frac{B^t \text{ at } 450 \text{ nm}}{B^o \text{ at } 450 \text{ nm}}$$

Although values of BP have no units, the duration of exposure is usually stated. Thus values of BP serve as numerical estimates of the susceptibility of a particular yam tuber to the browning reaction. For example a yam tuber with a BP of three will show more browning than one with a BP of two at the same duration of exposure. The determination of BP for each yam cultivar was replicated five times and repeated two times thereafter. All data were subjected to significance tests at the 5 per cent probability level.

Extraction of polyphenol oxidase

Extraction of PPO was carried out using acetone powder of yam tissues as described by Ikediobi and Obasuyi (9). A suspension of 5 g of the acetone powder in 20 ml of 0.1 M potassium phosphate buffer 6.0 was constantly stirred for one hour while standing in an ice bath. The mixture was centrifuged at 2000 rev/min for ten min. The supernatant was stored in a refrigerator and used as the crude enzyme without further purification.

Assay of polyphenol oxidase activity

Polyphenol oxidase activity was assayed for the catecholase action by the spectrophotometric method of Adamson and Abigor (1). Enzyme activity was indicated by the absorption of the product formed in the third minute of reaction at 450 nm on a Secam SP 1800 UV spectrophotometer. Determination of PPO activity from each yam cultivar was replicated five times and repeated two times thereafter. An analysis of variance at the 5 per cent significance level was also carried out to determine significant differences between fertilizer-grown and fertilizer-free yams. Enzyme activity data were also correlated with BP data.

RESULTS AND DISCUSSION

In all the four cultivars of *D. rotundata* considered, BP of fertilizer-grown yams were significantly higher than those of the fertilizer-free yam (Fig. 1). Similarly PPO activity was significantly enhanced in fertilizer-grown yam tubers (Fig. 2). Linear correlation tests for BP and PPO activity yielded "r" values which had probability levels greater than 10 per cent in both fertilizer-grown and fertilizer-free yam. There is therefore no correlation between PPO activity and BP in the

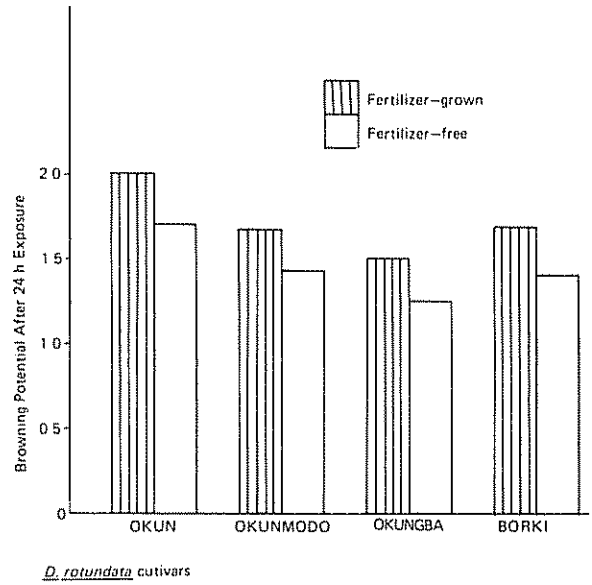

Fig 1. Browing potential in fertilizer-grown and fertilizer-free yam tuber of *D. rotundata* cultivars after 24 hours exposure time at ambient temperature.

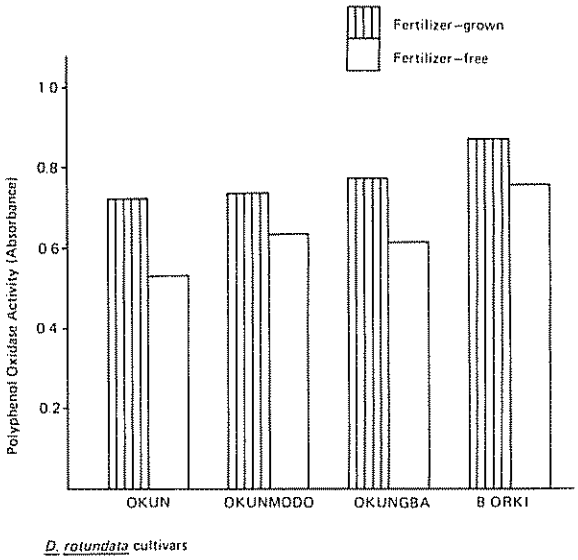

Fig. 2. Polyphenol oxidase activity in fertilizer-grown and fertilizer-free yam tubers of *D. rotundata* cultivars

four cultivars studied. The absence of correlation between enzyme activity and browning has been observed previously in potato tubers (12). This observation implies that the browning reaction in yam tubers when cut, is not due substantially to PPO activity. Presumably, the non-enzyme browning process may be the major contributor to the browning phenomenon in cut tubers of *D. rotundata*. The crucial reactions which lead to non-enzymic browning in food products have been shown by Reynolds (18) to be those between aldoses and free amino acids or free amino groups of proteins. Crude protein levels ranging from 6.3-13.4 per cent reported to be present in various *Dioscorea* species and cultivars (13) are believed to be high enough to promote a significant non-enzymic contribution to the browning phenonon.

This study has clearly established that NPK fertilizer-grown yam has a higher tendency to undergo the browning reaction. The enhancement effect of NPK fertilizer on BP could therefore be attributable to the increase in tuber concentration of protein amino acids and other chemicals such as phenolic compounds, that may serve as substrates for the non-enzymic browning reaction as well as enzyme proteins (5, 10).

Although poisoning resulting from browning reactions in cut tubers have not been reported, the enhancement of PPO activity by NPK fertilizer is significant since, according to Osagie and Opoku (16), its activity may lead to modification in the properties, palatability, resistance and usability of most products derived from yams.

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