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Changes in the Activity of Ascorbate Peroxidase under Anaerobiosis in Cocoyam (*Colocasia esculenta*)

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Abstract: This study was conducted to determine the activity of ascorbate peroxidase in the cormels of cocoyam (*Colocasia esculenta* var. antiquorum) immediately after harvest and in storage under anaerobiosis for one and three weeks, respectively. During stress condition in plants, hydrogen peroxide is released and mechanisms to detoxify it must be maintained. The cocoyam tubers that were neither damaged nor affected by disease were harvested from a local farm in Ugbogui, Ovia North Local Government Area in Edo State, Nigeria. The selected cocoyam tubers were peeled manually, washed with ice cold water and cut into pieces. The root tissues (50 g) were homogenised with 100 mL of ice cold 0.05 M phosphate buffer. The extract obtained was clarified by centrifugation for 15 min at 8000 g at 4°C. Ascorbate-peroxidising activity was assayed using the initial rate of decrease in ascorbate concentration as measured by its absorbance at 290 nm using Milton Roy Spectron 21D. Results showed the weight of the cormels decreased all through during storage. Immediately after harvest the activity of ascorbate peroxidase was 15.49 unit mL^{-1} with a significant increase ($p<0.05$) after one week to 73.05 U mL^{-1}. Thereafter there was a significant decrease in activity of the enzyme after three weeks of storage to 33.33 U mL^{-1}. This increase in activity of ascorbate peroxidase after three weeks of storage may be related to increase in response to various biotic stresses. Therefore, manipulation of the capacity of cocoyam to tolerate anaerobiosis is a function of its ability to modulate the antioxidant enzymes' armory in case of need.

Key words: Biodegradation, cormels, detoxifying enzyme, storage

INTRODUCTION

Cocoyam (*Colocasia esculenta*) belongs to the family Araceae and it is an important food crop in Nigeria and many other countries of the tropics and subtropics. This crop grows perennially and it is cultivated for its cormels and leaf which serve as vegetable. Cocoyam is not edible when raw. It contains calcium oxalate crystal. Calcium oxalate is highly insoluble and contributes to kidney stones.

Ascorbate peroxidase is an enzyme that acts on hydrogen peroxide using ascorbate as its substrate. It is required in higher plant metabolism as it detoxifies hydrogen peroxide produced during stress conditions by plants.

Postharvest losses on storage of tropical tuber crops are enormous because of their inherent storage qualities (Osagie, 1992). The traditional methods of preserving tubers in the ground, in trenches, barns or heaps have serious problems of autolytic deterioration and microbial attack. Also, attacks by insects, rodents and higher animals lead to irreparable losses in the available tubers during storage have been documented (Eka, 1998). However, Tindall (1983) reported that cocoyam can be stored effectively in pits.

The best way to improve storage life of crops is to store in conditions of lower temperature and high humidity. The length of storage is increase if the storage temperature is maintained at 11-13°C. Cocoyam can be stored conveniently for up to 6 weeks at low temperature of 15°C and high humidity of 85% (Agbor-Egbe and Rickard, 1991).

This research is aimed at determining the effect of postharvest storage on the activity of ascorbate peroxidase enzyme in the cormels of cocoyam.

MATERIALS AND METHODS

The cocoyam tubers were harvested from a local farm in Ugbogui, Ovia North Local Government Area in Edo State, Nigeria. The tubers for this study were neither damaged nor affected by disease.

Preparation of plant extract: The selected cocoyam tubers were peeled manually, washed with ice cold water and cut into pieces. The root tissues (50 g) were homogenised with 100 mL of ice cold 0.05 M phosphate buffer. The extract obtained was clarified by centrifugation for 15 min at 8000 g at 4°C. The supernatant obtained was used for the determination of the level of ascorbate peroxidase.

Assay procedure: A 2.5 mL of the 0.05 M phosphate buffer (pH 7.4) was added to 1.5 mL of ascorbic acid. To the mixture was added 1.5 mL 30 mM hydrogen peroxide. The loosely stoppered tubes were thoroughly mixed and an aliquot (0.5 mL) of the supernatant (cocoyam extract) was added. Absorbance of the turbid supernatant was determined at 290 nm for 1 min.

Determination of ascorbate peroxidase level: Ascorbate-peroxidising activity was assayed according to the method of Nakano and Asada (1981) using the initial rate of decrease in ascorbate concentration as measured by its absorbance at 290 nm. The reaction mixture contained 0.05 M sodium phosphate buffer (pH 7.0), 0.6 mM ascorbate, 30 mM hydrogen peroxide in a total volume of 6 mL. The reaction was started by adding hydrogen peroxide and the change in absorbance was measured using Milton Roy Spectron 21D. One unit of ascorbate-peroxidising activity was defined as the oxidation of 1 μmL of ascorbate per min at 25°C under the above condition.

Statistical analysis: The values are reported as Mean±SEM. Statistical differences for the biochemical values were determined using analysis of variance (ANOVA) and differences in the means were tested by Duncan's multiple range test (Sokal and Rohf, 1969).

RESULTS AND DISCUSSION

The results of the study are presented in the Table 1 and 2. There was significant increase in the activity of ascorbate peroxidase after one week of storage and thereafter, the activity decreased after three weeks of storage. There was a significant level of ascorbate peroxidase in the newly harvested cormels as against three weeks of storage under anaerobiosis.

Environmental stress such as pathogenic attack cause oxidative stress which modulate the activities of antioxidant enzymes (Yu and Rengel, 1999). H_2O_2 is generated during pathogenic attack which can be detoxified by the ascorbate-glutathione cycle, which includes ascorbate (Noctor and Foyer, 1998). During normal aerobic metabolism, the leakage of electrons from the mitochondria respiratory chain produces superoxide radicals (Frei, 1994). Under the physiological conditions of anaerobiosis, the production and destruction of reactive oxygen species are well regulated in the cell metabolism.

Table 1: Weight of cocoyam in gram before and after storage

Weight of cocoyam (g)	Test
Before storage	31.84±2.03^b
After 1 week of storage	26.40±0.07^a
After 3 week of storage	18.20±0.24^a

Results are expressed as Mean±SEM, Means of the same row with different letters as superscript are significantly different ($p<0.05$)

Table 2: Activity of ascorbate peroxidase in the cormel of cocoyam during storage under anaerobiosis

Duration of storage	Enzyme activity (units mL^{-1})
Immediately after harvest	15.49±0.32^b
1 week after harvest	73.05±0.03^a
3 weeks after harvest	33.33±5.45^b

Results are expressed as Mean±SEM, Means of the same row with different letters as superscript are significantly different ($p<0.05$)

Results showed that the weight of the cormels decreased during storage. Before storage, the weight was 31.84 g; after one week of storage the weight decreased to 26.40 g and further decreased to 18.20 g after three weeks of storage (Table 1). This decrease is associated to the degradative action of catalytic enzymes present in the cormels of cocoyam. The activity of ascorbate peroxidase before storage was 15.49 U mL^{-1} while at one week of storage, the activity was 73.05 U mL^{-1}. At three weeks of storage, the enzyme activity of ascorbate peroxidase decreased to 33.33 U mL^{-1} (Table 2). The decrease in the activity of ascorbate peroxidase after one week could be a general rearrangement of the H_2O_2 detoxification cycle due to decreased oxidative threat. The decrease in the activity after one week of storage may also arise from reduced synthesis, enhanced degradation or inactivation of ascorbate peroxidase or all these factors. The increase in activity of ascorbate peroxidase after three weeks of storage may be related to increase in response to various biotic stresses (Kwak *et al*., 1996).

CONCLUSION

As a plant response is triggered by a stress factor, the activities of antioxidant enzymes usually increase in the stage of the stress, providing a certain degree of protection from oxidative damage. Therefore, manipulation of the capacity of cocoyam to tolerate anaerobiosis is a function of its ability to modulate the antioxidant enzymes' armory in case of need.

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