Phytochemistry, Vol. 23, No. 4, pp. 893–894, 1984.
Printed in Great Britain

0031-9422/84 $3.00 + 0.00

SHORT REPORTS

α-D-GALACTOSIDASE DEFICIENCY IN COCONUT ENDOSPERM: ITS POSSIBLE PLEIOTROPIC EFFECTS IN MAKAPUNO*

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(*Received* 9 *August* 1983)

Key Word Index—*Cocos nucifera* L; Palmae; coconut; α-D-galactosidase; ontogenetic activity; enzyme deficiency; mutant endosperm.

Abstract—α-D-Galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) activity in the normal endosperm increased with age continually up to endosperm maturity. In contrast, makapuno α-D-galactosidase was hardly detected in almost all stages of development except the last, where its activity was 8300-fold lower as compared to the normal. The possible role of α-D-galactosidase in the formation of the makapuno endosperm is discussed.

INTRODUCTION

In our previous paper [1], we reported the isolation of a significantly high amount of galactomannans in makapuno coconut endosperms, and suggested that the accumulation of these viscous components could have caused the aberrant cellular behavior and properties of makapuno. Galactomannans constitute 61% of the total carbohydrates in mature coconut kernel [2] and their role in the development of the cell wall of coconut and other palm seeds has been forwarded [2, 3]. The detection and comparison of galactomannan-degrading enzymes between the normal and makapuno endosperms may elucidate the altered pathway of galactomannan degradation in makapuno. As a first step, α-D-galactosidase, which is one of the three enzymes implicated in galactomannan degradation [4–7] was investigated. In general, the enzyme catalyses the hydrolysis of α-D-galactosyl groups from α-D-galactose-containing oligo- and polysaccharides [8]. In this paper, we report the detection and comparison of α-D-galactosidase activity in the normal and makapuno coconut endosperms at various stages of development.

RESULTS AND DISCUSSION

Ontogeny of α-D-galactosidase activity in the normal endosperm

The α-D-galactosidase activity at various stages of normal endosperm development is presented in Table 1. α-D-Galactosidase activity increased with age continually up to endosperm maturity (Stage VI). The lowest activity was noted at Stage II with 0.07 units/mg protein. The activity was highest at Stage VI with 1.71 units/mg protein. The pattern of α-D-galactosidase activity is inversely correlated with the amount of galactomannans obtained at similar developmental stages from the normal endosperm [1]. Hence, it is possible that this enzyme plays a major role in the *in vivo* degradation of coconut endosperm galactomannans. McCleary and Matheson [9] reported similar findings on the depletion of galactomannans in the germinating seeds of lucerne, guar, carob and soybean which was accompanied by a rapid increase and then a decrease in α-D-galactosidase levels.

α-D-Galactosidase deficiency in makapuno

α-D-Galactosidase activity was not detected in the makapuno endosperm when the usual kinetic assay procedure which was used in the detection of enzyme activity from the normal endosperms was employed. However, activity was detected when the enzyme was incubated in the reaction mixture for 18–24 hr at 30°. Using this equilibrium or incremental assay procedure,

Table 1. α-D-Galactosidase activity in normal coconut endosperms at various stages of development

Stage	Age after pollination (months)	Specific activity* (Units/mg protein)
II	7–8	0.07[d]
III	8–9	0.23[d]
IV	9–10	0.59[c]
V	10–11	0.99[b]
VI	11–12	1.71[a]

*Average of six replications. Means followed by the same letter are not significantly different from each other at 0.05 level (Duncan's Multiple Range Test).

*Sixth of a series of papers on the genetics and biochemistry of makapuno coconut endosperm. For part 5 see ref. [1].

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the activity of makapuno α-D-galactosidase was detected in the 11 to 12 month old endosperms. The total enzyme activity was 8300-fold lower as compared to the activity in the normal. Despite partial purification with $(NH_4)_2SO_4$, makapuno α-D-galactosidase was not detected by the kinetic assay method. However by using the incremental assay procedure, the activity was detected in the 40–70% $(NH_4)_2SO_4$ cut. The enzyme was not subjected to further purification steps because of its very low activity.

The detection of enzyme activity in makapuno ruled out the possibility of a mutation in or absence of the structural gene for α-D-galactosidase in this endosperm. The fact that the enzyme exhibited catalytic properties almost similar to those from the normal [10] suggests that either a continuous repression of enzyme synthesis or the presence or absence of specific effectors caused the deficiency in enzyme activity.

The deficiency of α-D-galactosidase activity coincided with the significantly high level of galactomannans in makapuno [1]. Possibly, the normal degradation pathway is disturbed in this tissue, consequently leading to the accumulation of large amounts of galactomannans. In turn, the abnormally high level of the polysaccharide could have caused the expression of other altered characteristics, such as loss of intercellular adhesion, highly elongated cells, amitosis etc. Balasubramaniam [2] suggested that galactomannans play a structural role in the formation of the primary cell wall in coconut endosperms. Likewise, Kooiman [3] suggested that during the transition of the endosperm from the hydrated, gelatinous phase to the dehydrated, solid mature state most of the galactose groups are removed from the cell wall galactomannans. It was observed that galactomannans were predominant in the cell walls of unripe palm seeds whereas those of the ripe palm seeds consist chiefly of mannans, containing at most a few percent of galactose residues [11].

The failure of makapuno nuts to germinate under natural environmental conditions could be due also to α-D-galactosidase deficiency. Normally, the enzyme is required for the degradation and mobilization of reserved polysaccharides in the seeds during the onset of germination [8]. It is possible that in makapuno, the reserved energy required for germination is not mobilized.

The expression of the various altered phenotypes suggests the pleiotropic nature of the makapuno α-D-galactosidase gene. The pleiotropic effect could be manifested also in the activities of specific enzymes involved in other metabolic pathways. In particular, the activities of peroxidase [12] and tryptophan aminotransferase [unpublished] were significantly different between the normal and makapuno endosperms at specific stages of development. In addition, the level of cyclic AMP is persistently low in makapuno [13]. It is possible that they are all a consequence of the pleiotropism of α-D-galactosidase gene. Thus, it is worth investigating other key pathways, particularly those related to cell wall biosynthesis, cell–cell interaction, intracellular transport and hormone metabolism, in order to elucidate the mechanism responsible for the final expression of the mutant phenotype.

EXPERIMENTAL

Endosperms. Makapuno (mmm) coconut endosperms at five stages of development were collected from embryo-cultured makapuno trees (*Cocos nucifera* L. var Laguna) at the Department of Horticulture, College of Agriculture, UPLB. Likewise normal (MMM) coconut endosperms were obtained from true-breeding normal trees. Three nuts each from two makapuno and two normal trees were collected at each stage of endosperm development. The classification of developmental stage was based on the age of the nut in months after pollination, thus: Stage I, 6–7 months after pollination; Stage II, 7–8 months; Stage III, 8–9 months; Stage IV, 9–10 months, Stage V, 10–11 months and Stage VI, 11–12 months. The endosperms were cut into small cubes, placed inside a perforated plastic bag and frozen immediately. They were lyophilized to constant weight and stored desiccated at −10° until use.

Enzyme extraction. The freeze-dried coconut endosperm (5 g) was homogenized with 50 ml 0.05 M NaOAc buffer, pH 4 for 5 min in a pre-chilled Waring blendor cup. The slurry was centrifuged at 15000 rpm for 20 min at 4° and the supernatant was filtered by suction through Whatman filter paper no. 3. All crude extracts were stored at −10° overnight prior to determination of α-D-galactosidase activity.

α-D-Galactosidase activity was determined spectrophotometrically by measuring the increase in A at 402 nm. The reaction mixture was prepared by adding 0.2 ml of 1×10^{-2} M *p*-nitrophenyl α-D-galactoside in 0.10 M KPi buffer, pH 7.5 with 0.7 ml of the same buffer. The reaction was started by mixing 0.1 ml of the enzyme soln. One unit of enzyme activity is defined as the hydrolysis of one μmol of *p*-nitrophenyl α-D-galactoside/min at 30° and pH 7.5 under specified conditions. Sp. act. is defined as the enzyme unit activity/mg protein.

Protein determination. Total protein was determined by the method of ref. [14] but with some modifications.

Acknowledgement—This research was initially supported by a grant from the Philippine Coconut Authority and latterly from the Philippine Coconut Research and Development Foundation.

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