GALACTOMANNANS IN DEVELOPING NORMAL AND MAKAPUNO COCONUT ENDOSPERMS

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Galactomannan was isolated and purified from normal and makapuno coconut endosperms at different stages of development. In makapuno endosperm, galactomannan increased from 3.54% at 7-8 mo to 8.56% at 9-10 mo and slightly decreased to 8.34% at 10-11 mo. In normal endosperms, galactomannan was 5.73 and 5.80% for the first two stages and declined to 4.01% in the more mature stage.

Similar mannose:galactose ratios of 3.0 were obtained for the galactomannan from both normal and makapuno endosperms at the three stages.

The intrinsic viscosity of galactomannan from normal increased 3-fold with age. However, the intrinsic viscosity of galactomannan from makapuno increased only in the first two stages studied and levelled off in the third stage. At 10-11 mo, the makapuno galactomannan had lower intrinsic viscosity than that of normal (2.25 vs 3.25 dL/g).

Fractionation of the coconut polysaccharides also showed an increase in galactomannan for makapuno and a decrease for the normal in maturing endosperm as in above. On the other hand, the mannan polymer increased in the normal and decreased in makapuno.

INTRODUCTION

The makapuno mutant coconut endosperm is soft, fluffy, and viscous, while the normal coconut endosperm is firm and compact. A jelly-like precipitate can be obtained from both, but previous studies have shown that makapuno yields up to 10 times more than the normal. Acid hydrolysis of the precipitate gives galactose and mannose, indicating that the viscous component is galactomannan.

The enzyme D-galactosidase has been detected at increasing levels in maturing normal coconuts, while present only in mature makapuno at levels 8300-fold lower than the normal. This enzyme helps in removing galactose from galactomannan.

These results suggest that some of the abnormal cellular properties of makapuno are a direct consequence of an

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altered galactomannan metabolism. As part of our efforts to elucidate the mechanism of the abnormal cell growth of makapuno, this study was done to (i) determine the identity of the viscous polysaccharide, (ii) determine the levels of galactomannan in developing and maturing normal and makapuno endosperms, and (iii)partially characterize the polysaccharides in terms of viscosity and solubility.

MATERIALS AND METHODS

Makapuno (mmm) coconut endosperms at four stages of development were collected from embryo-cultured makapuno trees (*Cocos oucifera L. var Laguna*) at the Dept. of Horticulture, College of Agriculture, UPLB. Likewise, normal (MMM) coconut endosperms were obtained from true-breeding normal trees. For each type of coconut, three nuts were sampled from two different trees at each stage of endosperm development. The developmental stage was classified based on the age of the nut in months after pollination, thus: Stage II, 7-8 months; Stage III, 8-9 mo; Stage IV, 9-10 mo; Stage V, 10-11 mo. For lack of samples, Stage I (6-7 mo) and Stage VI (11-12 mo) were not utilized.

The harvested nuts were dehusked, deshelled, and the testa adhering to the endosperm removed. The white coconut meat was then sliced into small pieces, frozen and dried under reduced pressure. The freeze-dried endosperms were stored in the freezer until further processing.

Galactomannans were isolated and purified by the Aspinall method (1). One hundred grams of freeze-dried endosperm were desugarized and defatted by extraction with absolute ethanol, followed by a Sohxlet diethyl ether extraction. The defatted sample was shaken with hot water and centrifuged. Absolute ethanol was added to the supernate, and the precipitate (crude galactomannan) was collected in long fibers.

Galactomannan was purified by first dissolving in 4% NaOH, and then adding Fehlings solution. The precipitate formed was removed by centrifugation and redissolved in 0.5N HCl. The solution was poured into ethanol, and the precipitate collected and washed with a mixture of acetone-water and finally with absolute methanol. The purified galactomannan was dried in vacuo and stored in the freezer.

The extraction of galactomannan was done in three trials, each trial representing a different nut per type of nut per stage.

Purified galactomannan was hydrolyzed using the Albershein procedure (2). Ten mg of the material were mixed with 2 mL 2 N trifluoroacetic acid. The mixture was heated at 121°C for 1 h in a sealed tube. The resulting solution was evaporated in vacuo.

Derivatisation of the sugar samples was done using the McGinnis procedure (3). Hydroxylamine-HCl stock solution (0.4 mL) was added to the freeze-dried hydrolysates. The mixture was put in a heating block for 10 min at 80°C, then 1 mL of acetic anhydride was added to the mixture. After 5 min, 1 mL chloroform was added and the solution injected in the gas chromatograph (GC-6 AM Shimadzu), using an OV-225 100/120 mesh column at 195°C. Three trials per stage were performed. Two injections were done per trial.

To determine viscosity, different amounts of purified galactomannan (0.1, 0.2, 0.3, 0.4 g/dL) were dissolved in a boiling water bath, and continuously stirred until gelatinized. The resulting solutions were poured separately in an Ostwald viscometer, and the viscosity determined in a water bath at 40°C. Three trials per type of nut per stage were performed.

The 8-mo-old and 10-mo-old nuts were fractionated with NaOH following the procedure of Balasubramaniam (4). About 50 g of the freeze-dried endosperm was defatted and extracted with

water. The slurry was centrifuged and precipitated in ethanol. The residue was extracted with 4% NaOH and centrifuged. The supernate was neutralized with acetic acid. The neutralized supernate was precipitated in ethanol. The same procedure was done for the 17% NaOH extractions.

All precipitated polysaccharides were washed with acetone-water mixture and absolute methanol before freeze-drying. Dried fractions were stored in the freezer until further analysis. Two nuts per type of nut per stage were analyzed.

RESULTS AND DISCUSSION

Galactomannan Content. Galactomannan in makapuno endosperm increased 2.4-fold from 3.54% at Stage II to 8.56% at Stage IV, decreasing slightly to 8.34% at Stage V (Table 1). In contrast, the amount of galactomannan in normal endosperm remained in the 5.73-5.80% range at Stages II to IV, decreasing to 4.01% at Stage V.

The 8.5% galactomannan level in mature makapuno is lower than that reported for some leguminous seeds. For example, carob bean seeds consist of 38% of galactomannan (based on weight of milled seeds) and guar bean seeds, 35% (6).

Table 1. Amount of galactomannan in normal and makapuno coconut endosperms.

Stage of Development	Age of Coconut+ (mo)	Galactomannan (%) Normal Fresh	Normal Dry	Makapuno Fresh	Makapuno Dry
II	7-8	0.46	5.73	0.36	3.54
III	8-9	0.68	5.29	1.50	7.31
IV	9-10	1.07	5.80	2.58	8.56
V	10-11	1.05	4.01	3.10	8.34

+ After pollination.

Mannose:Galactose (M:G) Ratio. Galactomannan from either normal and makapuno endosperm had M:G ratios of 3 at different stages of development (Table 2). Balasubramaniam reported a similar M:G ratio of 3.00 for coconut galactomannan, while Kooiman (7) and Rao *et al.* (8) reported 2.57 and 2.0, respectively. Conditions in the purification of the crude galactomannan could account for the differences in the ratios observed. Obtaining only mannose and galactose from acid hydrolysates of the isolated material confirmed the identity of the polysaccharide as galactomannan.

Table 2. Mannose: galactose ratio of normal and makapuno galactomannans at different stages of development+.

Stage of Development	Normal	Makapuno
II	2.92 ± 0.06	2.94 ± 0.01
III	3.00 ± 0.03	2.93 ± 0.02
IV	2.74 ± 0.02	2.72 ± 0.03
V	2.90 ± 0.04	2.99 ± 0.05

+ Average of two trials in 2 injections per trial.

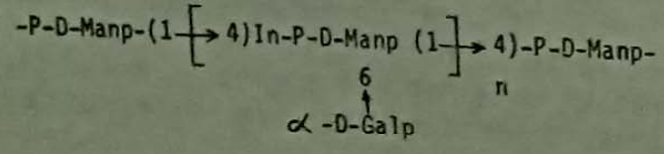
The mannose:galactose (M:G) ratio of galactomannans vary. Galactomannans from *Medicago sativa L.* (alfalfa, lucerne) have M:G of 1.0-1.25; from *Cyamopsis tetranogonolaba* (guar), 1.3-7.0; from *Ceratonia siligua* (carob, locust bean), 1.2-5.25 (9,10). The M:G ratio determines hydration properties. The higher the M:G ratio, the less capable is the substance of forming thick and viscous solutions with water. Also, the higher this value, the less soluble is the polysaccharide in water because of lessened solute-solvent interaction. Thus, it can be noted that in the extracted polysaccharides, the M:G ratios obtained are the same. Primarily this is because they are extracted by the same solvent.

Viscosity. Since the viscous character differentiates the normal endosperm from that of makapuno, the intrinsic viscosity of the isolated and purified galactomannan was determined. The intrinsic viscosity of galactomannan from normal endosperm increased from 1.05 to 3.25 dL/g at Stage III to V, respectively, while that of makapuno increased from 0.85 to 2.25 dL/g at Stage III to IV only, and remained at 2.2 dL/g until Stage V (Table 3). At Stage V, the intrinsic viscosity of galactomannan from normal was higher than that of makapuno, indicating shorter and less coiled galactomannan polymers in makapuno than in normal. However, due perhaps to the much greater level of galactomannan in makapuno than in normal, makapuno seems more viscous than normal. The shorter form of galactomannan may be due to the higher specific activity of β-mannase in makapuno than in normal (11).

Table 3. Intrinsic viscosities of purified galactomannan of normal and makapuno coconut endosperms.

Stage of Development	Intrinsic viscosity (dL/g) Normal	Makapuno
III	1.05	0.85
IV	1.58	2.25
V	3.25	2.20

Plant galactomannans consist of linear chains of (1⟶4) linked β-D-mannopyranosyl groups joined by (1⟶6) linkages by α-D-galactose along the chain (12).



The ability of polysaccharides and other macromolecules to form viscous solutions is capitalized in the industries. These galactomannans are used as thickeners, adhesives, emulsifiers, detergents, binder for explosives, etc. Physiologically, the viscosity of galactomannans is used by the plant for retaining water in its systems.

Polysaccharide Fractionation. The preceding results on galactomannan content indicate the increase of galactomannan in makapuno and decrease in normal as the endosperm matures. To determine what happens to galactomannan in the normal, the polysaccharides were further fractionated according to solubility in solvents of increasing severity. This fractionation showed that the makapuno endosperm contained 2 and 8 times more water-soluble galactomannans than the normal endosperm at 8 and 10 mo, respectively (Table 4). The polysaccharide which dissolved in 4%and 17% NaOH was of comparable levels in both normal and makapuno. These 4% and 17% NaOH extracts consisted of galactomannans but with lower amount of galactose (or higher mannose:galactose ratio). However, the hot 17% NaOH extract consisting of mannan polymers was highest in the normal endosperm at 10 mo. In makapuno this fraction decreased.

The enzyme α-D-galactosidase activity has been reported to increase continually up to mmaturity in normal endosperms (13, 14). In contrast, α-D-galactosidase was hardly detected in makapuno at almost all stages of development except at Stage VI where its activity was 8300-fold lower than in normal. These indicate that in normal, the cleavage of galactose from galactomannan proceeds at a high rate and results in the formation of the water-insoluble mannan polymer. On the other hand, the low level of α-D-galactosidase in makapuno results in the accumulation of galactomannan and not the mannan. Our results confirm these predicted trends and show the impor-

Table 4. Polysaccharide fractionation of 8 and 10 mo-old normal and makapuno endosperm.

	Polysaccharide (%)						
	8 mo			10 mo			Type of
	Normal		Makapuno	Normal		Makapuno	polysaccharide
Hot-water (2)***	3.77*		6.97	1.64		11.10	
4% NaOH (2)	3.68	73**	4.76 61	2.12	33	4.68	72 galactomannan
17% NaOH (1)	1.00		1.80	1.89		0.89	
(2)	0.45		5.02	1.30		3.02	
Hot 17% NaOH (1)	0.91	17	1.77 32	7.91	64	0.16	14 mannan
(2)	0.57		0.33	1.43		0.10	
Nonextractable Portion	1.18	10	1.45 7	0.42	3	3.34	14 cellulose
Total	11.56		22.10	16.71		23.47	

* Expressed as percent of polysaccharide in freeze-dried endosperm.
** Based on results of Balasubramaniam (1976); these are being verified in our laboratory.
*** No. of extractions in parentheses.

tance of galactomannan metabolism in the formation of the makapuno phenotype.

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