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DISTRIBUTION OF NONCELLULOSIC β-D-GLUCANS IN GRASSES AND OTHER MONOCOTS

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Key Word Index—*Saccharum officinarum*; *Phragmites australis*; *Zoysia japonica*; *Streptochaeta sodiroana*; *Hydrochloa caroliniana*; *Lithachne paucifolia*; Gramineae; chemotaxonomy; hemicellulose; β-D-glucans.

Abstract—Cell wall specimens from nonendospermic tissues of six grasses and representatives of nine other monocot families were treated with a specific glucanase in order to liberate wall-bound noncellulosic β-D-glucans. Gel filtration chromatography profiles of the oligosaccharides released from all grass species indicated the presence of a mixed linkage β-(1→3):(1→4)-glucan. The results also indicate that this glucan was not present in the other monocots examined. The evidence from this and previous studies indicates that the mixed linkage glucan may be restricted in the monocots to the Gramineae.

INTRODUCTION

Noncellulosic β-D-glucans comprised of mixed (1 → 4) and (1 → 3) glucosidic linkages have been identified as cell wall components in nonendospermic tissues of the following grasses: *Zea* [1–3], *Hordeum* [3, 4], *Sorghum* [3], *Triticum* [3, 5], *Panicum* [6], *Arundinaria* [7], *Secale* [3, 8] and *Avena* [9–12]. β-D-Glucans have also been detected in the caryopsis endosperm of *Lolium* [13] and numerous cereal grasses, most notably *Avena* and *Hordeum* [14–16]. These glucans have not been reported in species other than grasses, with the exception of the dicot *Phaseolus aureus* [17]; however, the presence of the glucan in *Phaseolus* was not confirmed by Kato and Matsuda [18] and the earlier report [17] suggests that the glucan has at most a transient existence in this dicot. Thus, the available literature indicates that the specific mixed linkage glucan is restricted to the Gramineae. A closely related but nonidentical polysaccharide, lichenin [19], has been found in the thallus of certain lichens [20, 21]. The present study extends the list of glucan-containing plants, and considers the chemotaxonomic significance of noncellulosic β-D-glucans in the grasses.

RESULTS AND DISCUSSION

Purified, α-amylase-treated cell walls from immature, nonendospermic tissues of the grasses *Zoysia japonica*, *Saccharum officinarum*, *Hydrochloa caroliniana*, *Streptochaeta sodiroana*, *Phragmites australis*, and *Lithachne paucifolia*; and the nongrass monocots *Juncus greenei* (Juncaceae), *Cyperus alternifolius* (Cyperaceae), *Rhoeo spathacea* (Commelinaceae), *Belamcanda chinensis* (Iridaceae), *Rhapis excelsa* (Palmae), *Billbergia nutans* (Bromeliaceae), *Aglaonema modestum* (Araceae), *Curculigo capitulata* (Amaryllidaceae), *Typha latifolia* (Typhaceae) and *Asparagus sprengeri* (Liliaceae) were incubated with a *Rhizopus* endo-β-1,3-glucanase solution. The liberated oligosaccharides were separated on a column of Bio-Gel P-2 and measured by the phenol–H_2SO_4 method.

The P-2 elution profiles for all grass species reveal that tri- and tetrasaccharides were released in molar rations of *ca* 2:1 to 3:1 (Table 1). The specificity of the *Rhizopus* enzyme and the similarity of the profiles to those obtained from the enzymatic degradation of known glucans [3, 11, 12] indicate the presence of a mixed linkage β-(1 → 3):(1 → 4)-glucan similar to those observed in other grasses [3]. The absolute quantity of oligosaccharides released (Table 1) most likely varies with tissue age (McClellan, M. K., unpublished results) and the growing conditions of the plants and was not explored in this study. The profiles for all nongrass species examined reveal that no tri- and tetrasaccharides were released, and it appears that the glucan is not a cell wall constituent in these species.

The taxonomic range of grasses covered in this study (five subfamilies), in conjunction with the members of the subfamily Pooideae examined in previous studies [3–5, 8–12], extends the coverage to the six subfamilies of the Gramineae. The presence of noncellulosic β-D-glucans in such a wide range of grasses, and its absence in the other monocots studied, provide evidence that the glucan may be restricted to the Gramineae. This observation may provide the basis for further chemotaxonomic studies of cell wall polysaccharides in the grasses, and serve to clarify uncertain taxonomic relationships within the Gramineae. In addition, the apparent confinement of the glucan to one family demands a close scrutiny of the hypotheses that cell growth and elongation

Table 1. Comparison of β-D-glucans derived from cell walls of six grass species

Subfamily; species	Trisaccharide yield (μg/mg wall)	Tetrasaccharide yield (μg/mg wall)	Molar ratio Tri/tetra
Chloridoideae; *Zoysia*	5.6	2.3	3.2
Panicoideae; *Saccharum*	27.2	9.7	3.7
Oryzoideae; *Hydrochloa*	2.9	1.3	2.9
Arundinoideae; *Phragmites*	13.7	8.7	2.1
Bambusoideae; *Lithachne*	3.9	2.0	2.6
Bambusoideae; *Streptochaeta*	6.5	3.1	2.8

specifically involves the metabolism of the glucan. More extensive studies should be performed to confirm these observations.

EXPERIMENTAL

Wall preparation. All plants were grown in the Botany Department greenhouses at Iowa State University except *Typha latifolia*, which was collected from a local marsh. Samples of elongating subapical tissue were collected and homogenized with ice in a Waring blender. The homogenates were collected on Miracloth and washed with cold Me_2CO (−20°) to remove cell contents. The insoluble material (cell walls) was dried *in vacuo* over P_2O_5.

Amylase digestion. Portions of each wall sample (20–50 mg) were incubated with 1.5 ml of an α-amylase soln (150 units of Sigma Type 1A porcine pancreatic α-amylase per 1 ml 25 mM pH 7.0 K-Pi buffer) at 30° for 24 hr to degrade any residual starch that may have adhered to the walls during preparation [11]. NaN_3 (0.02%) was included to suppress microbial activity. After amylase treatment, the wall–enzyme mixtures were heated at 100° for 10 min to inactivate residual enzyme. The walls were collected on Whatman glass microfiber paper (GF/C) and washed with H_2O.

Glucanase degradation of walls. Endo-β-1,3-glucanase was purified from a crude *Rhizopus* glucoamylase preparation (Sigma Chemical Co.) employing the procedure of ref. [3]. The walls were incubated with 1.5 ml of glucanase soln (5 μg *Rhizopus* endo-β-1,3-glucanase per 1 ml pH 4.6 McIlvaine buffer) at 30° for 24 hr; 0.02% NaN_3 was included. After glucanase treatment, the walls were collected on glass microfiber paper and rinsed with several 1 ml portions of H_2O. The filtrates and rinse solutions were combined and heated to 100°. Liberated oligosaccharides were fractionated on a 1.2×55 cm or a 1.6×85 cm Bio-Gel P-2 column (−400 mesh) at 40°. Fractions (1 or 2 ml) were collected and the carbohydrate content determined by the phenol-H_2SO_4 method [22].

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