NATURE AND DISTRIBUTION OF XYLAN POLYMERS IN SEED HAIRS FROM *Lygeum Spartum* PLANTS GROWING IN TWO DIFFERENT BIOTOPES

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Résumé

Les parois des poils des infrutescences de *Lygeum spartum* L. appartenant à 2 populations (hauts plateaux du sud ouest algérien et littoral oranais) ont été extraites et analysées. Dans les deux cas, l'ose majoritaire est le xylose (40%). Les parois des poils prélevés sur les infrutescences récoltées dans les hauts plateaux contiennent deux xylanes: une glucuronoarabinoxylane soluble et une xylane ramifiée fortement liée à la trame cellulosique. Dans la population littorale, seule la xylane ramifiée a été détectée dans les parois qui sont, par ailleurs, caractérisées par leur faible extensibilité. Les observations immunocytochimiques ont révélé que les xylanes sont absentes dans les parties apicales du poil. Elles apparaissent progressivement au cours de la maturation. L'augmentation des composés riches en xylanes pourrait contribuer à la perte du potentiel d'élongation des poils.

***Mots clés**: parois de poil, Lygeum spartum, polysaccharides, poils d'infrutescence, xylane.*

Abstract

Cell wall oligosaccharides were extracted from epidermal hairs of *Lygeum spartum* seeds harvested in two different algerian geographical biotopes. In both cases, xylose represented about 40% of the cell wall monosaccharides. Samples collected in arid high plateaus contained two kinds of xylan polymers, a highly soluble d-glucuronoarabinoxylan-like one and a relatively unbranched xylan tightly bound to the apoplastic network. In contrast, only the latter fraction was detected in seed hairs of the semi-arid saline coastal zone previously characterized by their low extensibility. In both batches of material immunocytochemical data revealed that xylan, absent from growing hair tips, appeared progressively during hair cell wall maturation. The increase in xylose rich compounds would probably contribute to the loss of potential elongation of hairs.

***Keywords**: cell wall, Lygeum spartum, polysaccharides, seed hairs, xylan.*

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ملخص

جدران خلايا الشعيرات لثمار نبات السنغا *Lygeum spartum* L. المقطوفة من منطقتين مختلفتين: الهضاب العليا والساحل الوهراني، استخلصت وكان السكر المهيمن هو سكر xylose بنسبة 40 .

جدران الشعيرات المنزوعة من ثمار الهضاب العليا تحتوي على نوعين من xylan : إحداهما glucuronoarabinoxylan ذائبة وأخرى xylane متفرعة شديدة الإلتصاق بالسللوز.

بالبنسبة لجدران شعيرات الساحل الوهرانب، فإن الـ xylane المتفرعة كانت تتص بالجدران قليلة التمدد. ملاحظات المناعة الكيموخلوية immunocytochimique بينت بأن الـ xylane غائبة في المناطق القيمة للشعيرات، وتظهر تدريجيا مع نمو الشعيرة. وعليه، فإن زيادة كمية المواد الغنية بالـxylose قد يكون عائق أمام إمكانية الإستطالة والتمدد للجدران.

***الكلمات المفتاحية:** جدران الشعيرات، سكرية الجدار، Lygeum spartum ، شعيرات البذور، كسلان،*

Lygeum spartum is one of the predominant species growing in Algeria arid high-plateau and semi-arid coastal regions. This perennial grass is known to be economically important for ecological reasons since it prevents the extension of desertic areas. Moreover the leaves can be used for pulping and forage [6,7]. The mature seeds might also be of interest. Indeed they are enclosed in spikelets covered with long (0.5-1.7cm) silky hairs morphologically rather similar to cotton hairs.

We have therefore begun to investigate samples harvested in their two natural biotopes. Cytological and genetic analyses suggested that the plants represented two different ecotypes [1. We could also demonstrate [11] that the spikelet hairs from coastal specimens were characterized by a higher mechanical resistance and a lower extensibility. Morover, cell wall composition depended on the geographical origin of the seeds. In the coastal region samples, hemicelluloses represented 52% of the cell walls whereas in high plateau samples the same polysaccharides accounted for 43% only. In contrast the neutral sugars associated with the few uronic acids detected in the so-called pectic fraction were less abundant in coastal samples than in high plateau material (3% and 18% respectively).

We intended, in the present study, to further analyze the main cell wall polysaccharide fractions. Using biochemical tools we investigated their composition and their localization across the cell wall of both kinds of specimens.

MATERIAL AND METHODS

Plant material

Mature spikelets from *Lygeum spartum* L.(Poaceae) contain one to four caryopses enclosed in a lignified hair tube resulting from the partial

coalescence of the lemmas. Spikelets and hair samples were collected from stands growing either in an arid zone from the high plateau region of Algeria (Naâma Wilaya) or in a semi-arid, saline zone near the coast in Oran Wilaya.

Analysis of cell wall polysaccharides

Sequential extractions of cell wall polysaccharides were performed on cell walls isolated from spikelet hairs according to Goldberg, Morvan and Roland [4]. Pectins, hemicelluloses and cellulose were then solubilized as previously described [11]. Due to the very poor amount of sugars recovered in the EDTA soluble fraction, only the pectins solubilized with boiling water were analyzed.

The extracted polysaccharides were hydrolyzed with SO_4H_2 (1h, 110°C, 1N for pectins, 1h, 100°C, 3N for hemicelluloses 1h30, room temperature 27N followed by 4h, 100°C, 1N for cellulose), then neutralized with $SrCO_3$, filtrated on millipore membranes 0.45 µm. The solution was then evaporated, resuspended in 100µl H_2O and released monosaccharides estimated by HPLC on a Spectra Physics (San Jose, U.S.A.) liquid chromatograph (model SP 8750) equipped with a Spherisorb-NH_2 column (200 x 4.6 mm; Thermo-separation Products, France). Sugars were eluted with a mixture of acetonitrile-water (80:20) and detected with a Spectra Physics SP 6040 refractive index detector connected to a SP 4290 integrator.

Immunodetection of xylan

Small specimens were cut from the hairy tubes enclosing the caryopses and fixed in a mixture of 4% paraformaldehyde and glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 2h. They were then washed in buffer and dehydrated through an ethanol series before embedding in LR white resin (London Resin Co, London U.K). Transverse semi-thin (1µm) sections for light microscope observations were collected in multiwell glass slides and incubated for 5 min with 0.05M Tris-HCI buffer, containing 0.9% NaCl, 0.1% bovine serum albumin and 0.01% Tween 20. Sections were treated instead with polyclonal antibodies cross-reacting with xylose polymers kindly provided by Dr.E.Grenet (dilution 1/40 or 1/80(v/v)). The antibodies raised against oat arabinoxylan have been successfully used to localize xylan in wheat straw and maize stems [8,9]. Visualisation of antibody binding using a second antibody coupled to fluorescein isothiocyanate did not give satisfactory results due to the strong autofluorescence of spikelet with hairs. Sections were then treated with immunogold-silver intensification and observed through epipolarisation [5].Thus, Incubation was made in goat anti-rabbit IgG conjugated to 15 µm colloidal gold probes (EMGAT 15, BioCell, Cardiff, U.K.). After intensification with the BioCell silver enhancement kit, sections were observed with a light microscope equipped with an epipolarisation filter (Apol filter 487960, Zeiss, Asnières, France).

Studies at the electron microscope level were carried out on transverse ultrathin sections collected on nickel grids and treated following the procedure described above for immunogold labeling, omitting the silver enhancement step. Grid were then stained with 1% uranyl acetate followed, in some instances with lead citrate.

Ultrathin sections were examined with a JEOL, JEM-100CXII electron microscope.

For both light and electron microscopy, controls were prepared by omitting the incubation step with the antibody.

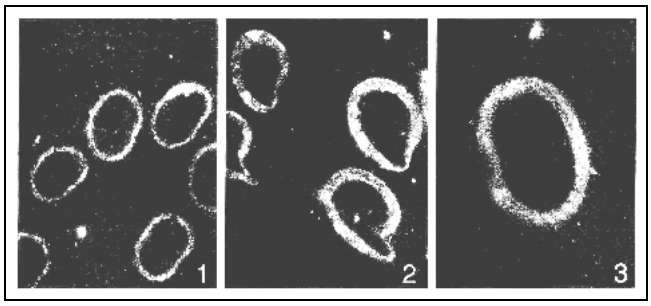
RESULTS AND DISCUSSION

Analysis of cell wall polysaccharides

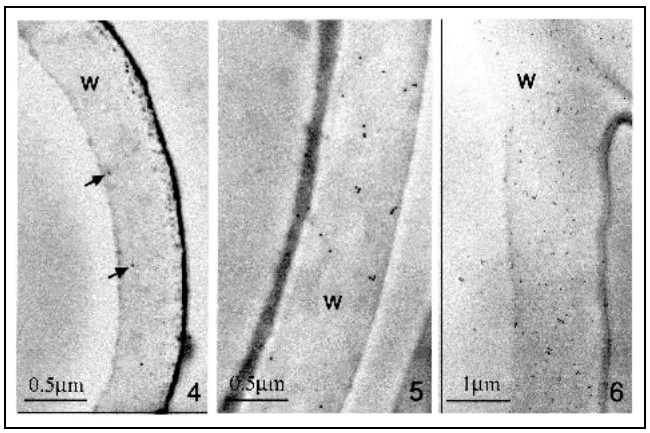
Table 1 shows the sugar composition of the different polysaccharide fractions extracted from hair cell walls of seeds harvested either in arid high plateaus or in coastal regions. In both cases, xylose represented 40% of the sugars obtained after acid hydrolysis. Such a high content is in agreement with the known fact that grasses are rich sources of xylan [3,10]. Although the total amount of extracted xylose was similar in all samples, the relative amount present in the several polysaccharide fractions varied according to the origin of the simple. In samples collected in high plateaus, the amounts of xylose (in percent of all extracted sugars) were respectively 7.8% in the pectin fraction, 29,2% in the hemicellulose and only 3% in the hemicellulose II fraction. In contrast, in samples collected in the coastal zone, xylose was found only in the hemicellulose fractions (32.5% in hemicellulose I and 74% in hemicellulose II. In these samples, the soluble xylan are then probably more tightly bound to the cell walls where they reinforce the cohesion of the apoplastic network. Actually, it was previously reported that the cell wall mechanical properties of this material were specially low [11]. On the other hand high plateau specimens exhibiting a higher extensibility were characterized by the presence of highly soluble xylose polymers associated to arabinose and galactose residues together with a few uronic acids. These polysaccharides might be related to the highly soluble glucuronoarabinoxylan, known to be abundant in grass cell walls [3]. The galactose residues might originate from the arabinogalactoproteins located at the inner surface of the wall [2].

	Geographical region	
Polysaccharide fractions	High plateau	Coastal region
Pectin		
*** arabinose**	1.7	
*** galactose**	8.6	3.0
*** xylose**	7.8	0.2
Hemicellulose (HC I)		
* Arabinose	5.0	6.2
* Galactose	3.6	5.7
* xylose	29.2	32.5
Hemicellulose (HC II)		
* Arabinose	0.6	-
* Glucose	1.3	-
* xylose	3.0	7.4
Cellulose		
* glucose	34.2	40.4

Table 1: Monosaccharide composition of the different fractions extracted from spikelet hair cell walls.
Data as percent of all sequentially extracted compounds.



Figures 1-3: Transverse sections of spikelet hairs collected either in the coastal region (Figs 1 and 2) or in the arid, high plateau zone (Fig.3) were observed with an epipolarisation filter after immunogold labeling with antixylan antibobody. Reaction product was present over the whole walls whether the section was made through the thin walls in the median part of the hair (Fig.1) or through the thick walls at hair basis (Fig.2 and 3). (x 85).



Figures 4-6: Transverse sections of hair cell walls from material collected in the coastal zone. Wall outer part is on the right. Immunogold labeling with the same antibody observed with the electron microscope. Post staining with uranyle acetate and lead citrate . Fig. 4: hair apical region, just below the tip; Fig.5: hair median region; Fig.6: hair basis.

Xylan immunolabelling

Similar results were obtained either at the light microscope (Figs 1-3) or at the electron microscope level (Fig. 4-6). Control sections were devoid of reaction product whether observed with the electron microscope or through epipolarisation. Observation of labeled samples revealed that the staining intensity increased in parallel with cell wall thickness from the tip to the basis of epidermis hairs. No gold deposits appeared in the walls at some distance near the growing tip (Fig.4-5), their density becoming rather high in the basal part of the hairs still inserted among other epidermal cells (Fig.5). Using the same antibody, an increase in xylan labeling was also observed during fiber and xylem differentiation in maize stem where it was related to an increase in cell wall hemicellulose content [8]. In apical growing epidermal hairs, the increase in xylose epitope is probably related to loss of elongation potential. At the hair basis a random distribution of gold particles was observed in all sections suggesting a homogenous distribution of xylose epitopes in the cell walls. In wheat straw also, xylan have been located within the entire cell wall [9]. No differences were observed between samples collected in arid or semi-arid zones (compare figures 1-2 and figure 3).

CONCLUSION

In conclusion, the above results confirm that the seed hairs harvested from two different biotopes exhibit significant differences in the distribution of xylose residues in their walls as well as in their mechanical properties. The occurrence of soluble polymers in samples from the high plateau region might entail the higher extensibility previously reported [11]. These findings are further evidence of the influence of the geographical origin on the phenotypic characters of plant. They back up previous results from our group, based on cytological and genetical analyses witch lead us to suggest the existence of at least two distinct ecotypes of *Lygeum spartum.*

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