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Research article

Quantification and monosaccharide composition of hemicelluloses from different plant functional types

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ABSTRACT

Hemicelluloses are the second most abundant polysaccharide in nature after cellulose. So far, the chemical heterogeneity of cell-wall hemicelluloses and the relatively large sample-volume required in existing methods represent major obstacles for large-scale, cross-species analyses of this important plant compound. Here, we apply a new micro-extraction method to analyse hemicelluloses and the ratio of 'cellulose and lignin' to hemicelluloses in different tissues of 28 plant species comprising four plant functional types (broad-leaved trees, conifers, grasses and herbs). For this study, the fiber analysis after Van Soest was modified to enable the simultaneous quantitative and qualitative measurements of hemicelluloses in small sample volumes. Total hemicellulose concentrations differed markedly among functional types and tissues with highest concentration in sapwood of broad-leaved trees (31% d.m. in *Fraxinus excelsior*) and lowest concentration between 10 and 15% d.m. in leaves and bark of woody species as well as in roots of herbs. As for total hemicellulose concentrations, plant functional types and tissues exhibited characteristic ratios between the sum of cellulose plus lignin and hemicelluloses, with very high ratios (>4) in bark of trees and low ratios (<2) in all investigated leaves. Additional HPLC analyses of hydrolysed hemicelluloses showed xylose to be the dominant hemicellulose monosaccharide in tissues of broad-leaved trees, grasses and herbs while coniferous species showed higher amounts of arabinose, galactose and mannose. Overall, the micro-extraction method permitted for the simultaneous determination of hemicelluloses of various tissues and plant functional types which exhibited characteristic hemicellulose concentrations and monosaccharide patterns.

1. Introduction

Cell-walls are by far the largest pool of organic carbon in plants. They consist of a variety of different compounds, mainly structural polysaccharides, pectins, lignin, proteins and other minor components. Structural polysaccharides are cellulose and hemicelluloses with the ratio of cellulose:hemicelluloses commonly varying between 2:1 to 1:1 [1]. Hemicelluloses are thus the second most abundant polysaccharide group in plants and, depending on the tissue, generally account for 10–30% of a tissue's dry biomass. They are defined as those cell-wall polysaccharides that are insoluble in water but can be extracted with aqueous alkali and hydrolysed into its component monosaccharides with diluted sulphuric acid [2]. Hence, hemicelluloses are rather defined methodically, than by structure and they comprise different classes of polysaccharides, of possibly different physiological and mechanical functions. Despite their quantitative importance, hemicelluloses are often neglected in ecological studies, mainly due to analytical difficulties associated with their chemical heterogeneity. Therefore, our current knowledge about hemicelluloses derived largely from structural research, dietary fiber analyses and the paper industry, but only few studies investigated the ecophysiological significance of these important plant compounds [references cited in [1,3]].

Hemicelluloses are grouped into four classes according to their main types of sugar residues present: xyloglucans, xylans, mannans and mixed-linkage β-glucans (for a detailed review on hemicellulose diversity see Ebringerova et al. [4]). Xyloglucans occur in primary cell-walls of all higher plants and seem to be tightly bound to cellulose fibrils by hydrogen bonds [5]. Hemicelluloses of the xylan type are the most abundant hemicellulose class in secondary cell-walls of hardwood species and herbaceous plants [6,7]. Glucomannans and galactomannans are the main representatives of the mannan-type hemicelluloses, which are the predominant hemicelluloses in secondary cell-walls of conifers [8] and seeds of *Leguminosae* [9]. Finally, mixed-linkage $(1\rightarrow3, 1\rightarrow4)$-β-D- glucans occur exclusively in *Poales* [10] and some pteridophytes (*Equisetum*, [11]).

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The great chemical diversity of hemicelluloses is the biggest challenge for quantitative and qualitative analyses of these cell-wall compounds. Many published methods isolate hemicelluloses by complex sequential extraction [12–14]. Using multiple extraction steps allows for a good separation of the cell-wall polysaccharides from low-molecular-weight water-soluble compounds, proteins, lipids and lignin. Hemicelluloses are then usually extracted with aqueous alkaline solutions and further hydrolysed with acid into their monosaccharides which are subsequently measured by chromatographic methods [12]. However, these multi-step extractions are very labour-intensive preventing large-scale screenings over a multitude of samples. In contrast to these very elaborate methods one procedure, which was originally developed for quality analysis of forage in agriculture [15–17], has been established as the most promising avenue for large-scale determination of hemicelluloses. The basic principle of this method is a two step separation of bulk-hemicelluloses from all non-structural cell compounds (the protoplasmatic content) and cell-wall pectins on one hand, and cellulose and lignin on the other hand. Since hemicelluloses are hydrolysed into their corresponding monosaccharides during the extraction process, the hemicellulose-containing extract can be directly used for the qualitative determination of the hemicelluloses' monosaccharide composition (e.g. by HPLC). However, the standard procedure after Van Soest requires relatively large amounts of sample material which conflicts with the parallel analysis of many samples.

Here we report on a study in which total concentrations of hemicelluloses as well as their monosaccharide composition in different tissues from 28 plant species (comprising four different plant functional types) were analysed by a micro-extraction method. This method, which is based on the original Van Soest fiber analysis [15–17], decreases the required amount of plant material per sample (50 mg dry weight) and thus permits rapid screening for hemicelluloses in up to 80 samples within a single run. Particularly we substituted all filtering steps by sequential centrifugation steps, which enabled a drastic reduction of the required sample volume. Subsequent HPLC analyses of the hydrolysed monosaccharides permitted their identification. To our knowledge this is the first broad comparative analysis of hemicellulose concentrations and composition over different tissues of several plant functional types within a single study. The novel analytical procedure is a long awaited break through that will permit a better exploration of this important field of plant science. The results are compared with data from previous studies using different methods for the quantification and description of hemicelluloses. Finally, ecological implications of the functional type-specific differences in hemicellulose content and composition are discussed.

2. Results

2.1. Hemicelluloses in different plant functional types

The gravimetric analyses of total hemicelluloses showed characteristic differences among plant functional types and tissues with the highest concentrations in sapwood of broad-leaved trees and roots of grasses (both about 25% d.m. (dry matter)) followed by conifer sapwood (approximately 20% d.m., Fig. 1). Smaller hemicellulose concentrations of around 15% d.m. were present in leaves of grasses and herbs with high variation between species. Leaves and bark of broad-leaved trees as well as needles and bark of conifers and roots of herbs exhibited the lowest mean hemicellulose concentrations of 10–12% d.m.

Depending on plant functional type and tissue, the sum of cellulose plus lignin accounted for 14–70% of the dry biomass. Overall, the 'cellulose and lignin fraction' was 1.5–3.5 times greater than the gravimetric hemicellulose pool (Fig. 2). Among the analysed tissues, leaves of broad-leaved trees, grasses and herbs exhibited relatively smaller ratios of 'cellulose and lignin' to hemicelluloses, with leaves of two herbaceous species (*Silene flos-cuculi* and *Urtica dioica*) having even slightly more hemicelluloses than cellulose and lignin. In contrast, exceptionally high ratios were found in the bark of broad-leaved trees and conifers, which resulted rather from low hemicellulose than high cellulose and lignin concentrations.

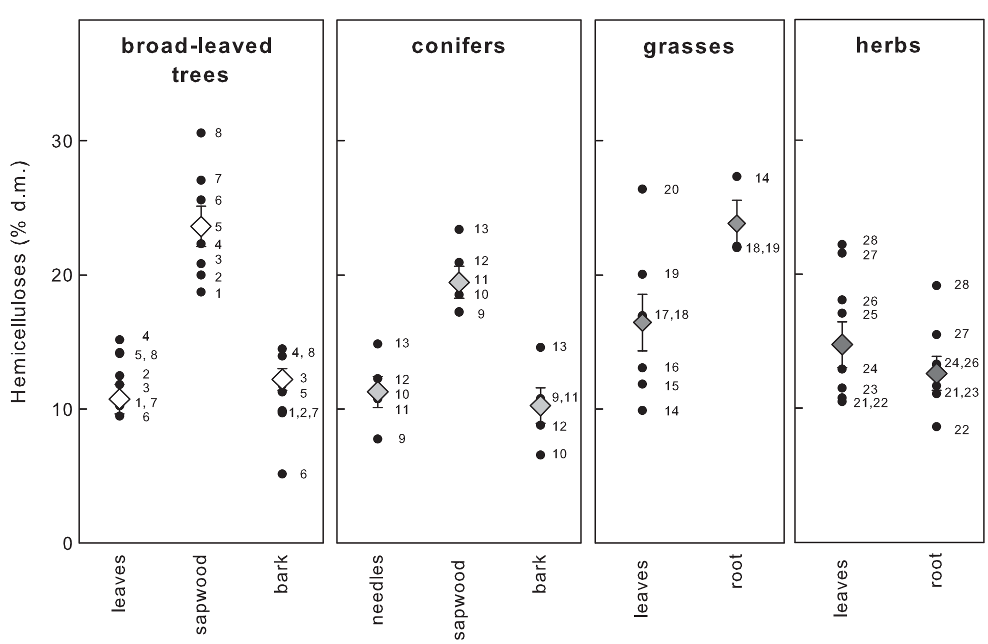

Fig. 1. Total hemicellulose concentrations [gravimetrically calculated as total cell-wall fraction – cellulose and lignin fraction] in % dry matter in different tissues of four functional plant groups. The black dots are values for single species; open diamonds are means for the respective tissue ± standard error. The numbers given beside the black dots indicate the species identity as listed in Table 1.