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Spectroscopic characterization of genetically modified flax fibers

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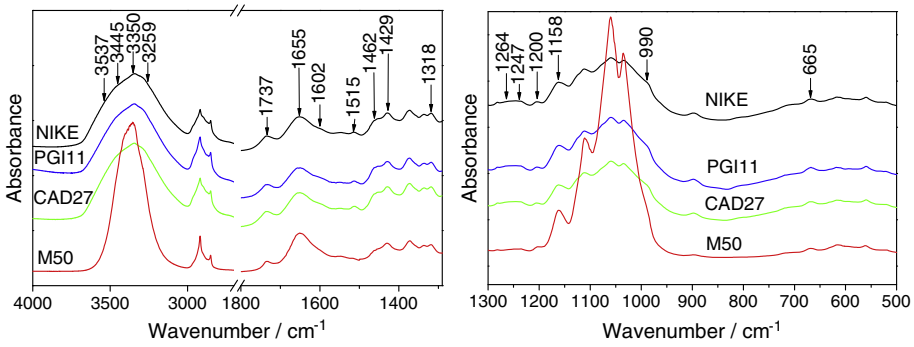
HIGHLIGHTS

- The content of bio-chemicals in GM and natural flax fibers has been compared.
- FT-IR spectra of fibers have been reported and analyzed.
- XRD studies have been used to characterize the cellulose fibers.

GRAPHICAL ABSTRACT



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ABSTRACT

The principal goal of this paper is an analysis of flax fiber composition. Natural and genetically modified flax fibers derived from transgenic flax have been analyzed. Development of genetic engineering enables to improve the quality of fibers. Three transgenic plant lines with different modifications were generated based on fibrous flax plants as the origin. These are plants with: silenced cinnamyl alcohol dehydrogenase (CAD) gene; overexpression of polygalacturonase (PGI); and expression of three genes construct containing β-ketothiolase (*phb A*), acetoacetyl-CoA reductase (*phb B*), and poly-3-hydroxybutyric acid synthase (*phb C*). Flax fibers have been studied by FT-IR spectroscopy. The integral intensities of the IR bands have been used for estimation of the chemical content of the normal and transgenic flaxes. The spectroscopic data were compared to those obtained from chemical analysis of flax fibers. X-ray studies have been used to characterize the changes of the crystalline structure of the flax cellulose fibers.

Introduction

Several methods have been proposed for GMO identification and quantification. These are enzyme-linked immunosorbent assay analyses based on protein information [1], Western blot [2], immunoassay TRAIT test [3], and polymerase chain reaction analyses based on DNA information [4,5]. In recent years, rapid and inexpensive analyses have been developed. They include near-infrared (NIR) [6] and Fourier-transform (FT-IR) spectroscopies for the discrimination of genomics DNA from different genotypes [7]. The former method is often coupled with photoacoustic (PAS) cell in the studies of plants [8] and foods [9]. FT-IR-PAS method has been recognized as a rapid and nondestructive

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technique that allows to distinguish between transgenic and non-transgenic plants. This method is particularly effective when combined with canonical discriminant analyses [10,11].

The above described methods have been applied to several products derived from genetically modified plants, e.g. soybean seeds [10], tobacco [12,13], rice [14], and aspen [15]. In the present research we wanted to apply another method that allows to characterize differences between natural and GMO organisms. This method applies the deconvolution of the spectral contour into Lorentzian components and comparison of their integral intensities for the transgenic and non-transgenic flax fibers.

Flax (*Linum usitatissimum*) is an annual plant cultivated in temperate climate, particularly in Central Europe. Products obtained from this plant are useful in industry, being a valuable source of oil and fibers. Development of genetic engineering enables to improve the quality of these products. The genetical flax modification enhances the quality of flax fibers, oil properties, elevation of antioxidant level and creation of pathogen-resistant plants. These modifications made flax more useful and precious source for a broad range of products applicable in industry. The transgenic approach was used to give overexpression of fungal pectinase enzymes in flax PGI 11. The introduced modifications resulted in a pectin content decrease and thus an improved retting efficiency [16]. The reduction in the lignin content in CAD 27 plants resulted in an improvement in the elastic properties of flax plants; transgenic plants showed increased mechanical properties in comparison to nontransformants [17]. Significant modification in stem mechanical properties was accompanied by the PHB (poly-3-hydroxybutyric acid) accumulation in growing cells of fibers in the transgenic plants (M50) [18].

Properties of flax fiber depend strongly on the growing conditions and the applied fiber processing technique [19]. Among the methods used in the studies of these materials, FT-Raman and FT-IR spectroscopies were found to be very suitable to detect the major chemical components of the flax stems *in vivo* [20]. These techniques provide information on the molecular changes of the flax fibers caused by ageing [21], mechanical processing [22], and chemical treatment [23].

Many papers have been published on the vibrational spectra of the flax fibers and the components isolated from this plant [12,23–28]. The application of these methods allowed to recognise several important problems of the flax chemistry. For instance, they gave the information on polarization behavior of the IR bands for oriented cellulose fibers [28], structural changes of flax fibers in chemical treatment [23,27], the fiber content of flax stems, strain induced shifts of the Raman bands of natural cellulose fibers [29,30], effects of the enzymatic retting of flax stems [26], and the role of the hydrogen bonds in the different packing of the celluloses I and II [31]. These investigations resulted also in finding of the diagnostic way to differentiate two categories of the native celluloses, i.e. algal and bacterial celluloses I_A, and higher plant celluloses I_B [32].

The biochemical, mechanical, and structural properties of flax stems and fibers, derived from field grown genetically modified (GM) flax, were studied in our previous papers [33–36]. In the present work the results of FT-IR studies of native (NIKE) and transgenic flax (M50, CAD 27, PGI 11) fibers have been reported. The chemical content and structure of the fibers have been analyzed on the basis of the IR spectra. X-ray diffraction method has been used to compare the crystalline structures of the studied flax cellulose fibers.

Experimental section

Plant material

Three types of transgenic plant were generated based on fibrous flax plants as the origin. Cinnamyl alcohol dehydrogenase gene repression [17], fungal polygalacturonase overexpression [16], and PHB synthesising [18] transgenic flax plants were the source of fibers.

All modified plant types were generated using an Agrobacterium method [18,37–40] with the constructs described in Table 1. To avoid doubts that not all of the CAD isoforms were silenced, a conservative fragment of 480 nt was chosen to be used in the transformation. It has over 90% identity with a large number of CAD sequences from angiosperms. Briefly, cotyledons of 6-day-old seedlings were infected with an *Agrobacterium tumefaciens* strain containing a dedicated binary plasmid introduced by electroporation. After the transfer of cotyledons to callus induction medium and subsequently to shoot induction medium, the explants were collected and grown on root-inducing medium. The pre-selection and further selection of modified plants were carried out by means of PCR and Northern blot analysis as described previously [16–18]. From each transformation, one line that showed the expected effect of the introduced modification and normal phenotype was chosen for the field trial as a source of fiber to be used throughout this study.

Flax plants from all transgenic lines and the control were cultivated in the field in the vicinity of Wrocław (in the 2010 season). After harvesting 4-month-old plants, the straw was retted for 3 weeks using the dew method and then processed to obtain the fiber used for further analysis.

Cellulose content analysis

Cellulose amount was measured with a colorimetric method employing anthrone reagent. To remove all contaminants for further analyzing, the samples were incubated for 1 h at 100 °C in a mixture of HNO_3 and AcOH (1:8 v/v). After this the samples were centrifuged, the supernatant discarded, the pellet washed twice with distilled water and then dissolved in 67% H_2SO_4 (v/v). Cooled anthrone reagent was added to mixed samples. The cellulose content was measured spectrophotometrically at 620 nm [41].

Lignin content measurement

Total lignin content was measured by the 'acetyl-bromide' method. Briefly, dried and ground into powder tissue samples were heated for 1 h at 65 °C and next filtrated through GF/A filters washed several times with different organic solvents. The samples prepared in such a way were dried for 12 h and then, after adding 25% acetyl-bromide in AcOH, they were incubated for 2 h at 50 °C and further dissolved in 10 ml of 2 N NaOH mixed with 12 ml AcOH. After incubating the samples for at least 12 h at room temperature lignin content was measured spectrophotometrically at 280 nm. The results were given as an equivalent of coniferyl alcohol, for which calibration curve had been made [42].

Determination of pectin content

The measurements were conducted in three steps. Initially, the contamination from tissues was removed by extracting the samples in the following way, with 96% EtOH at 100 °C, 80% EtOH at 80 °C, chloroform:methanol solution (1:1 v/v) and then acetone at room temperature. After drying, the samples were hydrolyzed in an ice bath with concentrated H_2SO_4. Dilluted with water and centrifuged, the supernatant containing pectin was collected to new tubes and further the amount of pectin was determined spectrophotometrically by biphenyl method.

The hydrolyzate was supported in turn with 4M sulfamic acid potassium sulfonate solution, pH = 1.6; $Na_2B_4O_7$ in H_2SO_4, than incubated for 20 min at 100 °C. Finally, m-hydroxybiphenyl was