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Research article

Variability of seed traits and properties of soluble mucilages in lines of the flax genetic collection of Vavilov Institute

A. Pavlov [a], F. Paynel [b], C. Rihouey [b], E. Porokhovinova [a], N. Brutch [a], C. Morvan [b,*]

[a] Department of Oil and Fiber Crops, N.I.Vavilov Research Institute of Plant Industry, 190000, B. Morskaya, 42, Saint-Petersburg, Russia
[b] University of Rouen, Chemistry Laboratory PBS, UMR 6270, CNRS, FR 3038, 76821 Mont-Saint-Aignan, France

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ABSTRACT

Upon hydration, flax seeds secrete mucilages whose content and physico-chemical properties vary according to the genotype and environment. The aim of the work was to investigate the complex genetic relationships between the vegetative period, colour, size and production of seed, the composition (polysaccharides and proteins) and physico-chemical properties of soluble mucilages collected at 28 °C from seeds of 18 lines grown in St Petersburg area. The vegetative period duration was found to impact the size and production of seeds, the yield of mucilages, including the polysaccharides, and the galactosidase enzymes, as well as their composition (mainly the rhamnogalacturonan I moieties) and some of their properties (mainly viscosity). Data allowed to significantly distinguish 6 fibre lines with mucilages enriched in rhamnogalacturonan I, 6 lines with mucilages enriched in arabinoxylan including 5 linseeds and 1 mutated fibre-line, and 5 lines with mucilages enriched in homogalacturonan-like polymer including 4 fibre lines and 1 brown linseed. Seven fibre lines had mucilages particularly rich in galactose. High to very high variability was found for 14 traits. Relatively independent characters (form/shape, protein and galactosidase) were identified and could be combined by breeding, with a focus on mucilage yield, composition and properties. Main-component analyses of line characters showed a large diversity in linseeds mainly due to their different origin but small variation in Russian fibre lines with brown seeds.

1. Introduction

Flax (*Linum usitatissimum* L.) which belongs to the family Linaceae is a commercial crop grown for the production of i) cellulosic fibres, manufactured in textile and composite industries and ii) linseed oil, used in varnishes and paints. In addition to these technical applications, the benefits of flax seeds for human and animal nutrition have been abundantly documented (e.g. Ganorkar and Jain, 2013). Proteins (e.g. Oomah and Mazza, 1993; Rabetafika et al., 2011; Singer et al., 2011), lignans (Hyvärinen et al., 2006; Touré and Xueming, 2010) together with mucilages (soluble fibres) have been traditionally used for medical purposes to reduce gastritis, blood cholesterol, atherosclerosis, diabetes, nephritis, and/or hormone-dependent cancers (e.g. Rubilar et al., 2010; Wanasundara and Shahidi, 1998). The possible biological activity of peptides has also been investigated (Marambe et al., 2008). The emulsifying and foaming properties of proteins were shown to be enhanced by the presence of mucilages (Dev and Quensel, 1986; Mazza and Biliaderis, 1989; Susheelamma, 1989). Mucilages were also used as stabilizer or texture ingredients in food (Anttila et al., 2008).

According to the flax literature, flax-seed mucilages represent a few to ~10% of the seed mass. They can be purified into neutral and acidic polymers (for a review see Naran et al., 2008). Neutral polymers consist of arabinoxylans (AX) of high molar mass (Warrand et al., 2005). They are built of typical β-D-xylan backbones, with t-Ara residues attached to both the O-2 and O-3 of some xylose residues; AX may be weakly acidic, owing to t-GlcA residues also attached to the xylan chain (Naran et al., 2008). Several populations of AX-rich polymers have been distinguished according to their molecular size which might have similar Ara/Xyl ratio but vary in the apparent degree of substitution with additional Gal and Fuc side-chain residues (Warrand et al., 2005). Acidic polymers mainly consist of rhamnogalacturonan I (RGI) i.e. a backbone made of repeating dimers of →2)-α-L-rhamnose-(1→4)-β-D-galactosyluronic acid-(1 →, substituted by the rare sugar L-Gal

* Corresponding author. Tel.: +33 2 35 14 66 94; fax: +33 2 35 14 67 04.
E-mail addresses: a.pavlov@vir.nw.ru (A. Pavlov), Florence.paynel@univ-rouen.fr (F. Paynel), christophe.rihouey@univ-rouen.fr (C. Rihouey), e.porohovinova@gmail.com (E. Porokhovinova), n.brutch@vir.nw.ru (N. Brutch), claudine.morvan@univ-rouen.fr (C. Morvan).

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(Anderson, 1933) and L-Fuc residues attached to the O-3 position of rhamnose instead of the O-4 position (Naran et al., 2008). The ratio of Gal to Rha was specifically determined in a commercial yellow variety to be in the range of 0.54–0.81 (Warrand et al., 2003). Mucilages collected at moderate temperatures were particularly enriched in GalA (Fedeniuk and Biliaderis, 1994; Wannerberger et al., 1991) and some homogalacturonan-like structures (named HG) were identified (Paynel et al., 2013; Qian et al., 2012a).

With the aim to enhance the value of mucilages by breeding, several papers have dealt with the genotype influence on mucilage content, composition and/or rheological properties (Bhatty, 1993; Cui et al., 1996; Diederichsen et al., 2006; Fedeniuk and Biliaderis, 1994; Oomah et al., 1995; Wannerberger et al., 1991). Measuring the mucilage viscosity as soon as it was extracted (parameter named MIV for mucilage indicator value), Diederichsen et al. (2006) observed a large variability for MIV within the world flax seed collection, but no correlation could be evidenced with the mass or colour of seeds, nor with the geographic region of origin of the accession. On the other hand, Oomah et al. (1995) studying 109 accessions, reported a high variability of Rha/Xyl ratio. In a subsequent paper, Cui et al. (1996) pointed out that some yellow linseeds produced viscous AX enriched mucilages, while brown seeds might produce AX enriched mucilages with low viscosity properties. The difficulty to get a clear idea of a possible relation between composition and rheological properties of mucilage might originate from the high temperature used for the extraction of mucilages, leading to a mixture of soluble mucilages and mucilages more tightly linked with the epidermis cell-walls (Naran et al., 2008; Paynel et al., 2013). Indeed, Fedeniuk and Biliaderis (1994) clearly indicated that mucilages extracted either at 4 °C or at 80 °C were characterized by a high percentage of either GalA or Xyl. After purification on anion exchange chromatography of the former (4 °C extracted) mucilage, the neutral polysaccharide was shown to have a higher intrinsic viscosity than its acidic counterpart. When extracted at moderate temperature from 5 lines, soluble mucilages – without any further fractionation – were also shown to exhibit increasing viscosity with increasing xylose residues (Wannerberger et al., 1991).

For better understanding of the variability of biochemical data and evaluation of interactions between different characters we needed to use wide scale of flax genetic diversity. Since the beginning of the 20th century, Vavilov Institute in St Petersburg has been involved in the development and management of plant genetic resources. Among them, flax accounted for six thousands of accessions from all over the world. The objectives of the present study were (i) to investigate the genetic variability of mucilage composition and properties in 18 lines of flax selected under St Petersburg growth-conditions and (ii) to consider the mucilage features in relation to vegetative period, seed weight, plant height, colours and shapes of flowers and seeds. Following Fedeniuk and Biliaderis (1994) and Paynel et al. (2013) working at 4 °C or moderate temperature, we collected soluble mucilages at temperatures < 30 °C. Two characters, important for breeding, vegetative period and seed production, were considered for the line choice, in addition to the colour of seeds (and flower) and the plant use (oil or fibres). Colours of the flowers were noted as the trait which can help to determine the degree of purity of the seeds and they can also play a part in selection (Dubois et al., 1979). To our knowledge, no data are available concerning the impact of vegetative period (Vp), size of seeds, colours and shapes of flowers on the composition and properties of soluble mucilages. In this paper, we focused our study on the composition (in term of polysaccharides and proteins) and physico-chemical properties (molar mass and viscosity) of soluble early-released mucilages.

Thus, the data dealt specifically on soluble mucilages naturally extruded in water at moderate temperature. These mucilages are mainly located in the cell junctions and the outer tangential wall of the epidermial cells (Attoumbré et al., 2011) and on seed hydration, they adsorb water and diffuse to form a gel-like capsule around the seed. Over longer hydration time, the outer tangential cell-wall would be partly disrupted/hydrolysed and cell-wall cross-linked mucilages would be released (Naran et al., 2008). The former soluble mucilages are thought i) to help in dispersal by sticking to animals, and in the adhesion to soil, ii) to facilitate the seed hydration or to resist desiccation, iii) to attract microorganisms in the rhizosphere (Western, 2012; Yang et al., 2012a). Together with the lately released mucilages, they provide a nutrient reserve-material during germination (Yang et al., 2012). On the other hand, expanded mucilages prevent the seed burying into deep soil levels, interacting with fine soil particles of upper soil levels, where they can easily germinate. Such ecological/biological/agronomical significance of our results will be approached in the discussion.

2. Materials and methods

2.1. Plant material

A set of 18 lines was selected from accessions of Vavilov Institute collection (Russia) of flax genetic resources having different origin (Table 1). Self-pollination of individual plants with purity control was made for 6 generations. After this, the lines were multiplied and some lines passed through the genetic analyses of flower and seed colours (Brutch et al., 2001; Brutch and Porokhovinova, 2005; Brutch et al., 2005). These lines were also characterized by their differences in vegetative period (**Vp**) characterizing line earliness (**Vp** = 70–74 d) to lateness (**Vp** = 89–91d), and in use (fibre or oil; the corresponding lines being designated either as fibre line or linseed, respectively).

Ten lines have red-brown seeds, including 6 so-called “wild” type fibre-lines. One of these lines has chlorophyll mutation, two have dilution of their petal colour and one is of linseed type. Three lines have yellow seeds under different genetic control. The gc103 line has gene *s1* which inhibits all anthocyan pigmentation, and has pleiotropic effects from star shaped white flower, to yellow anthers and seeds. The gc159 line has dominant *YSED1* gene controlling yellow seeds. The third gc173 line has recessive *ysed2* gene, not allelic to *YSED1*. Five lines have modified brown seeds. The gc65 line has gene *ora1* which controls small yellow speckles on brown seeds and orange anthers. The gc124 line has gene f^e which controls yellow spot on red-brown seed. Lines gc141 and gc143 have two alleles *pf1* and $pf1\text{-}a^d$ respectively which control pink flower with orange anther and yellow hue of seeds. The allele *pf1* controls only dark yellow brown seeds while $pf1\text{-}a^d$ controls different yellow hues of seeds from yellow to dark yellow brown. The line gc176 is an inbred line from the hybrid gc-141 × gc-103 which has genes *s1* and *pf1* from parents' lines (Porokhovinova, 2011).

2.2. Plant growth conditions

Lines were sawn in May 2005, in field conditions, on plots of 1 m^2 in Leningrad region, Russia. Leningrad region is situated in latitude 60 North, near Baltic Sea. In June, the time of day light reach 20 h. During the vegetative period, from May to August, the average temperature was 14 °C, general precipitation was 350 mm. Plants were harvested between the end of July and end of August, after 70–91 days of vegetative period, when the seeds were mature, according to their earliness. Soils of this region are brown podzolic, light-adobe, and humus content is 3–4% with a pH in the range of 5.5–6.0 (Dimo, 1976).