Comparative Analysis of Polysaccharides from Two Ecological Types of *Leymus chinensis*

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Abstract *Leymus chinensis*(*Trin.*) Tzvel., widely distributed at eastern Eurasian steppe and divided into gray-green type and yellow-green type, has different stress resistance to environment. In the present study, the water-soluble polysaccharides from two ecotypes of *L. chinensis* were analyzed in detail, and the differences between polysaccharides from the two ecotypes of *L. chinensis* in the yield, monosaccharide composition, molecular weight and structure were clarified. The polysaccharides of *L. chinensis* were composed of both neutral and acidic polysaccharides. The neutral polysaccharides contained mannose, glucose, galactose, xylose and arabinose, and mainly consisted of *β*-1,4-Glc*p*, *α*-1,3-Gal*p* and *α*-1,2-Xyl*p* residues. The acidic polysaccharides contained mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, xylose and arabinose. However, the yields, monosaccharides contents and the molecular weights of the polysaccharides from the two ecotypes of *L. chinensis* were different. Moreover, the resistance type(gray-green type) of *L. chinensis* contained a number of *α*-1,3-Man*p* and reducing end of *β*-Glc*p* residues, and much more *O*-methyl groups than normal type(yellow-green type) of *L. chinensis*. The differences of the polysaccharides of the two ecotypes of *L. chinensis* might be due to the long-term environmental adaptability of plant, and the differences of the polysaccharides might influence the stress resistance of *L. chinensis*.

Keywords *Leymus chinensis*; Polysaccharide; Fractionation
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1 Introduction

Leymus chinensis(*Trin.*) Tzvel., a perennial rhizomatous C3 grass, is widely distributed at Songnen Plain and the eastern part of the Mongolian Plateau. There are two ecotypes of *L. chinensis* according to leaf color, including the gray green(GG) type and the yellow green(YG) type. The GG type of *L. chinensis* is distributed in an extensive range of environmental conditions, including forest brown soil, chernozem soil, meadow and harsh saline soil, where soil pH value can be higher than 12. While the YG type of *L. chinensis* can be only found at chestnut soil and mild saline meadow[1]. Therefore, researches on the intra-specific differences between the two types of *L. chinensis* have received much attention, such as geographic demography, gas exchange and genetic diversity, anatomical and physiological divergences and compensatory effects[1—6].

In recent years, more and more studies have revealed that the polysaccharides play an important role in the growth of plants[7,8]. However, to our knowledge, there has been no study on the polysaccharide divergences between the two ecotypes of *L. chinensis* in detail. Here we respectively analyzed the water-soluble polysaccharides from GG type and YG type of *L. chinensis*, and clarified the differences between polysaccharides from the two ecotypes of *L. chinensis* in the yield, monosaccharide composition, molecular weight and structure.

2 Experimental

2.1 Materials

The GG type and YG type of *L. chinensis* were collected from the Songnen Grassland Ecological System Research Station of Northeast Normal University(China), and dried at 40 °C in a drying oven to stable mass. The diethylaminoethyl (DEAE)-cellulose was purchased from Shanghai Hengxin Company(China). All the other chemicals were of analytical grade.

2.2 Extraction and Fractionation of Polysaccharides from *L. chinensis*

Dried material(1 kg) was ground, and then extracted with water(95 °C, mass ratio 1:15) three times(6 h each time). The

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aqueous filtrates were combined and concentrated, subsequently 95% ethanol was added to the aqueous filtrates up to 80% to precipitate the polysaccharides which were collected by centrifugation and dried in vacuum. The precipitate was dissolved in water(5%, mass fraction) and the insoluble substances were removed by centrifugation. The supernatant was treated with Sevag reagent[*V*(*n*-butanol):*V*(chloroform)= 1:4] to remove free proteins. After removing the remaining Sevag reagent by vacuum evaporation, the water phase was freeze-dried, giving rise to water-soluble *L. chinensis* polysaccharide(WLP). WLP was dissolved in water(10%, mass fraction) and further fractionated by DEAE-Cellulose ion-exchange chromatography, eluted with distilled water and 0.5 mol/L NaCl in turn, generating two fractions: WLP-N and WLP-A. Finally, the water-soluble polysaccharides were fractionated into 2 fractions of either material: the fractions G-WLP-N(yield 6.7%) and G-WLP-A(yield 11.1%) were from GG type of *L. chinensis*; the fractions Y-WLP-N(yield 5.9%) and Y-WLP-A (yield 4.0%) were from YG type of *L. chinensis*. The procedure for the extraction and fractionation of polysaccharides from *L. chinensis* is shown in Fig.1.

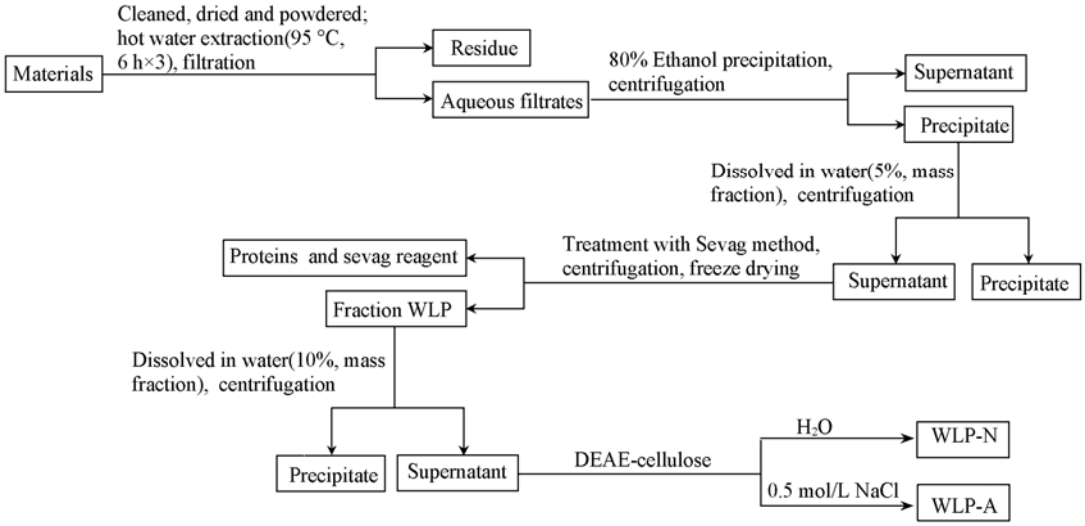

Fig.1 Extraction and fractionation procedures of polysaccharides from *Leymus chinensis*

2.3 General Methods

Total carbohydrate content was determined by phenol-H_2SO_4 method with monosaccharide mixture(according to the sugar composition) as standard[9]. All gel filtration chromatography tests were monitored by assaying carbohydrate content. Uronic acid content was determined by *m*-hydroxydiphenyl colorimetric method with galacturonic acid as standard[10]. Protein content was determined by the method of Sedmak and Grossberg[11], with Coomassie brilliant blue reagent with bovine serum albumin as standard. High performance gel permeation chromatography UV-Vis absorbance spectra were recorded with a UV-Vis spectrophotometer(Model SP-752, Shanghai Spectrum Instruments Co. Ltd., China). HPLC(high performance liquid chromatography) was carried out on a Shimadzu 10Avp HPLC system with a 10Avp HPLC Pump and an SPD-10Avp UV-Vis detector.

2.4 Determination of Homogeneity and Molecular Weight

Determinations of homogeneity and molecular weight were carried out on an high-performance size-exclusion chromatography(HPSEC)-linked gel filtration column of TSK-G3000 PWXL eluted with 0.2 mol/L NaCl at a flow rate of 0.67 mL/min at (35.0±0.1) °C. The gel filtration column was calibrated with standard dextrans(50000, 25000, 12000, 5000 and 1000) *via* linear regression. Sample concentration was 5 mg/mL and injection amount was 20 μL.

2.5 Determination of Monosaccharide Component

The monosaccharide component analysis was performed by the HPLC method as described by Honda *et al*.[12]. Briefly, the sample(2 mg) was first methanolyzed in anhydrous methanol(0.5 mL) containing 2 mol/L HCl at 80 °C for 16 h. Then the methanolyzed products were hydrolyzed in 2 mol/L CF_3COOH(0.5 mL) at 120 °C for 1 h. The hydrolyzed-products, monosaccharides, were derivatized to be 1-phenyl-3-methyl-5-pyrazolone(PMP) derivatives and subsequently analyzed by HPLC on a Shim-pak VP-ODS column(150 mm×4.6 mm i.d.) with a guard column on a Shimadzu HPLC system and monitored by UV absorbance at 245 nm.

2.6 NMR Analysis

The ^{13}C NMR spectra were obtained on a Bruker AV600 spectrometer at 600 MHz. The samples(20 mg) were dissolved in D_2O(1 mL, 99.8%) with overnight stirring at room temperature. The spectra were recorded at 25 °C after 57000 scans.

3 Results and Discussion

3.1 Isolation and Fractionation of Water-soluble Polysaccharides from *L. chinensis*

WLP were obtained from GG type and YG type of *L.*

chinensis by hot water extraction and Sevag reagent deproteination. After separating the neutral and acidic polysaccharides by DEAE-cellulose ion-exchange chromatography, the water-soluble polysaccharides were finally fractionated into 2 fractions of either material: the fractions G-WLP-N and G-WLP-A were from GG type; the fractions Y-WLP-N and Y-WLP-A were from YG type of *L. chinensis*.

The total carbohydrate, uronic acid, protein contents and yields of each polysaccharide fraction are listed in Table 1. It appeared that the water-soluble polysaccharides accounted for 10.2% of GG type of *L. chinensis*, dry mass and 13.3% of YG type of *L. chinensis,* dry mass. Owing to the process of removing protein by Sevag method, the protein contents of polysaccharide fractions were all below 0.7%, while the polysaccharide and uronic acid contents in resistance type(GG type) of *L. chinensis* were markedly less than those in normal type(YG type) of *L. chinensis*.

Table 1 Yield, total carbohydrate, uronic acid and protein contents of each polysaccharide fraction from two types of *L. chinensis*

Fraction	Yield*(%)	Total carbohydrate Content (mass fraction, %)	Uronic acid Content (mass fraction, %)	Protein Content (mass fraction, %)
G-WLP	10.2	91.2	7.7	0.7
G-WLP-N	6.7	99.8	0.0	0.2
G-WLP-A	1.1	99.7	53.5	0.3
Y-WLP	13.3	90.7	16.9	0.5
Y-WLP-N	5.9	99.8	0.0	0.1
Y-WLP-A	4.0	99.8	42.2	0.2

* Yield calculated based on dried material mass.

3.2 Characterization of Water-soluble Polysaccharides from *L. chinensis*

To further analyze the relationship between stress resistance and polysaccharide of *L. chinensis*, the monosaccharide components were studied by PMP-derivation method. As shown in Table 2 and Fig.2, there were significant differences in the contents of mannose, glucuronic acid, galacturonic acid and glucose between polysaccharides from two ecotypes of *L. chinensis*. The mannose content of neutral polysaccharide and galacturonic acid content of acidic polysaccharide in GG type of *L. chinensis* were more than those in YG type of *L. chinensis,* while the glucose content of neutral polysaccharide and the glucuronic acid content of acidic polysaccharide in GG type of *L. chinensis* were less than those in YG type of *L. chinensis*.

Table 2 Monosaccharide compositions of each polysaccharide fraction from two types of *L. chinensis*

Fraction	Monosaccharide composition[a](mass fraction, %)							
	Mannose	Rhamnose	Glucuronic acid	Glucturonic acid	Glucose	Galactose	Xylose	Arabinose
G-WLP-N	**13.8**[b]	—	—	—	**44.4**[b]	16.7	9.0	16.1
G-WLP-A	**4.2**[b]	7.4	**2.4**[b]	**49.3**[b]	**11.9**[b]	7.3	7.8	9.7
Y-WLP-N	**6.4**[b]	—	—	—	**54.5**[b]	16.2	9.1	13.8
Y-WLP-A	—	7.2	**5.1**[b]	**32.8**[b]	**30.1**[b]	7.5	8.6	8.6

a. Sugar-PMP derivatives obtained after methanolysis combined with CF_3COOH hydrolysis followed by PMP precolumn derivation and analysed by HPLC; *b.* bold numbers represent significant differences.

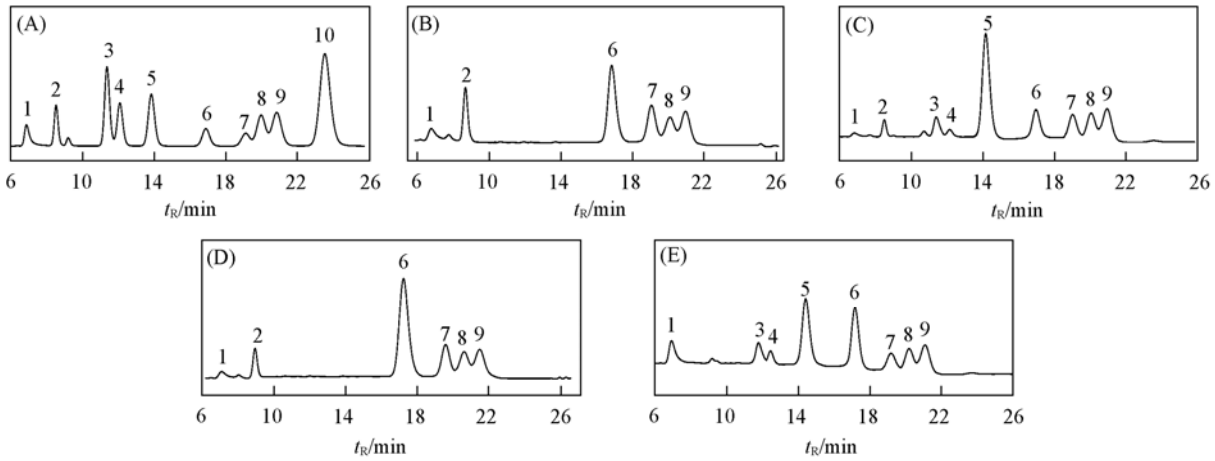

Fig.2 Monosaccharide composition analysis of polysaccharide fractions from *L. chinensis* by HPLC

(A) Standard monosaccharides; (B) fraction G-WLP-N; (C) fraction G-WLP-A; (D) fraction Y-WLP-N; (E) fraction Y-WLP-A. 1. PMP; 2. mannose; 3. rhamnose; 4. glucuronic acid; 5. galacturonic acid; 6. glucose; 7. galactose; 8. xylose; 9. arabinose; 10. fucose.

HPSEC(Fig.3) showed that the molecular weight distribution of G-WLP-N and Y-WLP-N were homogeneous and M_w were estimated to be 1.8×10^3(M_w/M_n=1.4) and 1.5×10^3 (M_w/M_n=1.2, respeclively). While, G-WLP-A and Y-WLP-A exhibited wide molecular distributions on gel filtration column of TSK-G3000 PWXL, indicating they were heterogeneous.

The structural features of G-WLP-N and Y-WLP-N were further analyzed by ^{13}C NMR(Fig.4), with the assignments of carbon atom signals listed in Table 3. According to NMR data in the literature[13—19], the anomeric carbon signals at δ 103.13 to 103.11 corresponded to the C1 of β-Glc*p*, the signal at δ 97.01 corresponded to the C1 of β-Glc*p* in the reducing end, the signals at δ 102.63 and 102.59 were assigned to the C1 of α-Gal*p*, the signals at δ 102.42 and 102.32 were assigned to the

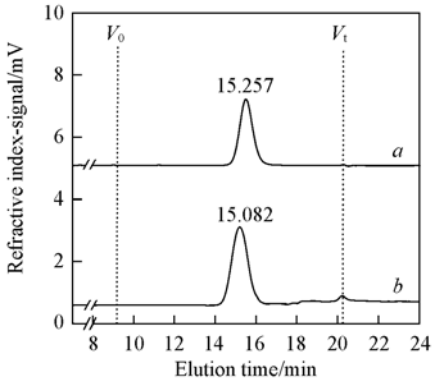

Fig.3 HPSEC elution profile of polysaccharide fractions from *L. chinensis*

a. Y-WLP-N from yellow-green type; *b*. G-WLP-N from gray-green type; V_0, void volume; V_t, total volume.

C1 of *α*-Xyl*p* and the signal at *δ* 100.75 was assigned to the C1 of *α*-Man*p*. The signals at *δ* 58.89—58.99 indicated the presence of *O*-methyl groups. Signal at *δ* 79.98 was the typical one of the C4 of *β*-1,4-Glc*p*. The signals at *δ* 80.10 and 80.03 were attributed to the C3 of *α*-1,3-Gal*p*. The signals at *δ* 79.23 and 79.22 were attributed to the C-2 of *α*-1,2-Xyl*p*. The signal at *δ* 75.89 was attributed to the C-3 of *α*-1,3-Man*p*.

The ^{13}C NMR data(Table 3) reveal that neutral polysaccharides from two ecotypes of *L. chinensis* were both mainly composed of *β*-1,4-Glc*p*, *α*-1,3-Gal*p* and *α*-1,2-Xyl*p* residues. GG type of *L. chinensis* contains a number of *α*-1,3-Man*p* and the reducing end of *β*-Glc*p* residues, and much more *O*-methyl groups than YG type. The absence of arabinose residues in ^{13}C NMR spectra shows that arabinose residues may be the position in the interior of polysaccharide molecules. Moreover, the number of *O*-methyl groups decreased with the decrease of

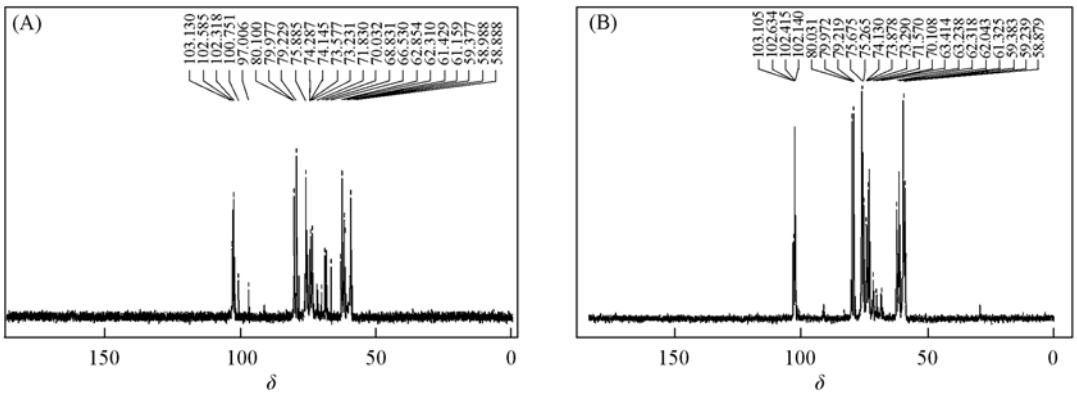

Fig.4 ^{13}C NMR spectra of G-WLP-N(A) and Y-WLP-N(B)

Table 3 ^{13}C NMR chemical shifts of neutral polysaccharides from *L. chinensis* in D_2O

Fraction	Residue	*δ*					
		C1	C2	C3	C4	C5	C6
G-WLP-N	→4)-*β*-Glc*p*-(1→	**103.13**^{a}	73.58	75.89^b	**79.98**^{a,b}	75.89^b	61.43
	→3)-*α*-Gal*p*-(1→	**102.59**^{a}	71.63^b	**80.10**^{a}	70.03^b	73.23	62.31
	→2)-*α*-Xyl*p*-(1→	**102.32**^{a}	**79.23**^{a}	74.29	70.03^b	62.85	
	→3)-*α*-Man*p*-(1→	**100.75**^{a}	70.02^b	**75.89**^{a,b}	68.63	71.63^b	59.38
	→4)-*β*-Glc*p*	97.01	74.29	75.69^b	**79.98**^{a,b}	75.69^b	61.16
Y-WLP-N	→4)-*β*-Glc*p*-(1→	**103.11**^{a}	73.88	75.68^b	**79.97**^{a}	75.68^b	61.33
	→3)-*α*-Gal*p*-(1→	**102.63**^{a}	71.57	**80.03**^{a}	70.11^b	73.29	62.32^b
	→2)-*α*-Xyl*p*-(1→	**102.42**^{a}	**79.22**^{a}	74.13	70.11^b	62.32^b	

a. Bold numbers represent glycosylation sites; *b*. these values may have to be interchanged.

the number of arabinose and mannose residues, so partial arabinose and mannose residues were *O*-methylated.

Summarizing the above results, polysaccharides from two ecotypes of *L. chinensis* are composed of neutral and acidic polysaccharides. The neutral polysaccharides both contain mannose, glucose, galactose, xylose and arabinose, mainly consist of *β*-1,4-Glc*p*, *α*-1,3-Gal*p* and *α*-1,2-Xyl*p* residues. The acidic polysaccharides both contain mannose, rhamnose, glucose, galactose, glucuronic acid, galacturonic acid, xylose and arabinose. However, there are many differences between the GG type of *L. chinensis* and the YG type of *L. chinensis* in the yield, monosaccharide composition, molecular weight and structure of polysaccharides. The contents of mannose and galacturonic acid in GG type of *L. chinensis* were higher than those in YG type of *L. chinensis* while the yield of polysaccharide and the content of glucose and glucuronic acid in GG type of *L. chinensis* were less than those in YG type of *L. chinensis*. The molecular weight of neutral polysaccharide in GG type of *L. chinensis* was higher than that in YG type of *L. chinensis*. Moreover, GG type of *L. chinensis* contains a number of *α*-1,3-Man*p* and the reducing end of *β*-Glc*p* residues, and much more *O*-methyl groups than YG type of *L. chinensis*. So, we deduced that the polysaccharide divergences between the two ecotypes of *L. chinensis* might be relevant to their different stress resistances to environment, in terms of the polysaccharide content, monosaccharide composition, molecular weight and structure feature. The differences between the two ecotypes of *L. chinensis* in polysaccharides might be due to the long-term environmental adaptability of plant.

4 Conclusions

In the present study, the water-soluble polysaccharides of two ecotypes *L. chinensis* were analyzed in detail for the first time. The results revealed that the two ecotypes *L. chinensis* polysaccharides are different on yield, monosaccharide composition, molecular weight and structure feature, and the polysaccharide divergences between them might be relevant to their different stress resistances to environment.

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