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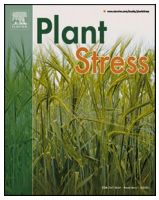

Cell wall composition and nutritional quality through seasons of the saltgrass *Distichlis laxiflora* growing in halophytic and mesophytic meadows

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ABSTRACT

The genus *Distichlis* comprises halophytic perennial C4 grasses which grow in very extreme conditions. It is distributed throughout almost the entire American continent. Despite these interesting characteristics, studies on these plants are relatively scarce, and their ecological and productive value deserves to be further explored. In this work, nutritional aspects, and composition of the cell walls of the aerial part (leaves and shoots) of *D. laxiflora* Hack were studied. Their changes over four samplings in different seasons, in two nearby sites: a halophytic meadow (H), and a mesophytic meadow (M), were analyzed. Photosynthetic efficiency and K/Na ratio were determined as stress indicators. Plants of sites H and M have similar cell wall content except for spring when plants from site H show lower values. Also, the spring samples present a markedly higher *in vitro* digestibility for site H. In agreement, samples from site H contain lower amounts of glucuronoarabinoxylans and β-glucans. The protein content is lower in site H, except for spring, and with a maximum in summer for site M. This work shows some changes in the cell walls of *D. laxiflora* in response to salinity, and these changes influence the digestibility of this material. Spring, coinciding with the regrowth cycle of this plant and with the worst stress indicators, is the season when plants of the halophytic site show the highest nutritional quality for grazing cattle.

1. Introduction

The Salado River Basin, in Argentina, is an extensive plain, where 60 % of the soils are affected by salinization with alternating periods of flooding and drought. This creates a heterogeneous ecosystem, with the preponderance of native grasslands (Cid et al., 2011). Soils in the experimental areas considered in this work are Natraquoll type, with poor organic matter and a natric B2t horizon (Lavado and Taboada, 1988). The impaired drainage, flooding, and dry pulses characteristic of this type of soil, result in the concentration of dissolved ions, such as Na^{+}, Ca^{2+}, Mg^{2+}, K^{+}, Cl^{-}, $SO_4^{=}$, HCO_3^{-}, $CO_3^{=}$ and NO_3^{-}, that limit the species able to grow on these environments (Cid et al., 2011; Paz et al., 2012). These conditions strongly restrict the development of large-scale agricultural activity, which is why most of this region is not cultivated,

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maintaining its natural or semi-natural vegetation used for the breeding of cattle (Nieva and Ruiz, 2020). In these environments, grasslands are characterized by the predominance of grasses and a high deficiency of legumes. Distichlis species are often predominant among the former, particularly in alkaline soils, while in non-alkaline soils the diversity of grasses is somewhat greater (Debelis et al., 2005; Cid et al., 2011). D. scoparia is among the main warm-season species in this area, with its peak aerial productivity in summer (Sala et al., 1981). Sustainable management strategies are needed for this type of environment, not only due to their economic importance but also to their potential as carbon sinks.

The genus *Distichlis* comprises perennial rhizomatous C4 grasses with the ability to thrive in conditions where few plants can survive. Some aspects of the adaptation of these plants to salinity are well known, like salt excretion by glands (Morris et al., 2019), proline accumulation (Ketchum et al., 1991), and stomatal responses (Kemp and Cunningham, 1981). But their cell walls and the possible relationship between their composition, and their halophytic character, have not been explored. Regarding the species studied in this work, *D. laxiflora,* there is even less information available. On the other hand, the characteristics of the cell wall and nutritional quality are closely related, given that this structure represents a high proportion of the plant biomass. The nutritional quality of *Distichlis* species has been considered between poor (Sala et al., 1981) and intermediate (Bustan et al., 2005; Brizuela et al., 1990). It has been highlighted that, unlike halophytes such as *Atriplex* or other tolerant grasses, *Distichlis* does not accumulate large amounts of salts in its leaves (Bustan et al., 2005). Thus, their contribution to marginal environments should not be underestimated. Besides, its peculiarities have led to its investigation also for soil remediation (Sabzalian et al., 2018) and treatment of aquaculture effluents (Lymbery et al., 2013), being the most studied species *D. spicata, D. palmeri,* and *D. scoparia*.

Regarding the cell wall composition, grass is expected to have primary cell walls of type II, with hemicellulosic polysaccharides, such as glucuronoarabinoxylans (GAX) and mixed linked glucans, and small amounts of pectins. Cellulose and phenolic compounds are also important components in these structures. These cell walls prevail in young and dynamic tissues. In addition, conduction vessels, fibers, and other tissues have secondary cell walls, which develop between the primary cell wall and the plasma membrane, characterized by xylans, mannans, cellulose of higher crystallinity, and, in most cases, important amounts of lignin. It is worth considering, that when the aerial part of a plant is analyzed, a mixture of different cell walls is involved (Albersheim et al., 2011). The present work proposes a first characterization of the cell wall material of D. laxiflora, both from a chemical and a nutritional approach, and its changes throughout the seasons. The objective is to contribute to the understanding of the particularities of the cell walls of halophytic grasses, growing in mesophytic and halophytic meadows, as well as contributing to the sustainability of the Salado River Basin, the largest area devoted to cattle breeding in Argentina.

2. Materials and methods

2.1. Sampling area and plant material

Specimens of *Distichlis laxiflora* were collected on INTECH land, close to the Chascomús Lagoon (*Av*. Intendente Marino Km. 8.2, B7130 Chascomús, Province of Buenos Aires, Argentina). Two collection sites were defined, based on the contrasting characteristics of the soil, and the evident differences in vegetation present in each one: halophytic meadow or site H (35°37'27" S, 57°59"51″ W) and mesophytic meadow or site M (35°37'26" S, 57°59"50″ W). The determination of the species was made based on vegetative morphological characteristics, and it was also possible to observe flowering specimens that allowed confirming the determination. Morphological determination is based on the herbarium specimens JMA-807 deposited at Instituto de Botánica Darwinion, San Isidro, Buenos Aires, Argentina (SI; acronym follows Thiers, 2016). The collected plants were identified as *Distichlis laxiflora*. Although it resembles to the sympatric species *D. spicata* (L.) Greene and *D. scoparia* (Kunth) Arechav, both widely distributed in South America, *D. laxiflora* **(Suppl. Fig. 1 B-C)** is characterized by its leaf blades, less than 2 mm wide (in *D. spicata*, leaf blades are usually $>$ 3.5 mm wide), and by its leaf blades wavy and inflorescences, with more than 5 spikelets (in *D. scoparia,* leaf blades are straight and inflorescences have less than 5 spikelets). *Distichlis laxiflora* Hack. is an endemic species from Argentina, that inhabits halophytic and mesophytic meadows in the Pampean region. This species belongs to a genus of perennial grasses from temperate and subtropical zones of the American continent. The sampling dates were October 2019 (spring), April 2021 (autumn), August 2021 (winter) and February 2022 (summer). On each date, three different samples corresponding to the aerial part (leaves and shoots) were collected and used for the nutritional, and sodium and potassium content determinations (see below). For the chemical analysis of cell walls, the material from each site and date was treated as a pool.

2.2. Soil analysis

Electrical conductivity was measured (Procheck GS3, Decagon Dev. Inc.) on each sample site and date. In summer, soil samples were collected and analyzed: pH, sodium adsorption ratios (RAS), anions, and cations by a volumetric method (AcNH4 1 N pH7) and by atomic emission spectroscopy (Espasandin et al., 2018).

2.3. Photosynthetic activity through chlorophyll a fluorescence emission kinetic analysis

On each sampling date, *in situ* recording of the chlorophyll a fluorescence emission kinetic was conducted, and subsequent analysis of each recording was used to determine different OJIP parameters, as outlined in Gazquez et al. (2015). This was executed using a HANDY PEA fluorometer (Hansatech Instruments® Ltd., King's Lynn, Norfolk, UK) following the manufacturer's instructions. To induce adaptation to darkness, blade sections of intact leaves were covered with a leaf clip for 20 min. Subsequently, the blade sections were exposed to a 3-s pulse of red light (650 nm, 3500 μmol photons m^{-2} s^{-1}). The raw fluorescence data obtained from the fluorescence emission kinetic were processed utilizing the PEA plus software (Hansatech Instrument, UK) for the determination of OJIP parameters: maximum quantum efficiency of the PSII (F_V/F_M) and performance index based on equal absorption (PI_{Abs}). F_V/F_M represents the maximum efficiency with which an absorbed photon results in Q_A reduction, and it is also denominated maximum photochemical efficiency of PSII. PI_{Abs}, is a very sensitive index to different types of stress, which incorporates F_V/F_M and two other parameters: a structural one, that estimates the amount of chlorophyll that makes up the reaction centers, and a biochemical one that estimates the efficiency of moving electrons from PSII to the electron transport chain.

2.4. Measurement of sodium and potassium content

The sodium (Na) and potassium (K) content of plant biomass was determined following the method outlined by Campestre et al. (2011). The material was frozen in liquid nitrogen and then homogenized. Each homogenate (10 mg) was mixed with 1 mL HCl 0.1 N in an Eppendorf tube, thoroughly vortexed, and incubated for 2 h at 60 °C. Then, the mixture was cooled for 20 min at 20 °C and centrifuged (10,000 × *g*, 5 min). The pellet was discarded and an aliquot of 250 μL of the supernatant was diluted in 5 mL of distilled water. The resultant solution was used to determine sodium (Na) and potassium (K) content in a flame photometer (2655–00, Cole-Parmer Instrument Co., Chicago, IL).

2.5. Nutritional parameters

Samples were analyzed for dry matter (DM) (65 °C until constant

weight), ash (600 °C during 2 h) (AOAC, 1990; #942.05), crude protein (CP) (Kjeldhal AOAC 1990; #984.13), neutral detergent fiber (aNDF) with α-amylase, and acid detergent fiber (ADF) sequentially on the same sample (Van Soest et al., 1991), with an ANKOM® equipment (Model 220, ANKOM™ technology, Fairport, NY, USA). Lignin content was obtained by sulfuric acid treatment (LDA (sa)). In the text, plain abbreviations were used expressed as g/kg DM (CP, NDF, ADF, and Lignin).

2.6. In vitro gas production technique

To assess the *in vitro* cumulative gas production, fermentation kinetics, and digestibility, samples obtained from shoots and leaves were incubated in dark brown, 100 mL bottles with bromobutyl septa caps (20 mm diameter) and sealed with aluminum caps. Ruminal liquor (solid: liquid mass ratio 50:50) was collected before the morning feeding from two cannulated ewes, fed to maintenance with a standard diet alfalfa pellet:maize grain, 70:30. Incubation medium was prepared by mixing one part of ruminal liquor with 10 parts of carbonate-bicarbonate buffer. According to the method of Theodorou et al. (1994), modified by Wawrzkiewicz and Danelón (2004), duplicate samples were incubated for 48 h and 72 h at 39 °C. Pressure changes inside the incubation bottles were measured using a pressure transducer (T443A, Bailey and Mackey Ltd, Birmingham, England) connected with a three-way valve. At 2, 4, 6, 9, 12, 17, 20, 24, 30, 36, 48, and 72 h of incubation, pressure values were corrected by the amount of substrate DM incubated. Volume was then regressed on pressure records, to fit a linear regression model, to calculate the actual volume record for every bottle and time. Raw gas production data were corrected by their respective blanks to calculate net cumulative gas production (NCGP), and data were expressed per gram of incubated DM basis (mL/g DM). The fermentation was terminated after 48 h of incubation on two bottles of each sample, by adding 2–3 drops of a saturated thymol solution, as a first step to evaluate the *in vitro* dry matter digestibility (ivDMD). The remaining bottles continued in the incubating bath until 72 h. Each bottle content was filtered through fiber filter bags (ANKOM #F57) before being sealed. The ivDMD was calculated from the residues of the filter bags, after being treated with neutral detergent solution. Neutral detergent fiber digestibility (NDFD) was analyzed (Goering and Van Soest, 1970). True DMD at 48 and 72 h of incubation was therefore calculated as follows: *ivDMD (%)* = 100 - *NDF residue* x 100/DM incubated.

2.7. Preparation of the alcohol and hot water insoluble residues

Dried plant material (65 °C until constant weight), from the aerial part of the plants (shoots and leaves) was milled. The material was sequentially extracted with organic solvents for 1 h (~100 mg/mL, two times with alcohol, two times with acetone, once with ethyl ether) and finally dried to obtain the alcohol-insoluble residue (AIR). Then two extractions with H_2O for 3 h at 90 °C (~50 mg/mL) were carried out to obtain the hot water insoluble residue (HWIR) (Fry, 1988). The residue was separated from the supernatant by centrifugation, then washed and freeze-dried.

2.8. Chemical analyses of cell wall material

Total carbohydrate content of samples was analyzed by the phenol–sulfuric acid method (Dubois et al., 1956). Uronic acids content was determined colorimetrically (Filisetti-Cozzi and Carpita, 1991). For both determinations, adaptation for insoluble material was followed (Ahmed and Labavitch, 1978). After saponification, the phenolic content in the supernatant was estimated using the Folin-Ciocalteu technique (Shui and Leong, 2006). Gallic acid was used as standard, and results were expressed as gallic acid equivalents (GAE). To determine the composition of neutral monosaccharides using gas chromatography (GC), alditol acetates were obtained in triplicate by hydrolysis with TFA 13 M (37 °C, 1 h), followed by dilution of the acid to 11.5 M heating at 100 °C for 1 h, and further dilution to 2 M to achieve the regular hydrolysis conditions for insoluble polysaccharides (Morrison, 1988). The hydrolyzate was derivatized to the corresponding alditol acetates. GC of the derivatized samples was carried out on an Agilent 7890 A gas-liquid chromatograph (Avondale PA, USA) equipped with a flame ionization detector and fitted with a fused silica column (0.25 mm id × 30 m) WCOT-coated with a 0.20 μm film of SP-2330 (Supelco, Bellefonte PA, USA). Chromatography was performed as follows: (a) from 200 °C to 240 °C at 2 °C min^{-1} followed by a 15 min hold for alditol acetates. N_2 was used as the carrier gas at a flow rate of 1 ml min^{-1} and the split ratio was 25:1. The injector and detector temperature were 300 °C.

2.9. ATR-FTIR spectroscopy

Fourier Transform Infrared spectra were recorded from 4000 to 400 cm^{-1} with an iS50 Nicolet FT-IR spectrophotometer (Madison, WI, USA), equipped with a DTGS Attenuated Total Reflectance (ATR) detector. Thirty-two scans were taken with a resolution of 4 cm^{-1}. Spectra were processed using Spectragryph - optical spectroscopy software, version 1.2.15, 2020 http://www.effemm2.de/spectragryph/.

2.10. Statistical analysis

For photosynthetic, nutritional, yield, and chemical parameters, statistical significance ($p \leq 0.05$) was determined by unpaired Student's *t*-test using the Infostat statistical software package employed throughout the study. Principal components analysis (PCA) was performed on PAST software.

3. Results

3.1. Characterization of the sampling sites

The halophytic and mesophytic sampling sites are located in an area close to a highly eutrophic and very turbid shallow lake (Chascomús Lagoon) (Lagomarsino et al., 2015) **(Suppl. Fig. 1 A)**, being the halophytic meadow the lowest and closest to the lagoon. These sites were already characterized by Maguire et al. (2021). In accordance with their description, our results for soil samples (summer) showed a pH of 7.44 and 7.57, an electrical conductivity (EC) of 10.56 dS/m and 3.62 dS/m, a sodium adsorption ratio (SAR) of 13.14 meq/L and 7.25 meq/L for H and M, respectively **(Suppl. Table 1).**

3.2. Photosynthetic state of D. laxiflora plants

The differences described in 3.1 should condition the performance of plants that grow there. Photosynthetic activity, described through (F_V/F_M), remained mostly below optimal values (0.75–0.85) in both subareas, except during winter (Fig. 1A), when optimal F_V/F_M values were only recorded in subarea M, but not in H. Moreover, suboptimal values were lower in subarea H, particularly during spring and summer, compared to those recorded in M, except for autumn, when no differences were observed between subareas. The lower photosynthetic activity in H compared to M was also supported by the lower values of the photosynthetic performance index (PI_{ABS}) in all cases (Fig. 1B).

3.3. Ratio K/Na

The K/Na ratio is usually considered an indicator of salt tolerance (the higher the ratio, the greater the tolerance, since it implies the possibility of selectively retaining K^+ in a context of excess of Na^+). Excess of Na^+ in the environment can cause a deficiency of K^+ and a reduction of the K/Na ratio (Espasandin et al., 2018). In this case, regarding the K/Na ratio evaluated among sites, higher levels were found at the mesophytic site (M) in all four sampling dates (Fig. 2).

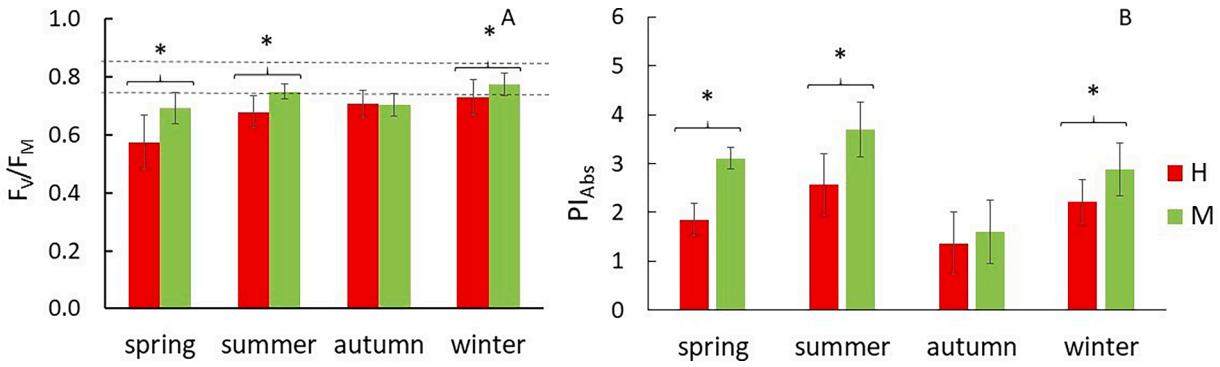

Fig. 1. Determination of the photosystem II state by the OJIP test technique for *D. laxiflora* growing at mesophytic (M) and halophytic (H) sites. The F_V/F_M (A) and the PI_{Abs} (B) were determined in leaf blades four times during the year. Dashed lines in (A) represent the maximum (0.85) and minimum (0.75) F_V/F_M optimum values. Asterisks represent significant differences between plants from M and H areas. Data represent mean ± *S*.E. (Student's *t*-test).

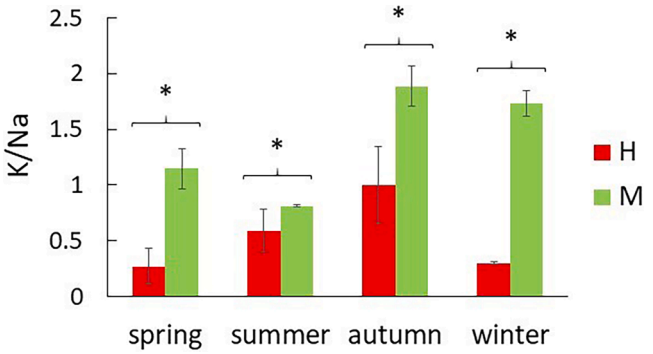

Fig. 2. K/Na molar ratio in aerial parts of *D. laxiflora* from the mesophytic meadow (M, green) and halophytic meadow (H, red). Asterisks represent significant differences between plants M and H areas. Data represent mean ± *S*.E. (Student's *t*-test).

Despite these differences, all the values obtained were well below the levels considered optimal (Kader et al., 2006). Similar results were reported before for *D. spicata*, with values between 0.38 and 0.69 for different genotypes (Marcum et al., 2007). A well-known mechanism to reduce the salt stress effects in plants consists in the exclusion of Na^+ or its compartmentalization in different organs. Plants also reduce the ionic phase of this stress by regulating toxic Na^+accumulation and enhancing K^+ uptake and accumulation, being the key factors for the survival of tolerant plants in saline environments (Munns and Tester 2008; Assaha et al. 2017). In places with high concentrations of Na^+, as the halophytic site analyzed in this work, homeostasis in plants can be severely affected. To alleviate in part this constraint, *Distichlis laxiflora* has the possibility to excrete Na^+ at leaves level (Bach Allen and Cunningham, 1983). Conversely, it has been suggested that this indicator may not be valid for halophytes, because compartmentalization within leaf cells can make the index at the tissue or organ level not reliable as an indicator (Dashtebani et al., 2014).

3.4. Nutritional characterization

The differences found in soil analysis in both marginal ecosystems described, and the functional photochemistry could affect the chemical composition of plant species, such as *Distichlis laxiflora*. Changes in chemical composition and specific changes in cell wall material may have important effects on nutrient availability for ruminants grazing in these environments.

Dry matter (DM) was variable throughout the seasons at both sites (Fig. 3A). In addition, significant differences between sites in spring and autumn were evident. Regarding aNDF, which represents most of the cell wall material, values were quite stable throughout the year, with a significant difference between sites in spring. Values of ADF were more variable, with the most marked differences in spring and winter (in both cases, again, with lower content of this fraction of the cell wall material in site H). In general, DM, ADF, and lignin showed a similar pattern regarding variations between sites and between seasons (Fig. 3B-D). Protein content (CP) remained within a narrower and lower range for H (58–74 g/kg DM), while for M the range was broader and higher (61–110 g/kg DM), with a clear maximum in summer (Fig. 3E).

3.5. In vitro digestibility

To evaluate feed digestibility, *in vitro* methods are usually preferred because they are easier, less time-consuming, and cheaper than those determined *in vivo* (Mc Donald et al., 2011). For this purpose, the dried aerial part of the plants was used as substrate and fermented with buffered rumen fluid. Gas production was measured at fixed time intervals. To evaluate digestibility, the residue obtained at the end of the fermentation process was used. Besides, total cumulative gas production and gas production kinetics were determined because they mirror the substrate digestion in the rumen and emulate the *in vivo* digestion kinetics (Mould et al., 2005). The gas produced *in vitro* depends on the nutritive composition of the feed analyzed (Wangui et al., 2022). Rymer et al. (2005) found a strong negative correlation between the feed NDF content and the gas production rate. Cell wall polysaccharides, such as pectin, hemicelluloses, and cellulose, and reserve carbohydrates as starch have different digestion rates.

Results from *in vitro* digestibility with gas production of the cell wall material of *D. laxiflora* showed differences between seasons, with more *in vitro* digestibility during spring and autumn **(Suppl. Fig. 2)** in coincidence with the plant's regrowth cycle, and in agreement with the lower values of DM, aNDF, and ADF presented in Fig. 3A-C. Regarding the gas production kinetics, significant differences were only found in spring (Fig. 3F, Suppl. Fig. 3), with a net cumulative gas production (NCGP) in the first 5 h of incubation of plants from site H, 50 % higher than plants from site M. However, the highest differences were found in the hourly gas production rate between 7.5 and 22 h of incubation. Changes in cell wall composition, mainly in the hemicellulosic fraction, could be related to the differences found in this range of incubation hours (Vago et al., 2021).

3.6. Cell wall characterization

As major components of any type of plant biomass, characteristics of the cell walls largely determine their nutritional quality. These characteristics can comprise the amount of their different polymeric components, as well as their chemical structure and the interactions between them.

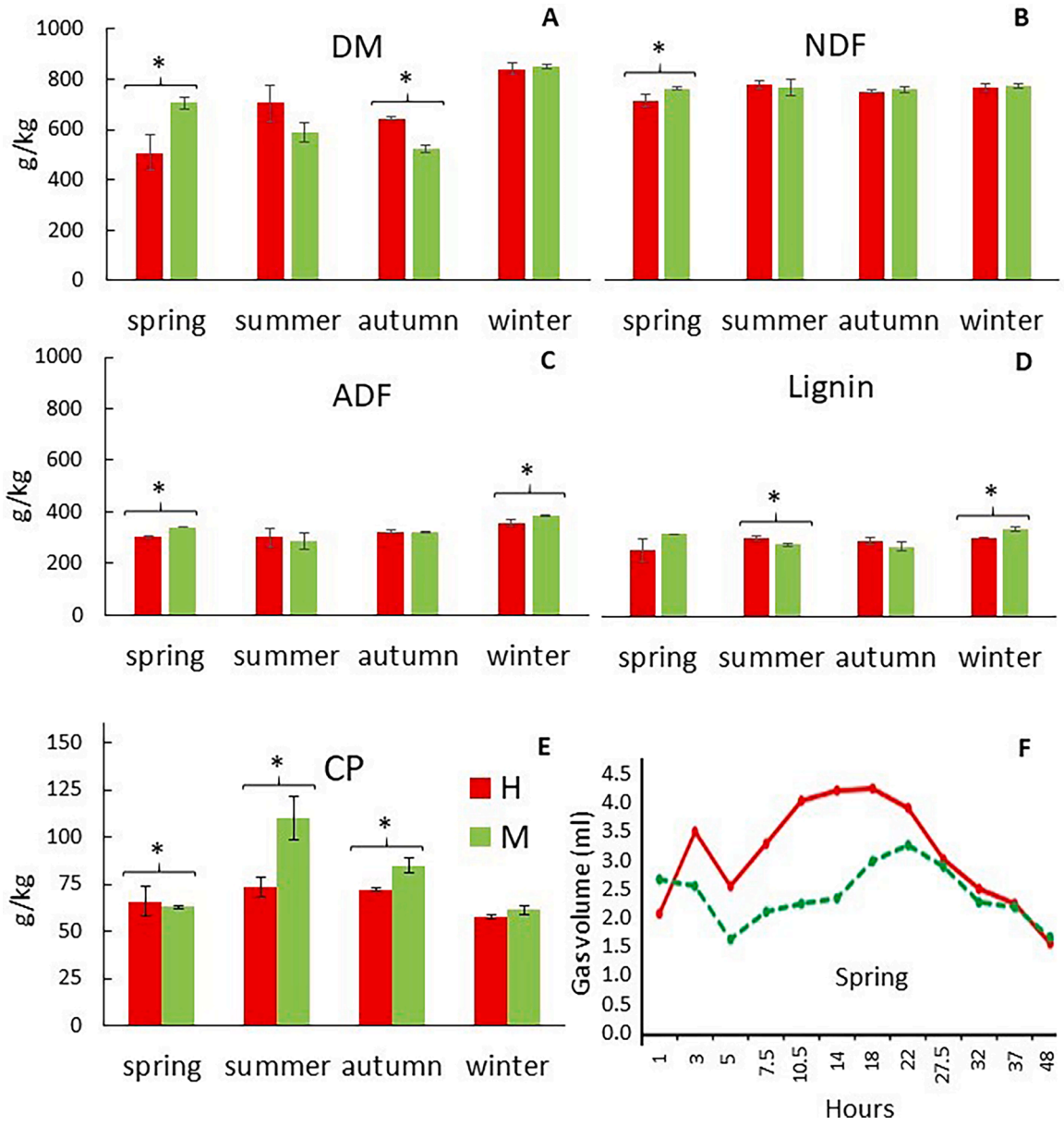

Fig. 3. Nutritional characterization related to the cell wall polysaccharides from the aerial parts of *Distichlis laxiflora* growing in a halophytic (H) and in a mesophytic (M) meadow in spring, summer, winter, and autumn samples (A-F). A: Dry matter content g/kg; B: Neutral detergent fiber (aNDF) (g/kg DM); C: Acid detergent fiber (ADF) (g/kg DM); D: Lignin (sa) (g/kg DM); E: Crude protein (CP) (g/kg DM); F: *In vitro* cumulative gas production kinetics from spring biomass materials (shoots+leaves). Asterisks represent significant differences between plants from areas M and H. Data represent mean ± *S*.E. (Student's *t*-test).

Yields of AIR (alcohol insoluble residue) for each site did not show significant variations throughout the seasons, although small differences between sites were observed for spring sampling, with a lower value for site H (Fig. 4B). On the other hand, yields of the hot water insoluble residue (HWIR) looked more variable, but these results showed greater dispersion, generating deviations that do not allow establishing significant differences when comparing sites and seasons.

FTIR spectroscopy is a widely used tool where the interaction of infrared radiation with the bonds and functional groups of the molecules generates spectra that provide valuable structural information. A first look at the ATR- FTIR spectra of the HWIR of the samples from each site for the different seasons indicated very similar results (Fig. 4A). Analysis was focused on the range of 1800–800 cm^{-1}, according to the usual criteria for this type of material (Liu et al., 2021, and references therein). This range of wavenumbers includes part of the region known as the fingerprint for polysaccharides (1400–800 cm^{-1}), and another wavenumber range with bands corresponding to the different functional groups (1800–1400 cm^{-1}), as that at 1733 cm^{-1}, corresponding to carboxyl groups of uronic acids, and those at 1634 cm^{-1} (C = O stretching vibration) possibly due to the amide group of peptidic linkages. The fingerprint region is very characteristic of each polymer, but at the same time, in complex samples, it is very difficult to analyze due to the overlapping of peaks. The spectra show a major peak originated by the high absorbance in the region of 1100–950 cm^{-1}, with its maximum intensity around 1030–1035 cm^{-1}, characteristic values of samples rich in cellulose and hemicelluloses (Liu et al., 2021). The peak around 896 cm^{-1} is associated with β-glycosidic bonds.

Regarding the composition of the cell walls (**Suppl. Table 2**), the major differences (both between seasons and sites) were observed in the content of xylose (Xyl), followed by glucose (Glc), uronic acids (UA), and arabinose (Ara) (Fig. 4C). The former derives mostly from glucuronoarabinoxylans (GAX); part of the arabinose and uronic acids constitute the side chains of the xylan backbone. Regarding glucose, it corresponds to cellulose and other minor β-glucans, as mixed-linkage

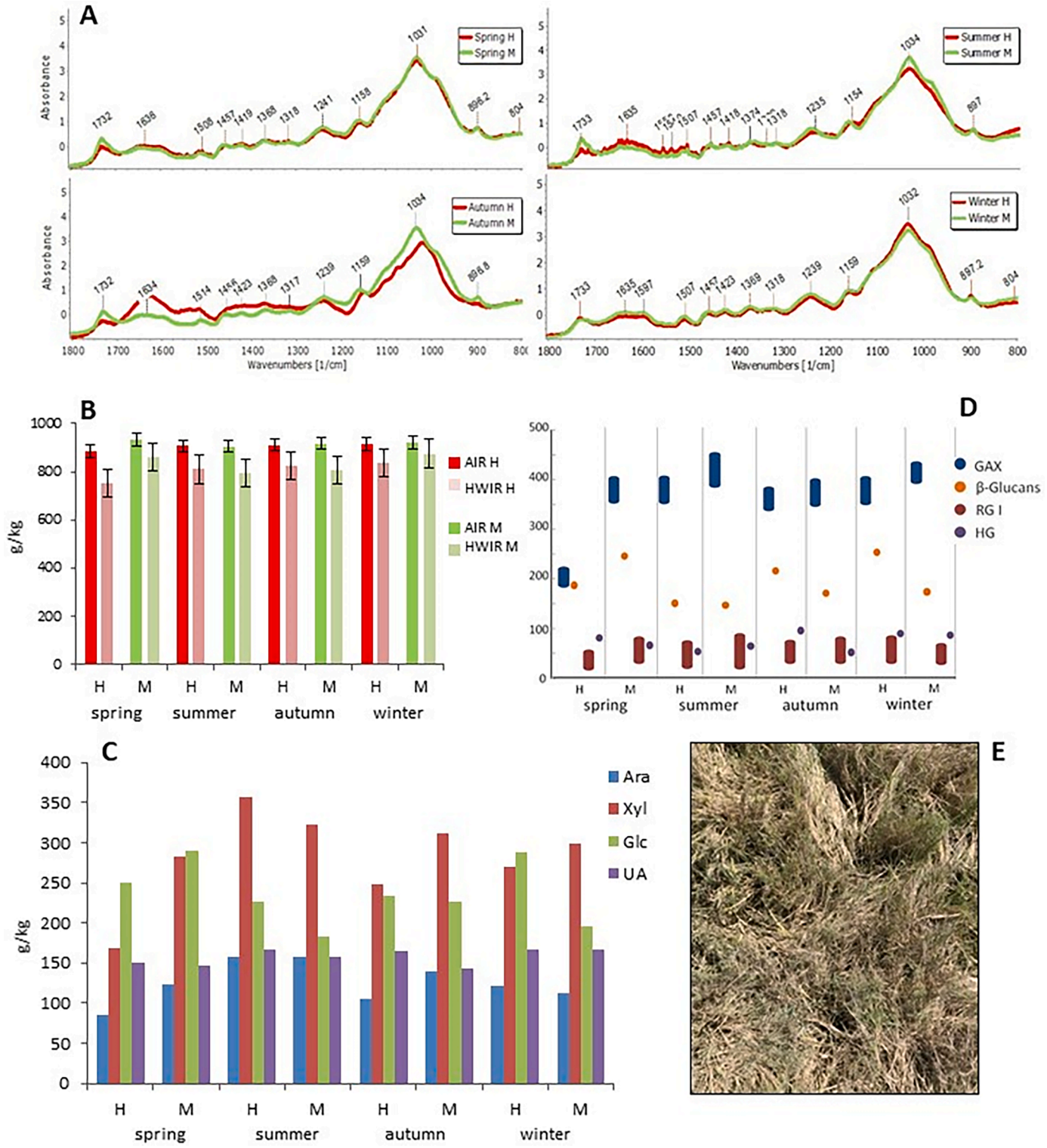

Fig. 4. Cell wall characterization of the aerial parts of *Distichlis laxiflora* growing in a halophytic (H) and in a mesophytic (M) meadow in spring, summer, winter, and autumn samples (A-E). H: halophytic meadow; M: mesophytic meadow. A: FTIR spectra pre-processed with Standard Normal Variate of the HWIR of the samples, comparing both sites (H and M) for each season. B: Yields of the Alcohol Insoluble Residue (AIR) and the Hot Water Insoluble Residue (HWIR), expressed as g/kg of dry matter. C: Major neutral monosaccharides (Ara: arabinose; Xyl: xylose; Glc: glucose) and uronic acids (UA), expressed as g/kg of HWIR. D: Estimated contribution of different cell wall polysaccharides in the dry matter of aerial parts of *D. laxiflora*. GAX = glucuronoarabinoxylans, ranges correspond to different degrees of substitution, with a ratio of 4:1 and 2:1 for xylose to arabinose, and a ratio of 6:1 for xylose to glucuronic acid (Carpita, 1996, and our unpublished data). β-glucans = almost all the glucose derives from cellulose, there could be a minimal contribution of MLG and XG. RGI = calculated as rhamnose + galacturonic acid (1:1) plus galactose and, for the upper range value, a portion of arabinose that is not part of GAX. HG: calculated as the total amount of UA minus the amount of GlcA in GAX and the amount of GalA in RGI). Small amounts of mannans were also detected (7–14 g/kg DM). E: Aspect of plants of *D. laxiflora* growing in H.

glucans (MLG) and xyloglucans (XG), as the content of α-glucans from starch that could remain in these residues should have little relevance. Conversely, the content of total phenolics did not show significant differences (except for spring samples) as well as the molar ratio Ara: Xyl, which accounts for the degree of branching of GAX. Remarkably, the spring samples from site H, which are the ones with the lowest yield of AIR and HWIR, that is, lower content of total cell wall, also showed lower content of arabinose and xylose, but slightly higher levels of phenolic compounds. The interaction between hemicellulose chains and of hemicelluloses and lignin through phenolic compounds can affect digestibility since it determines the accessibility of the enzymes to their potential target polymers. For instance, de Souza et al. (2018) analyzed the model C4 grass *Setaria viridis* and observed that a decrease in ferulic acid content on GAX determined greater digestibility by affecting the recalcitrance to enzyme attack.

Based on the current knowledge about the cell walls of this type of material, it is possible to establish an approximate range for each polysaccharide in the dry matter from the identified amounts of each monosaccharide (Fig. 4D). The ranges are variable, with no clear patterns allowing a simplified analysis, the most striking differences being the lowest value of GAX for site H in spring, which coincides, once again, with the highest *in vitro* digestibility observed. Regarding the ranges calculated for the pectic fraction, they are in line with those reported by Oliveira et al. (2020) for shoots and leaves of maize, which is another C4 grass affected by salinity. The authors studied in detail the changes observed in the cell wall polysaccharides of roots, shoots, and leaves of 12-day-old plants. Regarding the aerial part, they found that saline stress produced complex effects. In shoots, a decrease in crystalline cellulose, an increase in lignin, and few variations in arabinose and xylose; while in leaves, they found an increase in arabinose and glucose. In addition, changes in the phenolic profile were observed. It should be noted that in our case when taking the aerial part as a whole (Fig. 4E), this differentiated behavior between shoots and leaves could be masked.

4. Discussion

Comparison based on chemical and nutritional characterization between the aerial part of *D. laxiflora* from H and M showed that the spring samples are those that have the greatest contrast between sites. This was expressed in a lower yield of AIR (and even HWIR) and less content of GAX, along with lower values of DM, NDF, and ADF and higher content of protein. These differences were reflected in results from *in vitro* digestibility (IVD), with higher values for H and contrasting curves of gas production kinetics between the samples from H and M. This higher nutritional quality corresponds, surprisingly, to the moment in which the stress indicators reach the worst values, measured through the photosynthetic parameters and the K/Na ratio for site H. This is consistent with results previously reported for L. *tenuis* grown in greenhouse conditions (Vago et al., 2021), where shoots from salt-stressed plants showed lower NDF and ADF and higher IVD and gas production than control plants.

A multidisciplinary approach, such as the one developed in this work, allows us to discover differences that would otherwise be masked. From the point of view of the structure of the cell wall components, it would be necessary to deepen in search of these differences with the purification and fractionation of the individual types of polysaccharides of each part of the plants, and their detailed study. In particular, as *D. laxiflora* belongs to the Poaceae, which develop Type II cell walls, its hemicelluloses should be carefully studied. NDF is in the same range as the yields of HWIR (~700–850 g/kg). These results, in turn, agree with the reports obtained by Bustan et al. (2005) for *D. spicata* (NDF 720 g/kg) and by Klich (2020) for *D. spicata* and *D. scoparia* from Argentine Patagonia (NDF between 712 and 737 g/kg, and between 640 and 653 g/kg, respectively, with somewhat higher values in autumn *versus* spring for both cases). Additionally, for *D. palmeri*, about 320 g/kg of ADF were reported (Pearlstein et al., 2012), and for *D. spicata* and *D. scoparia*, between 240 and 340 g/kg (Klich, 2020), also in line with our results. Regarding protein content, the ranges described in the literature for the three species mentioned before varying between 50 and 190 g/kg (Bustan et al., 2005; Pearlstein et al., 2012; Lymbery et al., 2013; Klich, 2020). It is worth noting that Hansen et al. (1976) compared the CP values of *D. spicata* growing in alkaline soils (pH 8.3–8.8) from spring to autumn and found a maximum of 150 g/kg CP in spring, falling to 50 g/kg in summer. In our case, the maximum was observed at site M in summer, with the values at H being lower (except for spring) and more stable throughout the seasons. Finally, the IVDMD reported here, 30–60 %, with a maximum for the spring samples of site H, is close to those reported for *D. spicata* and *D. scoparia* (48–64 %). In the latter case, the highest values correspond to the material of *D. scoparia* collected in spring (Lymbery et al., 2013; Klich, 2020), as in this work. Without losing sight of the obvious limitations in nutritional quality, if this material is compared with other grasses and, even more so, if it is compared with legumes, it is worth noting that this saltgrass contributes with the greatest amount of biomass throughout the year in these marginal environments, improving the physical and chemical soil conditions, thanks to its powerful rhizome system (Sargeant et al., 2008). Several publications have proposed a revaluation of these plants as part of the diet of ruminants in these environments, in the context of rotational grazing and sustainable management proposals (Brizuela et al., 1990; Pelliza et al., 2005; Vecchio et al., 2019; Klich, 2015).

The stress conditions that were assumed from the soil characteristics and corroborated by data of photosynthetic efficiency and K/Na ratio, undoubtedly contribute to determining some of the differences observed in chemical and nutritional parameters. However, this relationship is complex, it is not unequivocal, and it can also be overlapped with the effects of seasonality and the phenological stages in *D. laxiflora*. Cell walls are very complex biological structures, and the chemical and nutritional methodological approaches shed light on different aspects, in agreement with the different objectives for which they were developed. None of them alone is enough to achieve a complete picture.

5. Conclusions

In this work, the greatest differences in digestibility between the halophytic and the mesophytic meadows were found during spring in the aerial parts of plants collected. These differences are related to a decrease in cell wall content, and in particular in the amount of GAX. Such changes could be associated with adaptations to high salinity and flooding conditions, which affect the composition and structure of the cell walls.

CRediT authorship contribution statement

Paula Virginia Fernández: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **María Elena Vago:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Juan Pedro Ezquiaga:** Investigation. **Santiago Maiale:** Methodology, Investigation, Data curation. **Andrés Rodriguez:** Writing – review & editing, Methodology, Investigation, Data curation. **Juan Manuel Acosta:** Writing – review & editing, Visualization, Methodology, Investigation. **Maximiliano Gortari:** Methodology, Investigation. **Oscar Adolfo Ruiz:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Marina Ciancia:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100519.

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