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Cellulose Content in Selected Plant species along the Dead Sea Coast and the Southern Desert of Jordan

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Abstract

Cellulose is the most abundant organic polymer in nature. It has many vital applications in the human life. The main objective of this study was to determine cellulose content in selected plant species along the Dead Sea coast and the southern desert of Jordan. To fulfill this objective, cellulose content was determined in plant samples according to Updergraff method with some modifications. The results showed significantly high cellulose content in: *Peganum harmala*, *Cleome amblyocarpa*, *Citrullus colocynthis*, *Rumex cyprius*, and *Capparis spinosa* (45.64, 36.44, 36.32, 32.64, and 32.8 % of dry weight, respectively). These values are comparable to cellulose content in biofuel crops such as wheat and barley. Moreover, these plant species are highly adaptive to harsh conditions of water deficiency and high temperature. Therefore, these plant species are promising alternatives in the field of cellulose industry.

Keywords: Cellulose; desert plants; extreme conditions; Dead Sea; Jordan

1. Introduction

There are four principal biogeographical regions in Jordan: Mediterranean, Irano-Turanian, Saharo-Arabian and Sudanian (Al-Eisawi 1996). Because of the diverse bioclimatic regions and subregions, Jordan has rich and highly diverse flora. In addition, different plant adaptations resulted in the characteristic vegetation of the different bioclimatic regions.

The Sudanian biogeographic region includes Jordan Valley to the north, the Dead Sea area, the whole area of Wadi Araba to Aqaba and the granite mountains to the south, including part of Wadi Rum. It has extremely low precipitation of 50 to 100 mm. The soil of this region is saline, sandy and granite. This region also has sand dunes. In this region, *Acacia* woodlands are characteristic of much of the Dead Sea Depression and Wadi Araba. Also, *Calotropis procera* is found in this region (Al-Eisawi 1996).

The Dead Sea is in the Jordan rift valley at the lowest point on Earth (407 m below sea level). It has extremely high salt content (8 times that in the world's oceans). Its name reflects the fact that a very few biological species can survive the extreme environment in this region. The Dead Sea area has two types of vegetations: the dry tropical and the halophytic (Taifour and El-Oqlah 2016). These vegetations types are found in the Saharo-Sindian regional zone. Examples of plant species of the dry tropical vegetation are: *Balanites aegyptiaca*, *Maerua crassifolia*, *Salvadora persica*, *Moringa peregrina*, and *Calotropis procera*. Examples of plant species of the halophytic vegetation are: *Tamarix tetragyna*, *T. macrocarpa*, *Anabasis setifera, Atriplex halimus*, *A. turcomanica*, and *Suaeda fruticosa*.

Drought problems have been exacerbating in the Mediterranean region including Jordan. More than 90% of Jordan area is desert. Hence, the exploitation of natural resources in the desert and saline environments for the benefit of the human communities is of high priority. Plant biomass is made of cellulose, hemicelluloses, lignin, extractives, lipids, proteins, simple sugars, starches, water, hydrocarbon components (HC), ash, and other compounds (Balat et al. 2009). The percentage of the different components of biomass varies among plant species (Bhat et al. 2015). Cellulose is the major component of plant cell wall. It is a polysaccharide made of β-glucose monomer (Mcfarlane et al. 2014). Historically, cellulose was used by humans for clothing, shelter, in medicine and food (Harris et al. 2010). Its ease of extraction resulted in its preference as a biofuel source in the biofuel industry (Abideen et al. 2014). Indeed, 46% of the renewable energy consumption in 2005 was from biomass (Demirbas and Demirbas 2010).

Many previous studies showed the significant negative effect of water deficit and salinity stresses on cellulose synthesis and consequently plant's biomass (Wang et al. 2016; Kesten et al. 2017). Therefore, the main objective of the present study is to test the natural variation in cellulose content in selected plant species along the Dead Sea area coast, and the southern desert of Jordan. Plant species with high cellulose content could be potential target for future applications of cellulose in the different sectors of industry. Indeed, the utilization of these stress adapted plants in the cellulose industry would be an efficient and cost-effective alternative for the growth of highly demanding plant species.

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2. Materials and Methods

2.1. Field Trip and Plant Collection

A field trip to the Dead Sea region and the southern desert of Jordan was conducted between 21 and 23 March 2019. Plant samples of the selected plant species were collected and identified following the Flora Palaestina (Zohary 1966) and were kept in paper bags (Table 1).

Table. 1 Sites of collection of the selected plant species along the Dead Sea coast and the southern desert of Jordan.

Plant species	Site of collection	GPS Coordinates
Prosopis farcta (Banks & Sol.) J.F.Macbr.	Dead Sea	31°33'30.9"N 35°28'30.9"E
Capparis spinosa L.	Dead Sea	31°29'04.2"N 35°34'22.2"E
Aizoon hispanicum L.	Dead Sea	31°29'16.4"N 35°34'13.0"E
Rumex cyprius Murb.	Dead Sea	31°36'06.4"N 35°33'44.7"E
Anabasis setifera Moq.	Dead Sea	31°35'59.3"N 35°33'42.6"E
Citrullus colocynthis (L.) Schrad.	Araba Valley	29°49'21.9"N 35°06'58.4"E
Zygophyllum coccineum L.	Araba Valley	29°49'21.9"N 35°06'58.4"E
Tetraena simplex L.	Araba Valley	29°49'21.9"N 35°06'58.4"E
Blepharis ciliaris (L.) B.L.Burtt	Araba Valley	29°49'21.9"N 35°06'58.4"E
Panicum turgidum Forssk.	Araba Valley	30°37'48.8"N 35°16'41.9"E
Cleome amblyocarpa Barratte & Murb.	Araba Valley	29°39'51.3" N 35°02'01.6" E
Peganum harmala L.	Wadi Rum	29°38'14.9"N 35°11'53.2"E
Pulicaria undulata (Forssk.) C.A.Mey.	Wadi Rum	29°34'21.23"N 35°25'7.04"E
Haloxylon persicum Bunge	Aqaba Customs Directorate	29°30'44.9"N 35°00'02.4"E
Zilla spinosa (L.) Prantl	Wadi Alyutum	29°35'46.7"N 35°09'52.8"E
Erodium crassifolium L'Hér. ex Aiton	Aqaba Customs Directorate	29°30'44.9"N 35°00'02.4"E
Atriplex holocarpa F.Muell.	Aqaba Customs Directorate	29°30'44.9"N 35°00'02.4"E

For each plant species, at least samples from three different plants were collected. The developmental stage for each plant species was recorded, and other relevant data about each plant species were retrieved from the Plant list (2013) and the Flora of Israel online by Prof. Avinoam Danin (https://flora.org.il/en/plants/) (Table 2).

2.2. Preparation of Plant Samples

Representative plant samples from at least 3 different plants of each plant species were wrapped in Aluminum foil and kept in the oven at 60 ºC for 2 weeks for the complete dryness of the samples.

2.3. Cellulose Analysis

Cellulose content was analyzed according to Updergraff (1969) with some modifications. A dry weight of 0.05 g of each plant sample was mixed with 1 ml of acetic: nitric reagent using a vortex mixer. The mixture was then placed in a water bath at 100 ºC for 3 min. After that, it was cooled and centrifuged at 10,000 rpm for 15 min. Then, the supernatant was discarded, and the residue was washed with water. Then, 1 ml of 67% H_2SO_4 was added to each sample, and samples were kept for 1 h at room temperature. One hundred (100) µl of the digested cellulose was mixed with 1 ml anthrone reagent (SC208057, Santa Cruz Biotechnology, TX, USA). The mixture was then kept in a water bath at 100 ºC for 10 min. The reaction tubes were then cooled. For each reaction tube that represents one plant of each species two technical replicates of 200 µl each were transferred to an ELISA plate. Then, the absorbance was read at 630 nm using the MultiScan Go spectrophotometer (Thermo Fisher Scientific, Finland). The blank was anthrone reagent mixed with water. A standard curve was prepared using cellulose powder (α- cellulose C6429, Sigma Aldrich, USA). Cellulose content was presented in $mg.g^{-1}$ dry weight (DW).

2.4. Statistical analysis

Data of cellulose content were first analyzed by one-way ANOVA. Then, Tukey pairwise comparisons were used to test the differences between the means of cellulose content for each plant species at a significance level of 0.05 (Minitab 17, Minitab Ltd, UK).

Table. 2 Description of selected plant species along the Dead Sea coast and the southern desert of Jordan.

Plant Species	Developmental stage	Family	Life form	Chorotype
Prosopis farcta (Banks & Sol.) J.F.Macbr.	Flowering	Leguminosae	chamaephyte, hemicryptophyte,phanerophyte shrub	Irano-Turanian
Capparis spinosa L.	Flowering	Capparaceae	chamaephyte,hemicryptophyte	Mediterranean
Aizoon hispanicum L.	Fruit formation	Aizoaceae	Annual	Saharo-Arabian
Rumex cyprius Murb.	Fruit formation	Polygonaceae	Annual	Irano-Turanian - Saharo-Arabian
Anabasis setifera Moq.	Vegetative	Amaranthaceae	Chamaephyte	Saharo-Arabian
Citrullus colocynthis (L.) Schrad.	Fruit formation	Cucurbitaceae	Hemicryptophyte	Saharo-Arabian
Zygophyllum coccineum L.	Flowering	Zygophyllaceae	Chamaephyte	Saharo-Arabian
Tetraena simplex L.	Flowering	Zygophyllaceae	Annual	Sudanian
Blepharis ciliaris (L.) B.L.Burtt	Vegetative	Acanthaceae	Chamaephyte	Irano-Turanian - Saharo-Arabian
Panicum turgidum Forssk.	Mature seeds	Poaceae	Chamaephyte, geophyte	Saharo-Arabian – Sudanian
Cleome amblyocarpa Barratte & Murb.	Fruit formation	Capparaceae	Annual	Saharo-Arabian – Sudanian
Peganum harmala L.	Vegetative	Zygophyllaceae	Hemicryptophyte	Irano-Turanian - Saharo-Arabian
Pulicaria undulata (L.) C.A.Mey.	Flowering	Compositae(Asteraceae)	Chamaephyte	Saharo-Arabian – Sudanian
Haloxylon persicum Bunge	Vegetative	Amaranthaceae	Phanerophyte shrub	Irano-Turanian
Zilla spinosa (L.) Prantl	Flowering	Cruciferae (Brassicaceae)	Chamaephyte	Saharo-Arabian
Erodium crassifolium L'Hér. ex Aiton	Mature seeds	Geraniaceae	Hemicryptophyte	Saharo-Arabian
Atriplex holocarpa F.Muell.	Fruit formation	Amaranthaceae	Annual	Australian

3. Results and Discussion

The analysis of variance one-way ANOVA showed a highly significant difference in the cellulose content of the different plant species analyzed in this study (P-value 0.000). A high cellulose content was found in *Peganum harmala*, *Cleome amblyocarpa*, *Citrullus colocynthis*, *Capparis spinosa*, and *Rumex cyprius* (22.82, 18.22, 18.16, 16.44 and 16.32 mg.g^{-1} DW, respectively) (Table 3). The lowest cellulose content was found in *Zilla spinosa* (1.73 mg.g^{-1} DW). The following plant species have a cellulose content less than 5 mg. g^{-1} DW: *Aizoon hispanicum*, *Zygophyllum coccineum*, *Blepharis ciliaris*, *Panicum turgidum*, *Haloxylon persicum*, and *Erodium crassifolium* (Table 3). In the present study, plant species were collected from their natural habitat at different developmental stages. Four out of 17 species were in the vegetative stage, 6 were in the flowering stage, 5 were in the fruit formation stage, and 2 have mature seeds. Cellulose content was found to significantly decrease in the advanced developmental stages (Choon and Ding 2017; Best et al. 2018). However, there was no correlation between the developmental stage and the cellulose content of the plant species with high cellulose content in this study. As some of these species were in the flowering and fruit formation stage, but still have high cellulose content such as: *Cleome amblyocarpa*, *Citrullus colocynthis*, *Capparis spinosa*, and *Rumex cyprius*. Yet, some were at advanced developmental stages and showed extremely low cellulose content such as: *Zilla spinosa* (flowering stage) and *Panicum turgidum* (mature seeds). In general, because in this study cellulose content was analyzed in different plant species, a consistent correlation between the developmental stage and the cellulose content cannot be shown. Nonetheless, the main objective of this study is to reveal plant species with high cellulose content regardless of the developmental stage.

The ploidy level of plant species was found to affect cellulose content in *Arabidopsis* (Corneillie et al. 2019). In *Arabidopsis*, the increase in the ploidy level negatively affected cellulose content. Most of the plant species in this study are diploid such as: *Capparis spinosa*, *Aizoon hispanicum*, *Anabasis setifera*, and *Zygophyllum coccineum* (Al-Turki et al. 2000). Nevertheless, this factor is not significant in our study because cellulose content was analyzed in different plant species regardless of their ploidy level.

The wastes of agricultural crops were used to extract cellulose for biofuel production (Abideen et al. 2011; Sharma et al. 2017). The composition of these wastes was revealed for different crops such as: rice, wheat, and barley (Saini et al. 2015). The percentage of cellulose in the straw of rice, wheat and barley is: 28-36, 33-38, 31-45 (%wt on dry mass basis), respectively. In the present study, some plant species were shown to have high cellulose content when compared to these crops. For example, the cellulose content (%wt on dry mass basis) of *Peganum harmala*, *Cleome amblyocarpa*, *Citrullus colocynthis*, *Rumex cyprius*, and *Capparis spinosa* is 45.64, 36.44, 36.32, 32.64, and 32.88, respectively (Table 3). *Tetraena simplex*, *Prosopis farcta*, and *Pulicaria undulata* also have good cellulose content of 27.98, 27.47, and 24.08, respectively. In general, these species show high cellulose content like that of the major biofuel crops. Moreover, these species are adapted to the extreme conditions of water deficiency and high temperature. Abiotic stresses were shown to significantly reduce cellulose content in different plant species (Wang et al. 2016; Kesten et al. 2017). Therefore, these species could be good candidates for the future applications in the biofuel industry and other cellulose based applications (Sharama et al. 2017).

Table 3. Cellulose content in selected plant species along the Dead Sea coast and the southern desert of Jordan.

Plant species	Cellulose content (mg g-1) ± SE	Cellulose content (%wt on dry matter basis)	Analyzed plant part	Number of analyzed plants
Prosopis farcta	13.73 ±2.84 BCD	27.47	Leaves	3
Capparis spinosa	16.44 ±1.43 ABC	32.88	Leaves	3
Aizoon hispanicum	3.30 ±0.63 EF	6.60	Aerial parts*	6
Rumex cyprius	16.32 ± 1.49 ABC	32.64	Aerial parts	3
Anabasis setifera	7.26 ± 2.29 DEF	14.52	Aerial parts	3
Citrullus colocynthis	18.16 ± 2.94 AB	36.32	Leaves	4
Zygophyllum coccineum	2.09 ± 0.28 F	4.18	Aerial parts	5
Tetraena simplex	12.04 ± 0.71 BCD	24.08	Aerial parts	5
Blepharis ciliaris	2.36 ± 0.33 EF	4.72	Leaves	4
Panicum turgidum	2.04 ± 0.17 EF	4.08	Leaves	4
Cleome amblyocarpa	18.22 ± 0.46 AB	36.44	Aerial parts	3
Peganum harmala	22.82 ±3.06 A	45.64	Aerial parts	3
Pulicaria undulata	13.99 ± 1.70 BCD	27.98	Aerial parts	3
Haloxylon persicum	4.16 ± 0.90 EF	8.32	Aerial parts	3
Zilla spinosa	1.73 ± 0.21 F	3.46	Aerial parts	5
Erodium crassifolium	4.08 ± 0.92 EF	8.16	Aerial parts	4
Atriplex holocarpa	9.29 ± 0.79 CDE	18.58	Aerial parts	3

Means of cellulose content that do not share a letter are significantly different.

*Aerial parts are leaves and soft stems

One important factor to be considered for the best utilization of plant species in the different applications of human life is the abundance of these species. According to the Jordan's Plant Red List, *Peganum harmala*, *Citrullus colocynthis*, and *Tetraena simplex* that showed significantly high cellulose content are abundant in Jordan (Taifor and El-Oqlah 2014). *Rumex cyprius* is moderately abundant, while *Cleome amblyocarpa*, *Capparis spinosa*, *Prosopis farcta*, and *Pulicaria undulata* are abundant (Taifor and El-Oqlah 2016). In general, these promising plant species are abundant in nature. Nonetheless, plant tissue culture technique could be used for the homogenous *in vitro* propagation of these promising plant species. In conclusion, the present study revealed new and promising sources of cellulose from the desert plant species of Jordan.

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