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Evaluation of phenolic compounds of two *Lygeum spartum* L. cytotypes

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***Lygeum spartum* represents a natural barrier in the Algerian high plateaus against the advance of sand and the desertification. The plant is of interest due to its tolerance to environmental stress. There are two levels of ploïdy in this species; the diploid cytotype 2n = 16 which is confined in the locality of Ain Benkhllil and the polyploid cytotype 2n = 40 which thrives in semi-arid climates and has a large ecologic plasticity. The aim of this study was the evaluation of the phenolic compounds and flavonoids in both cytotypes. The antioxidant activity was also measured with results showing differences in the level of phenolic compounds; thus, these compounds could serve as biomarkers to distinguish the two cytotypes.**

Key words: *Lygeum spartum* L, total phenols, flavonoids, 2,2-diphenylpicrylhydrazyl (DPPH), lipid peroxidation.

INTRODUCTION

Lygeum spartum L. (Poaceae) is a native species in Algerian steppe, widely distributed in semi-arid Mediterranean areas. The plant has considerable ecological importance due to its tolerance to environmental stress and represents a natural barrier against the advances of sand and desertification (Nedjimi, 2009). Harche et al. (1990) showed that as the Alfa (*Stipa tenacissima* L.), leaves of *L. Spartum* have fibrous walls rich in polysaccharide compounds which give the paper pulp. Studies have shown the existence of two levels of ploidy in this species in Algeria; a diploid with 2n = 16 located in arid regions and a polyploid with 2n = 40 present in the semi-arid zones of the littoral (Benmansour and Kaid-Harche, 2001; Djabeur et al., 2008; Boughanmi-Abdeddaim, 2010). Cytotype polyploid (2n = 40) is widespread in Algeria and is also present in Egypt (Ramanujam, 1938) and Spain (Lorenzo-Andreu and Garcia-Sanz, 1950). According to recent findings, the second cytotype diploid (2n = 16) seems to be located on the high Algeria trays, only in Ain Benkhellil and Kheiter (Benmansour and Kaid-Harche, 2001; Djabeur et al., 2008; Boughanmi-Abdeddaim, 2010). Djabeur et al. (2008) showed that the two cytotypes differ at the anatomical level in the spikelet morphology and reproductive capacity. The diploid cytotype has more vigorous fructifications with lemmas characterized by

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shorter distal parts, a character which can be used for an easy recognition of the cytotype in the field. Conesa et al. (2007) found that the *L. spartum* could be found in soils containing heavy metals, or soils rich in NaCl (Nedjimi, 2009) and this adaptation may be related to phenolic compounds and their antioxidant activity (Boscaiu et al., 2010). Phenolic compounds such as phenolic acids and flavonoids have been found to be the most widespread substantial groups of plant secondary metabolites produced from the shikimate-phenylpropanoid biosynthetic pathway (Torras-Claveria et al., 2012; Ma et al., 2014). These molecules have been described as markers of biotic and abiotic stress tolerance in plants (Lattanzio et al., 2006). Abiotic stresses cause changes at the morphological and molecular levels that adversely affect plant growth. Drought, high temperature, flooding, chilling, salinity, high light, and heavy metals are the major abiotic stresses that affect plants (Bita and Gerats, 2013). The present study is intended to identify the possible secondary metabolites present in the aerial part of both cytotypes by performing phytochimical screening of saponins, flavonoids, tannins, alkaloids and free quinines. An evaluation of total phenols and flavonoids by spectrophotometric assay was conducted. Antioxidant activity was measured by 2,2-diphenylpicrylhydrazyl (DPPH) radical inhibition test and lipid peroxidation was estimated by a 2-thiobarbituric acid reactive substances (TBARS) test.

MATERIALS AND METHODS

Plant material

The aerial parts (leaves, stems) of the two cytotypes (diploid and polyploid) were harvested at the Department of Biotechnology at the University of USTO in March 2014; the complete aerial part of the plant were then dried at 45°C for 48 h and ground with electric mill to give fine powder.

Preparation of plant extracts

The extraction of the grounded plant's aerial parts (leaves, stem) was carried using a Soxhlet apparatus. Six successive extractions were performed with the following organic solvents: Hexane, dichloromethane, chloroform, butanol methanol and ethyl acetate. In detail, 100 g powder of the plant was introduced into a filter cartridge adjusted to the size of the apparatus. The flask contained 1000 ml of the solvent; each solvent was brought to extraction for 48 h. The six different solvent extracts were obtained using a rotary evaporator and stored in a dry and dark place.

Determination of totals phenol contents

Assay for determination of total phenol contents of both populations in the butanolic, ethyl acetate and methanolic extracts was carried out using the colorimetric method of Folin-Ciocalteu (Singleton and Rossi, 1965). The amount of total phenolic compounds in each of the extracts was later extrapolated from a standard curve made with different concentrations of gallic acid.

Determination of flavonoids contents

The level of flavonoids was measured according to the method of Kim et al. (2003). The amount of flavonoids was determined by a standard curve made with different concentrations of catechin.

DPPH radical inhibition test

Evaluation of antioxidant activity was performed by the 2,2-diphenyl-1-picrylhydrazil free radical (DPPH) inhibition test (Brand-Williams et al., 1995). 50 µL of various concentrations of the extracts were added to 1950 µL of DPPH solution (0.025 g/L in methanol). After a 30-min of incubation at room temperature, the absorbance was read at 515 nm against a blank containing all reagents apart from the test compound. The ascorbic acid was used as positive control. Each sample was measured in triplicate. The results were expressed as percentage of scavenging activity (% I). I% = [(Abs blank - Abs sample)/ Abs blank] × 100. Extract concentration providing 50% inhibition (IC50) values were determined from the graph against extract concentrations.

Lipid peroxidation

Lipid peroxidation values (measured as levels of malondialdehyde, MDA) were estimated by a thiobarbituric acid reactive species (TBARS) test described by Hernandez et al. (2001). 50 mg of the sample (in dry weight) was ground and then homogenized in 2 mL of 1%w/v trichloroacetic acid (TCA). The homogenate was centrifuged at 15000 *g* for 10 min at 4°C and 0.5 mL of the supernatant was mixed with 1.5 mL of thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 90°C for 20 min. The absorbance was read at 532 nm using a spectrophotometer. The content of MDA was determined using the extinction coefficient at 155/mm/cm. All the experiments were conducted in triplicate and data presented as mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

The study focused on finding the major phenolic compounds and the antioxidant activity of both *L. spartum* L. cytotypes. The phytochemical screening revealed the presence of flavonoids, tannins, saponides and free quinones in both cytotypes (Table 1).

Estimation of total phenols showed that extracts from diploid cytotype were richer in phenolic compounds in the methanol and butanol extract, while the ethyl acetate fraction showed that polyploid cytotype contained more polyphenols. The quantitative estimation of total flavonoids by the trichloride aluminum method showed that the methanolic extract of both cytotypes was higher, but the diploid cytotype had a greater concentration of flavonoids. Previous studies have shown that extrinsic factors (such as geographic and climatic factors), genetic factors, and also the degree of maturation of the plant and the storage duration have a strong influence on the content of polyphenols (Aganga and Mosase, 2001). Quan et al. (2016) showed that phenolic acid plays a role in drought-tolerance of rice. In white clover, the high levels of flavonoids (flavonols, quercetin and kaempferol) contents were associated with enhanced stress tolerance

Table 1. Concentration of total phenolics, flavonoids, and antioxidant activity (IC_{50} values) of both *Lygeum spartum* L. cytotypes.

Extract	Butanolic extract	Ethyl acetate extract	Methanolic extract
Total phenols (mg GAE/ g of extract)*			
Diploid cytotype	82.85±0.04	25.66±0.002	79.63±0.08
Polyploid cytotype	27.55±0.01	48.68±0.005	57.61±0.05
Flavonoids (mg CE/ g of extract)**			
Diploid cytotype	14.16±0.01	7.24±0.02	38.49±0.08
Polyploid cytotype	11.43±0.02	20.63±0.13	21.58±0.02
IC 50 (µg DPPH /ml)			
Diploid cytotype	0.05±0.01	0.11±0.02	0.03±0.01
Polyploid cytotype	0.05±0.007	0.06±0.003	0.09±0.002

IC50 represents half maximal inhibition concentration. GAE, Gallic acid equivalent ; CE, catechin equivalent.

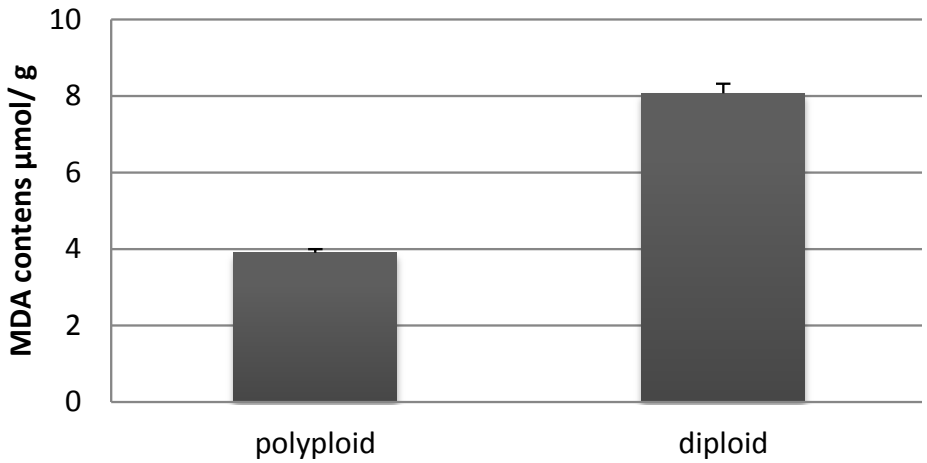

Figure 1. MDA content in µmol / g dry mass of the two *Lygeum spatum* L. cytotypes.

capacity of under UV-B radiation and drought conditions (Nichols et al., 2015).

Phenolic compounds may contribute directly to antioxidative action (Awika et al., 2003). The antioxidant activity of phenolic extracts shows variation depending on the solvent. Ethyl acetate fractions gave a higher antioxidant activity in diploid cytotype, while in polyploid cytotype, the methanolic extract gave a higher antioxidant activity. This can be explained by the fact that phenolic compounds vary between two cytotypes. The high antioxidant activities can be explained by the high reactivity of phenolic units, which may act as effective antioxidants (Jung et al., 2003). Many authors show that phenolic compounds and flavonoids have a protective effect against abiotic stress including antioxidant activity (Rice-Evans et al., 1997; Winkel-Shirley, 2002; Michalak, 2006). Indeed flavonols are able to inhibit the generation of reactive oxygen species (ROS) and as such they have a very important antioxidant function in cells (Brunetti et al., 2013).

The degree of lipid peroxidation was determined by the level of malondialdehyde (MDA), since its accumulation is an indicator of the damage caused by oxidative stress. Diploid cytotype showed a higher level in MDA contents compared to polyploid cytotype (Figure 1). Many authors have shown that high level of lipid peroxidation is caused by abiotic stress (Ben Youssef et al., 2005; Amor et al., 2006; Moradi and Ismail, 2007; Liang et al., 2008). The presence of trace solvent may influence the composition. Abiotic stresses are caused by complex environmental conditions such as high and low temperatures, freezing, drought, salinity, heavy metals, strong light, ultra-violet (UV) light, or hypoxia (Hirayama and Shinozaki, 2010). In this case, adaptive capacity of plants is mainly related to genetic evolution where the duplicated genome allowed for the new polyploid species to populate new ecological

niches or have a broader response in abiotic and biotic stress (Wood et al., 2009). This explains the restriction of the diploid population in small area and the high plasticity of polyploid cytotype. The results obtained indicate that there is a high interspecific variation of phenolic content and antioxidant activity between the two cytotypes of *Lygeum spartum* and these molecules can be further used as stress biomarkers for their distinction. A further investigation is warranted to understand the role of total phenols and flavonoids in plant protection against abiotic stress (drought, salinity, UV).

Conflict of Interests

The authors have not declared any conflict of interests.

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