Biochemical Systematics and Ecology 81 (2018) 12–16

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

The role of biochemical regulation on the adaptation of gypsophile and gypsovag species

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ARTICLE INFO

Keywords:
Antioxidant capacity
Carotenoids
Chlorophyll
Gypsum soils
Phenolics

ABSTRACT

There are two groups of plant species that spread in gypsiferous soils. While gypsophytes only grow on gypsiferous soils, gypsovags can grow on both gypsum and non-gypsum soil. Adaptation of plants to gypsum soils requires biochemical arrangements in addition to proper morphological and physiological characteristics. In this study, three gypsophyte species and on-gypsum and non-gypsum speciments of three gypsovag species were examined for antioxidant capacities. The average phenolic substance contents were 126.5, 30.5 and 37.6 μg g^{-1} DW in gypsophile, on-gypsum and in non-gypsum gypsovag species respectively. Gypsophyte, *Thymus leucostomus* var. *gypsaceus* species was identified as having seven different phenolic compounds and the highest phenolic substance content. However, phenolic substance compositions of gypsophyte and gypsovag plant species do not have common properties and show specific differences for each species. The total antioxidant capacity and carotenoid levels of gypsophyte were found to be quite high even though there was no significant difference between the chlorophyll values of the plant groups under investigation. Differences in car/chl and aox/chl ratios of gypsophiles and gypsovag species suggest that antioxidant compounds have a role in the adaptation of these plants. On the other hand, the values observed in on-gypsum and non-gypsum gypsovag species should be assessed as having no specific role in the formation of oxidative stress in gypsum soils.

1. Introduction

Gypsiferous soils are common geologic formations in the regions of arid and semi-arid climates. Gypsiferous soils having high sulfate content are hard-surfaced and low porosity. Massive gypsiferous soils in semi-arid regions cause high surface flow since they cannot absorb rain waters quickly. Gypsum can hinder growing of seedlings and seeds by packing soil surface as a tight crust (Escudero et al., 1999; Palacio et al., 2007). It is observed that most gypsiferous soils are poor of organic matters, cation exchange capacity goes down as gypsum content increases, and cation exchange capacity depends generally on organic matter content and the texture of soil (Casby-Horton et al., 2015). It has been well known that in the relation between macro-nutrients Ca, Mg, K, as Ca concentrations increases, Mg and K intakes are prevented, and the Ca:Mg ratio in tissues increases (FAO, 1990). In addition, it is long confirmed that high calcium content due to existence of gypsum may cause to Ca-Mg antagonism (Parsons, 1976; Mota et al., 2017). On the other hand, recent studies provide detailed results in the issues of soil fertility and the relation of gypsiferous soils and other soil types with heavy metal accumulation and toxic organic substances (Vereecken et al., 2016).

As it is known, taxa growing only in gypsiferous soils are named as gypsophytes and those plants growing in both gypsiferous and non-gypsiferous soils as gypsovags (Palacio et al., 2007; Cañadas et al., 2013). Presently, studies are still conducted on the mechanism of factors affecting distribution and performances of gypsophile and gypsovag species (Escudero et al., 2015). Recently, an inventory of the gypsophile flora of Palearctic and Australian territories was published. In the study area, 378 gypsophile species belonging to 52 families were reported (Pérez-García et al., 2018). Flora of gypsiferous soils is rather diverse, and generally contains rare and endemic species (Mota et al., 2017; Akpulat and Çelik, 2005). When conditions of gypsiferous soils unfavorable for growth of plants combine with drought, flourishing of endemism is inevitable. In the Eastern and Southeastern parts of Iberian Peninsula, 49 threatened taxa have been defined (Martínez-Hernández et al., 2011).

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https://doi.org/10.1016/j.bse.2018.09.007
Received 20 May 2018; Received in revised form 26 July 2018; Accepted 2 September 2018

There are various studies manifesting accumulation of phenolic substances in plants under stress. Even though there is a linear relation between amount of phenolic substances and antioxidant capacity, antioxidant capacities of phenolic compounds vary. Caffeic acid and p-coumaric acid have especially higher antioxidant activities. Antioxidant capacity is related to side groups of phenolic ring (Wojdyło et al., 2007; Kim and Lee, 2004). It is considered that stress conditions in gypsiferous habitats will cause oxidative stress in plants leading to increase of antioxidant capacity. Phenolic and flavonoid substances have been studied for their potential of being a biochemical marker for gypsum stress. Researchs show that phenolic ingredients exhibit great differences between taxa. Increase of phenolic substance content during summer time indicates that water stress is the principal stress factor, rather than sulfate ion toxicity, in gypsiferous soils (Boscaiu et al., 2010). Such findings are supported by the studies determining high accumulation of osmotic substances in plants growing in gypsiferous soils (Boscaiu et al., 2013).

The aim of this study is to determine biochemical parameters related to stress tolerance of gypsophile and gypsovag species. The elements of the antioxidant defense system are particularly emphasized because environmental stress conditions frequently trigger oxidative stress. In addition to total antioxidant capacity, basic antioxidant compounds such as phenolic compounds, carotenoids and chlorophyll content important indicators of oxidative stress, have been analyzed. Our research findings could contribute understanding of whether there are any differences in antioxidant substance compositions of plants under stress conditions of gypsiferous habitats.

2. Materials and methods

2.1. Material

Study material is composed of gypsophyte and gypsovag plant samples from gypsiferous and non-gypsiferous soils in Beypazarı and Sivrihisar, Central Anatolia Region of Turkey (Table 1). Plants were collected at the end of the vegetation period of 2015. After having collected as whole and brought to the laboratory, plants were rinsed with distilled water, short shoots (i.e. brachyblasts leaves) were picked, dried for 24 h at 70 °C and stored in a desiccator.

2.2. Climate of the research area

The research area is located in the west part of Central Anatolia. The data pertaining to Beypazarı and Sivrihisar stations were evaluated after having obtained from the Directorate General of Meteorology for studying the region's climate. Mean annual precipitation in the region varies from 391 to 402.8 mm. According to the method of Emberger, the region is under the influence of semi-arid lower cold and semi-arid upper very cold Mediterranean climate (Table 2). In the research area for both stations, the average maximum temperature of the hottest month (M) July varies from 29.0 °C to 32.2 °C. The average minimum temperature of the coldest month (m) January ranges from −1.8 °C to −3.2 °C. According to the method of Gaussen, a marked summer drought prevails from May to October (Table 2). Antecedent precipitation is W.S.A.S. (Winter, Spring, Autumn, Summer) in Beypazarı and characterized by the Eastern Mediterranean antecedent precipitation Type 1, while in Sivrihisar, it is S.W.A.S. and characterized by the Eastern Mediterranean antecedent precipitation Type 2. Accordingly, the common characteristic of these stations is that the season with the highest precipitation rate during any year is summer. It is observed that xerophilous steppe vegetation prevails in the region as result of insufficient precipitation and summer drought.

2.3. Analysis of phenolic compounds

Qualitative and quantitative analyses of phenolic compounds were performed by HPLC (Agilent 1200) according to Caponio et al. (1999). 0.1 g of leaf samples were homogenized in methanol (HPLC grade), and centrifuged at 10,000 g for 10 min and filtered with 0.45 µm filters. The samples were separated by reversed-phase column Supelco LC18 (250 × 4.6 mm^2, 5 µm) using an injection volume of 20 µL and a flow rate of 0.8 ml min^{-1}. The samples were detected at 278 nm. 2% acetic acid (A) and methanol (B) were used as mobile phase and applied with gradient as described by Caponio et al. (1999). Quantifications were calculated by comparing the peak surface areas with phenolic compounds standards (Rosmarinic, p-coumaric, benzoic, chlorogenic, hydroxybenzoic, caffeic, syringic, sinapic, t-cinnamic, t-ferulic, gallic acids, catechin, epicatechin, hesperidin and quercetin).

2.4. Total antioxidant capacity

0.5 g plant sample was crushed in porcelain mortar with 5 ml methanol (96%) for determination of total antioxidative capacity. The extract was centrifuged for 5 min at 5000 g and supernatant was taken. A reactive containing 6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate was prepared. 150 µl of supernatant was mixed with the reactive in a test tube so that last volume would be 3 ml. The tubes was maintained at 95° C for 90 min. and then cooled to room temperature and the absorbances were measured at 695 mm. Total antioxidative capacity was calculated as the equivalent of ascorbic acid (Prieto et al., 1999).

2.5. Pigment contents

Chlorophyll extraction from dry material (0.2 g) was carried out with 80% acetone. Chlorophyll contents were calculated according to Porra et al. (1989). Carotenoids analyse were done dry material (0.2 g) that ground in pre-chilled mortar in 5 ml aceton containing 200 mg Na_2SO_4 and then filtered through glass fiber disks (Whatmann GF/A). The volume of the aceton extracts was reduced in rotary evaporator and then resuspended in 1 ml of chloroform. Fifty microliters of the extracts and standarts were applied to slica gel TLC plates (20 × 20, 0.25 mm thickness). The chromatograms were developed with hexane, dietil eter, aceton, 60:30:20, v:v:v) (Moore, 1974). Xantophyll and β-carotene

Table 1
Gypsophyt and gypsovag plant species and their life strategies (G on gipsum, NG non gypsum).

Code	Species	Soil	Life Strategy
Ar	*Acantholimon riyatguelii* Yıldırım *(Plumbaginaceae)*	Gypsum	Gypsophyte
Tlg	*Thymus leucostomus* Hausskn. & Velen. var. *gypsaceus* Jalas *(Lamiaceae)*	Gypsum	Gypsophyte
Vg	*Verbascum gypsicola* Vural & Aydoğdu *(Scrophulariaceae)*	Gypsum	Gypsophyte
Fp G	*Fumana procumbens* (Dun.) Gren. & Godr *(Cistaceae)*	Gypsum	Gypsovag (on gypsum)
Fp NG	*Fumana procumbens*	Non-Gypsum	Gypsovag (on non gypsum)
Oa G	*Onobrychis armena* Boiss. & Huet. *(Fabaceae)*	Gypsum	Gypsovag (on gypsum)
Oa NG	*Onobrychis armena*	Non-Gypsum	Gypsovag (on non gypsum)
Al G	*Astragalus lydius* Boiss. *(Fabaceae)*	Gypsum	Gypsovag (on gypsum)
Al NG	*Astragalus lydius*	Non-Gypsum	Gypsovag (on non gypsum)