The influence of gypsiferous substrata on bryophyte growth: are there obligatory gypsophilous bryophytes?

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ABSTRACT In this paper the effects of gypsum (CaSO₄ x 2H₂O) in the growth medium were tested on two non-gypsophilous selected bryophyte species: Bryum argenteum Hedw. and Atrichum undulatum (Hedw.) P. Beauv. With aim to test if some bryophytes are exclusive for gypsum enriched substrates, the difference in gypsum effect on moss development were examined. In vitro cultures of two selected species were initiated from nearly mature spores within unopened capsules. Half strenght Murashige and Skoog media with added gypsum in concentrations of 50, 350 and 500mM were used to test bryophyte development. Plants were grown on media with gypsum for 3 days or 3 weeks to compare short and long term effect of salt exposure. Tested bryophytes, non-adapted to gypsum and exposed to various gypsum concentrations in MS medium, did not show to have a problem in surviving the new environment conditions. Moss species selected in this study and exposed to gypsum enriched MS medium showed variation in morphological parameters to some extent (index of multiplication, secondary protonemal production and survival rate) and chlorophyll content and slightly altered chlorophyll a/b ratio. In general, both tested species could survive gypsum enriched medium: secondary protonema was developed and new shoots were formed in both species, but slight differences were recorded between short and long term exposure to gypsum. According to data obtained, gypsum is not the stressor (at least not alone) which can separate some interesting bryophytes into the ecological group of gypsophytes.

Key words: bryophytes, mosses, gypsum, gypsophytes, Atrichum undulatum, Bryum argenteum

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INTRODUCTION

Gypsiferous substrata (i.e. soils and outcrops) are known to be present around the Mediterranean in different degree. The origin of the mineral gypsum is precipitation by sea water evaporation some 100 to 200 million years ago. During Earth history the great variation in the Mediterranean Sea level caused many areas to be under and above sea level. The later Miocene regression caused so called “Messinian salinity crisis” in the Mediterranean Sea, since it left behind in isolated or poorly communicated basins. In these hyper-saline basins the deposition of gypsum and other evaporate stone occurred, since water evaporated and the salt precipitated. This is the origin of many gypsiferous outcrops and soils around Mediterranean (eg. Sakinc & Yaltırak 2005; Roveri et al. 2008). However, the gypsum

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origin in different regions of the world can be transported by superficial or groundwater, eolian or mass movements (Porta 1998).

Substrata rich in gypsum (CaSO₄ x 2H₂O) ie. gypsosols are characterized by a gypsum (hydrous calcium sulfate) content over 5% (Escudero et al. 2000). The presence of gypsum as a widespread soil component in semi-arid and arid regions is due to its solubility. Although having a relatively low solubility in aqueous systems, gypsum can be dissolved and its ions translocated to the substrata. Further precipitation of Ca⁺² and SO₄⁻² as gypsum leads to the formation of a lenticular gypsum in the soil even there where there are no gypsum outcrops (Porta 1998).

Ecological group of plants namely gypsophytes represents one of the most conspicuous sets of arid soil endemics (Johnston 1941; Parsons 1976; Meyer 1986; Meyer & Garcia-Moya 1989).

These plants grow exclusively on gypsum soils or gypsosols, which extend over 100 million ha in the world (Verheye & Boyadgiev 1997). The peculiarity to adjust and survive these soils gives them competitiveness against other plants in such a site and thus they are mainly composed of narrowly distributed and threatened species (Meyer 1986).

Some authors considered the existence of a hard surface gypsum crust to inhibit seed germination of non gypsophytes, whereas gypsophytes may be able to surpass the soil crust (Meyer 1986; Verheye & Boyadgiev 1997), with rare experimental proofs (eg. Escudero et al. 1999, 2000). In addition, the ability of adult gypsophytes to persist under certain chemical restrictions of gypsum soils has also been stressed (Duvigneaud & Denaeyer-De Smet 1966, 1968; Cannon 1971; Boukhris & Loissant 1975), but also rejected by other authors due to little evidence (Meyer, 1986; Meyer et al. 1992; Verheye & Boyadgiev 1997). Escudero et al. (2000) highlighted the need of other biotic and abiotic effects in this habitat to be tested (i.e. soil properties and microhabitat features like litter, lichens, bryophytes, bare fraction, and gypsum crystals cover on earlier life stages of gypsophytes).

Gypsiferous substrata bear special vascular flora often called gypsiferous flora. Some vascular plants like Centaurea hyssopifolia Vahl. (Asteraceae) or Lepidium subulatum (Brassicaceae), are obligate gypsophytes treated as endemics of Iberian peninsula strictly appearing in gypsum rich substrata (eg. Escudero et al. 1997; Escudero et al. 2000).

There are some reports, these sites to be refuges of interesting, rare, vulnerable and endangered bryophytes and lichens (Guerra et al. 1995), but no real test on the gypsum effects on bryophytes were done. Although, gypsum stress can be treated as halo-effect to some extent, plants growing exclusively on gypsum including bryophytes can not be considered as typical halophytes (Sabovljević & Sabovljević 2007).

In this study, the effects of gypsum in the substrata were tested on two selected bryophyte species: Bryum argenteum Hedw. and Atrichium undulatum (Hedw.) P. Beauv.

MATERIAL AND METHODS

Two moss species were used in the experiment: Bryum argenteum Hedw. and Atrichium undulatum (Hedw.) P. Beauv. that were established as in vitro cultures. The cultures were initiated from nearly mature spores within unopened capsules (Bijelović et al. 2004; Sabovljević et al. 2002, 2003, 2005, 2006). With aim to test the difference in gypsum effect on moss development, B. argenteum was chosen as the representative of pioneering, ubiquitous species, known to grow in many different bare and inhospitable environments, while in contrast A. undulatum was chosen as widespread species but rather restricted to temperate boreal climate type, and wet brown clayly soils.

Plants were grown on half strenght MS (Murashige & Skoog 1964) medium with added CaSO₄ 2H₂O (gypsum) in concentrations of 50, 350 and 500mM. The pH was adjusted to 5.8 before autoclaving at 114°C and 118 kPa for 25 minutes.

To study the effect of gypsum, 10 mm long apical parts of shoots were used. For each concentration of salt and control, 90 transplants of A. undulatum and 130 transplants of B. argenteum were cultivated in petri dishes. The cultures were grown at 25±2°C and 60-70% humidity under cool-white fluorescent light (47 µmol/m²s irradiance) and a day/night regime of 16/8 h.

Plants were grown on media with salt for 3 days or 3 weeks to compare short and longterm effect of salt exposure. In case when plants were grown for three days on salt enriched medium, after that period they were trasfered to MS medium up to three weeks. After the given time, morphological parameters (survival, presence of buds and secondary protonema, protonemal radius and index of multiplication) as well as concentration of chlorophyll a, b and total chlorophyll, were measured. The index of multiplication represents the number of newly grown shoots originating from one shoot transplant. Pigment analyses followed Arnon (1949). Chlorophyll was extracted from frozen plant material with 80% acetone and the absorbance of acetone extract was measured with UV - visible Spectrophotometer Agilent 8453 at 4 wave lengths - 645 nm, 652 nm, 663 nm and 720 nm. The amount of chlorophyll a, b and total chlorophyll, were measured. The index of multiplication represents the number of newly grown shoots originating from one shoot transplant. Pigment analyses followed Arnon (1949).

\[
\text{Ch}_{a,b} = \frac{(A_{552} - A_{720}) \times 1000}{32.5}
\]

\[
\text{Ch}_{a} = 14.92 A_{663} - 2.90 A_{645}
\]

\[
\text{Ch}_{b} = 25.21 A_{645} - 5.15 A_{663}
\]
Chlorophyll amount was recalculated as ratio to total dry weight of the start material, to avoid the problems in weight measuring due to relative water content and water saturation in the bryophyte plants.

Data were analyzed using statistic-graphic programme Microsoft Office Excel and OriginPro, version 8.0, using a multiple range test with significant level at P < 0.05. Mean values and standard errors were calculated for at least 3 replicates for each measurement.

RESULTS AND DISCUSSION

Comparing the secondary protonemal radius of both tested species in various concentrations of gypsum (Fig. 1), it can be figured out that short term exposure (three days) on B. argenteum, as well as long term exposure (21 days) in lower concentration (50 mM), have positive effect, while on higher gypsum concentrations in substrate (350 or 500 mM) protonemal radius significantly decreased.

Substrate enriched with gypsum decreased significantly protonemal growth of A. undulatum in all concentration tested compared to control substrate with no gypsum added (Fig. 1).

Multiplication index (Fig. 2), i.e. new shoot development per explants tested, shows in B. argenteum, the opposite trend to protonemal growth. Thus, long term exposure stimulates new shoot development, and no significant difference among different concentration has been marked. In short term exposure of B. argenteum, new shoot development significantly decreased. In A. undulatum, new shoot development was better on short term exposure and even slightly increased from control to higher concentration values of gypsum in substrate. A. undulatum exposed for 21 days to gypsum in substrate, slowly but constantly decreased new shoot production up to the highest gypsum concentration.

Long term exposure to substrate gypsum decreased chlorophyll a, b and total chlorophyll content in A. undulatum (Figs. 3, 4, 5). However, with increase of gypsum content in media ratio of chlorophyll a and chlorophyll b in this species slightly increased (Fig. 6). Some mosses retain all pigments when dehydrated and they are called homoiochlorophyllous desiccation-tolerant (HDT) mosses (Proctor 1982, 1984; Tuba et al. 1998; Hamerlynck et al. 2002).

In such bryophytes, light as a stressor can cause changes in chl a/b ratio. Although the chlorophyll ratio can indicate stress stage, some data documented that in mosses only some stresses and peculiar bryophyte types can be related to chlorophyll ratio (eg. Valanne 1977; Aro & Valanne 1979; Valanne et al. 1982; Martin 1980; Martin & Cherchill 1982; Martin & Walter 1984). Thus, there are only scattered data on this phenomenon in bryophytes.

In short term exposure, A. undulatum has not shown any trend in chlorophyll content. In 50mM gypsum enriched MS substrate for three days, the ratio of chlorophylls a/b increases, while in higher concentration the chlorophyll ratio remain close to control ratio.

B. argenteum expresses similar chlorophyll content pattern in both short and long term exposure. Compared to control plants, any chlorophyll type (chl a, chl b and chl a+b) decreased in 50mM, continued decreasing in 350mM and increased significantly in 500mM gypsum substrate. The increase in the highest concentration overpasses controlled plant chlorophyll content in short termed exposure, while in long term exposure even if significantly increased, it never overpasses controlled plants.
It seems to be that higher concentrations influence chlorophyll production stimulatory in *B. argenteum*. However, the chlorophyll a/b ratio decreased with gypsum concentration increase and is higher in plants exposed to long term effect compared to plants shortly exposed.

Generally, chlorophyll content in bryophytes is much lower comparing to vascular plants (Lovelock & Robinson 2002). Also, the difference in pigment contents among different moss populations in nature has already been reported, mainly due to the growing condition (Marschall & Proctor 2004).

Many regulators required to induce a controlled chlorophyll breakdown remain to be uncovered (Hörtensteiner 2006), and even fewer are known in bryophytes. These results presented in the paper towards elucidations of chlorophyll retention processes should give direction in more investigations of chlorophyll eco-physiology and biochemistry in bryophytes, which share general biological patterns with vascular plants, but also have many peculiarities. Hörtensteiner (2006) states that chlorophyll binding protein is in close interconnection between chlorophyll and apoproteins.

In bryophytes, which have low matter turnover and energy flow, chlorophyll retention is a very important process since quick loss of chlorophyll demands a lot...
of energy for new synthesis, and makes the plant less competitive in harsh environment. Moss plants are known as resurrection plants, so the inactivation vs. activation of metabolic processes is quick and not clear, especially not for such important systems like chloroplasts. On the other side, breakdown of chlorophyll qualifies as detoxification mechanism, which is also vitally important for further plant development and survival.

A rapid chlorophyll content response to internal as well as external factors is widely accepted and demonstrated. It is known for a long time that shaded plants exhibit a higher chlorophyll pigments content per dry mass weight unit but a lower chlorophyll a/b ratio (about 2.5 – 2.9), as opposed to sun plants (ca. 3.2 – 4.0, Lichtenthaler 1987). Ramalho et al. (2000) add information on lower values of chlorophyll a/b ratio according to seasons (eg. at the end of summer (2.6) than obtained in the spring (3.6)).

Thus, our plants, in controlled conditions, expressed chlorophyll a/b ratio like plants grown in shade and warmth similarly to those conditions when plants are metabolically active and where they grow in nature. Also, relationship between plant nutrition and chlorophyll content values is very complicated and strongly modified by other internal and external conditions.

Nowadays, the quantitative determination of chlorophyll in different experimental plant material and investigation objects is especially recommended as a valuable characteristic of light harvesting capacity under stress (Ferus & Arkošiova 2001).

It was expected that with stressing mosses by growing them on increased content of gypsum, the ratio of chl a/b will decrease but there was no significant variation. This implies that gypsum was not a strong stressor if any.

It is documented that non-gypsophilous moss-species can easily survive on gypsum although with slightly changed parameters observed from controls. These results open the question if it is gypsum substrate which gives the moss species living in such environment peculiarity or this is just a synergism with other environmental conditions, above all the drought.

Since, non-gypsum species totally survived, it can be concluded that there are no obligatory gypsophilous bryophytes, but the species which their uniqueness (even though growing on gypsum ourcorps and soils) owe to some other environmental condition(s) rather than gypsum like vascular plants. Anyway, having in mind that the moss plants were grown in controlled condition in vitro i.e. ex situ, further investigation in situ are needed.

Fig. 7. A. undulatum grown under various conditions of substrata enriched with gypsum A-three days and than subcultured to gypsum free media, B-21 days (C-controlled plants, 1-50mM, 2-350mM and 3-500mM).

Fig. 8. B. argenteum grown under various conditions of substrata enriched with gypsum A-tree days and than subcultured to gypsum free media, B-21 days (C-controlled plants, 1-50mM, 2-350mM and 3-500mM).
to confirm previous hypothesis, considering various parameters excluded from laboratory environment which can be significant in synergistic effect in situ.

The results presented here are important in bryophyte layer establishment on gypsum bare spaces, in regard of restoration of gypsum exploitation sites, and for vascular gypsophyte seedlings establishments (eg. ESCUDERO et al. 1999).

CONCLUSION

Bryophytes, non-adapted to gypsum and exposed to various gypsum concentrations in substrate did not show to have a problem in surviving the new environment conditions.

Moss species selected in this study and exposed to gypsum showed variation in index of multiplication, secondary protonemal production and chlorophyll content and slightly altered chl a/b ratio which could lead to reduced photosynthetic efficiency and impaired moss growth. However, since the ratio of chl a/b was not significantly changed under various substrate gypsum concentrations, it can be assumed that gypsum did not represent strong stressor for these two moss species. Elucidation of physiological response of bryophytes to gypsum needs further investigation.

The synergism of conditions in gypsiferous soils and outcrops (where so called gypsophilous bryophytes are present) with drought above all, is rather the environment which dictates the peculiarity of mosses in such a harsh condition than gypsum itself.

The experiments conducted on two selected bryophyte non-gypsophile species show that these bryophytes can survive gypsum supstrata and/or even develop well on it to some extent.

These results open the question of bryophytes addicted to gypsum substrata to be obligatory gypsophiles or not. However, other factors like substrate moisture, radiation, interaction with other organisms (algal and fungal) and biochemical peculiarities of each species should be studied as well, in order to get precise ideas on this issue.

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REFERENCES


U ovom radu ispitivan je uticaj gipsa \((\text{CaSO}_4 \times 2\text{H}_2\text{O})\) u hranljivom medijumu na dve odabrane ne-gipsofilne vrste mahovina: \textit{Bryum argenteum} Hedw. i \textit{Atrichum undulatum} (Hedw.) P. Beauv. Sa ciljem da se dobije ideja da li su neke briofite u prirodi obligatne gipsofite, ispitivan je efekat gipsa na razviće mahovina. \textit{In vitro} kulture dve odabrane vrste uspostavljene su iz gotovo zrelih spora iz sterilnih uslova neotvorene kapsule. Da bi se ispitalo razviće mahovina korišćen je Murashige i Skoog medijum sa upola manjom koncentracijom mineralnih soli i saharoze, a sa dodatkom gipsa u koncentracijama od 50, 350 i 500mM. Biljke su gajene na medijumu sa gispom tokom 3 dana ili 3 nedelje radi poredjenja kratkotrajnog i dugotrajnog efekta soli. Ispitivane briofite, neadaptirane na gips i izložene njegovim različitim koncentracijama u medijumu, neometano su preživljavale novonastale uslove sredine. Vrste mahovina izabrane za ovo istraživanje i izložene gipsu u hranljivom medijumu pokazale su, do određene mere, variranje morfoloških parametara (indeksa multiplikacije, produkcije sekundarne protoneme i stope preživljavanja) i sadržaja hlorofila kao i blago izmenjen odnos a/b hlorofila. Uopšteno, obe vrste su bile sposobne da prežive uticaj podloge obogaćene gipsom, sekundarna protonema i izdanci su se razvijali kod obe vrste, te je zabeležena mala razlika izmedju kratkotrajnog i dugotrajnog tretmana gipsom. Prema dobijenim podacima, gips se ne može označiti kao stresni faktor (bar ne kada deluje autonomno) koji bi svrstavao neke briofite sa gipsa u ekološku grupu gipsofita.

\textbf{Ključne reči:} briofite, gips, gipsofite, \textit{Atrichum undulatum}, \textit{Bryum argenteum}