Enhancing seedling production of native species to restore gypsum habitats

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ABSTRACT

Gypsum habitats are widespread globally and are important for biological conservation. Nevertheless, they are often affected by human disturbances and thus require restoration. Sowing and planting have shown positive results, but these actions are usually limited by the lack of native plant material in commercial nurseries, and very little information is available on the propagation of these species. We address this issue from the hypothesis that gypsum added to a standard nursery growing medium (peat) can improve seedling performance of gypsum species and, therefore, optimise the seedling production for outplanting purposes. We test the effect of gypsum on emergence, survival, and growth of nine native plant species, including gypsophiles (exclusive to gypsum) and gypsovags (non-exclusive to gypsum). We used four treatments according to the proportions, in weight, of gypsum:standard peat, i.e. high-g (50:50), medium-g (25:75), low-g (10:90), and standard-p (0:100).

Our results showed that the gypsum treatments especially benefited the emergence stage, gypsophiles as group, and *Ononis tridentata* as a taxon. In particular, the gypsum treatments enhanced emergence of seven species, survival of three species, and growth of two gypsophiles, while the use of the standard peat favoured only the emergence or growth of three gypsovags. Improving emergence and survival in the nursery can provide a reduction of costs associated with seed harvesting, watering, and space, while enlarging seedlings can favour the establishment of individuals after outplanting. Thus, we suggest adding gypsum to a standard growing medium for propagating seedlings in species from gypsum habitats, thereby potentially cutting the costs of restoring such habitats. Our assessment enables us to provide particular advice by species. In general, we recommend using between 25 and 50% of gypsum to propagate gypsophiles, and between 0 and 10% for gypsovags. The results can benefit not only the production of widely distributed species commonly affected by gypsum quarrying, but also of narrow and threatened endemic species that require particularly efficient use of their seeds. In addition, our study shows the importance of using an appropriate growing medium to propagate plants characteristic of special soils such as gypsum soils.

***Keywords*:** growing medium, gypsum treatment, gypsophiles, gypsovags, gypsum species, seedling production

1. Introduction

Gypsum soils are widespread, with more than100 million ha worldwide, almost exclusively in arid and semi-arid regions (Boyadgiev and Verheye, 1996). These soils host very rare and narrow endemic flora that includes many endangered species, making them priority sites for biological conservation (Anonymous, 1992; Parsons, 1976; Mota et al., 2011; Sosa and De-Nova, 2012). However, gypsum habitats are often impacted by human disturbances such as quarrying, ploughing or grazing (Al-Harthi, 2001; Mota et al., 2004; Pulido-Bosch et al., 2004; Pueyo and Alados, 2007; Ballesteros et al., 2013). Therefore, recovery plans for these environments need to be addressed, and proactive measures need to be considered (Ballesteros et al., 2012, 2014), because natural succession has proved inefficient over the short term (Mota et al. 2003, 2004; Dana and Mota, 2006).

The recovery of gypsum areas has been satisfactorily approached through hydroseeding (Matesanz and Valladares, 2007), sowing (Ballesteros et al., 2012) or planting (Sharma et al., 2001; Blignaut and Milton, 2005; Ballesteros et al., 2014). Nonetheless, one of the main problems in restoring these environments is the lack of native plant material (seeds and seedlings), even though some studies report that this is a key factor (e.g. Matesanz et al., 2006). Thus, despite the successful use of planting as a restoration technique for gypsum habitats (e.g. Ballesteros et al., 2014), it is difficult to find seedlings of native species for gypsum substrates (gypsum species, hereafter) in commercial or public nurseries. In fact, little information is available for producing these native species. In addition, many of the gypsum species are narrowly endemic and/or endangered species and require specific harvesting efforts and efficient use of their seeds, for which the development of effective propagation methods constitutes a priority. In this sense, testing methods are required in order to enhance the emergence and survival of seedlings. Moreover, promoting early growth of seedlings during the nursery phase is particularly relevant for better outplanting performance (Kormanik, 1986; Thompson and Schultz, 1995; Jacobs et al., 2005).

In this context, we studied seedling production in gypsum species, starting from the premise that most of these are highly specialized in gypsum substrates. In this regard, several field experiments have demonstrated that the selection of a suitable substrate, composed mainly of native gypsum, effectively contributes to the success in sowing and planting (Ballesteros et al., 2013, 2014). Also, other experiments evidence that the presence of gypsum in the growth medium can be a key factor for gypsum species at the initial stages (e.g. Escudero et al., 1999, 2000; Cañadas et al., 2014), but this has never been verified for seedling production. Thus, we hypothesised that the addition of gypsum to a standard growing medium could enhance seedling performance and, therefore, the production of native plants in the recovery of gypsum habitats. To test this, we designed a manipulative factorial experiment to produce seedlings of nine gypsum species in a growth chamber, adding different gypsum proportions to a nursery growing medium commonly used for plant production. We monitored three key stages in plant production: emergence, survival, and early growth. Therefore, in this study, we determine whether gypsum treatments affect seedling performance, with the final aim of gaining insight into the propagation of gypsum species for habitat-restoration purposes.

2. Materials and methods

2.1. Target species and seed collection

Nine characteristic species of the EU priority habitat “Iberian gypsum vegetation, *Gypsophiletalia”* (Anonymous, 1992) were selected, including gypsophile (i.e. restricted to gypsum soils) and gypsovag plant species (i.e. occurring commonly on both gypsum and non-gypsum substrates; *sensu* Meyer, 1986). The gypsophiles were *Helianthemum squamatum* (L.) Dum. Cours. (*Cistaceae*), *Lepidium subulatum* L. (*Brassicaceae*), *Gypsophila struthium* L. subsp. *struthium* (*Caryophyllaceae*), *Ononis tridentata* L. subsp. *crassifolia* (Dufour ex Boiss.) Nyman (*Leguminosae*), and *Santolina viscosa* Lag. (*Asteraceae*). The first three gypsophiles are widely distributed in gypsum outcrops in the Iberian Peninsula and some localities in North Africa, and the last two arenarrow endemic species restricted to specific gypsum outcrops in south-eastern Iberian Peninsula and considered threatened (Vulnerable; Ballesteros et al., 2013). The four remaining species were gypsovags: *Helianthemum syriacum* (Jacq.) Dum. Cours. (*Cistaceae*), *Frankenia thymifolia Desf.* (*Frankeniaceae*), *Rosmarinus officinalis L. (Lamiaceae), Stipa tenacissima* L. (*Poaceae*), all with a Mediterranean distribution (see Blanca et al., 2009 and Mota et al., 2011 for further details on the selected species).

Seeds were collected in gypsum outcrops in south-eastern Spain (37.17°N, 2.84°W), under a semiarid and dry Mediterranean climate (rainfall ranging from 200 to 500 mm). Seeds were harvested from at least 50 individuals per species in natural populations. Subsequently, seeds were cleaned, discarding any visually malformed seed, and stored in darkness in paper bags under ambient conditions (c. 20°C and c. 30% relative humidity) until the experiment started.

2.2. Experimental design

We performed a manipulative experiment in a full factorial design including two factors: species (specified above) and gypsum treatments. To apply gypsum treatments, we prepared four different mixtures of standard nursery growing medium, i.e. peat (composition: organic matter= 85.4 %, pH=6-7, N=260 mg/kg, P=389 mg/kg, K=2000 mg/kg, Mg=678 mg/kg, Fe=15 mg/kg) and powdered gypsum ($CaSO_4·2H_2O$). According to the gypsum:standard peat in weight, we established four treatments, called: high-g (50G:50S), medium-g (25G:75S), low-g (10G:90S), and standard-p, (0G:100S, which represents the control treatment, because it is customarily used to propagate nursery plants).

We filled completely 450 pots of 250 cm^3 (6 cm x 5.6 cm x 8 cm) with each gypsum treatment (50 pots per species), and then in each pot 10 seeds of the same species were sown. Thus, a total of 1800 pots were placed, in a completely randomized array (9 species x 4 gypsum treatments x 50 replicates), in a growth chamber on three aluminium tables equipped with controlled spray-irrigation systems set to water every three days. The chamber was kept at 25ºC (ETN® thermostat, Carrier España, S.L.), under 14 h light/ 10 h darkness (FAEBER® lighting system,TIGER®, including 400w E40/ES OSRAM® lights, and a MicroRex D11 timer, LEXIC, LEGRAND®), reproducing favourable conditions for optimal plant development in the habitat (photoperiod and temperature from June to September).

2.3. Data collection

Pots were monitored for 21 weeks recording weekly emergence and survival. We visually checked cotyledon protrusion for emergence and marked the first seedling to emerge in each pot, or a randomly selected one if several seedlings emerged the same week (first individual, hereafter), for survival monitoring. Following the same criteria, a second seedling was marked to ensure that enough individuals were available to assess growth, in case of early death of the first individual. When each pot had two seedlings, new emerging plants were immediately removed after recording emergence. The second marked seedling in each pot was also removed after 4 weeks if the first individual survived, in order to avoid competition between seedlings.

After 21 weeks, the seedlings were harvested and washed with distilled water. Subsequently, we separated the shoots from roots and dried them in an oven (70ºC for 48 h). We weighed the samples in a precision scale (0.0001 g), after stabilization at room temperature, recording shoot and root biomass separately. These data were used to evaluate gypsum effects on growth.

2.4. Data analyses

The effect by species of gypsum treatments on emergence (measured as the percentage of emerged seedlings and as the time to emergence of the first individual) and growth (in terms of shoot and root biomass) was modelled by fitting generalized linear models (GLMs). Emergence was modelled by specifying a binomial error distribution and logit-link function for the percentage of emerged seedlings, and a poisson error distribution and a log-link function for the time to emergence of the first individual. The growth data were submitted to logarithmic transformation. To assess the effect of the different gypsum treatments on seedling survival, we fit Cox proportional hazard models by species as well as the Kaplan-Meier function to plot differences in survival among treatments (R “survival” package; Therneau, 2013). Despite that pots were monitored for 21 weeks, only individuals that emerged before the ninth week were used to assess the time to death in the survival analysis, ensuring an individual monitoring of 12 weeks at least (first week being the week of emergence). Also the biomass of the surviving individuals emerged before the ninth week was used to evaluate gypsum effects on growth.

3. Results

3.1. Emergence

Gypsum proved to have a significant effect on emergence for most species, with at least one gypsum treatment being positive compared to the standard-p for all gypsophiles and two gypsovags (Tables 1 and 2, Appendix A; Table A.1). In particular, emergence of the two threatened endemic species (*O. tridentata* and *S. viscosa*) was significantly higher in any of the gypsum treatment than in standard-p. The highest emergence rate of *G. struthium* was recorded in 25G:75S while high-g negatively influenced emergence. Moreover, the highest number of emerged seeds was found in high-g for *F. thymifolia*, 25G:75S for *L. subulatum*, and low-g for *H. squamatum* and *H. syriacum*. Standard-p was a better treatment for emergence only in the case of *S. tenacissima* and *R. officinalis.* Gypsum treatments had no effect on the emergence time of the first individual in any case (Appendix A: Table A.2).

3.2. Survival

Gypsum treatments positively affected the survival of three species after 12 weeks (Tables 1 and 2, Fig. 1, Appendix A: Table A.3). In particular, the survival of *O. tridentata* subsp. *crassifolia* and *F. thymifolia* seedlings proved significantly higher with any of the gypsum treatments than in standard-p. Thus, *O. tridentata* survival rose from 20.7% in standard-p to 83.3% in the high-g. *F. thymifolia* survival was 26.2% in standard-p but increased to 58.0% in the low-g. The highest survival values for *H. squamatum* seedlings were recorded in high-g (78.0%), while the lowest survival (42.6%) was in standard-p. Also, significant differences among treatments were found for *L. subulatum*, although differences between the highest survival in low-g (41.9%) and standard-p (25%) were not significant. For the remaining five taxa, the survival was high in both standard-p and gypsum treatments (higher than 72.9% in all cases), with no significant effects among treatments.

3.3. Early growth

Gypsum had a significant effect on seedling growth for some of the species (Tables 1 and 2, Appendix A: Table A.4). In particular, we found no negative effects of gypsum on early growth in plants of the gypsophile group, except for *S. viscosa* at high-g. By contrast, gypsum had a significantly positive effect on *O. tridentata* growth, with the effect of high-g being particularly positive on shoot and root. Shoot growth of *H. squamatum* was also significantly higher in all gypsum treatments than in thestandard-p. Concerning the gypsovag group, no significant positive effects of gypsum were found. On the contrary, the effect of gypsum treatments on *F. thymifolia* growth was negative. *H. syriacum* growth was significantly lower at high-g than in standard-p, but medium and low-g did not negatively affect growth. In addition, medium-g and high-g reduced root growth of *R. officinalis* compared to standard-p, and no significant response was recorded for *S. tenacissima.*

Table 1. Summary of the results by stages, species, and treatments. Treatments according to weight proportions of gypsum:standard growing medium; High-g (50G:50S), Medium-g (25G:75S), Low-g (10G:90S), Standard-p (0G:100S).

Species	Gypsum level	Mean Emergence (% ± SE)	Survival (%)	Mean shoot biomass (mg± SE)	Mean root biomass (mg± SE)
Ononis tridentata L. subsp. *crassifolia*	Standard-p	12.6 ± 1.7	20.7	18.3 ± 1.8	7.9 ± 0.9
	Low-g	17.1 ± 2.2	51.6	32.1 ± 1.8	17.3 ± 1.2
	Medium-g	17.3 ± 1.9	76.3	36.1 ± 7.1	18.1 ± 3.5
	High-g	17.4 ± 1.4	83.3	147.8 ± 32.5	43.5 ± 7.2
Gypsophila struthium subsp. *struthium*	Standard-p	54.4 ± 3.2	81.6	128.6 ±16.0	28.1 ± 4.0
	Low-g	54.0 ± 2.6	86	125.1 ± 15.8	24.1 ± 3.4
	Medium-g	56.6 ± 2.5	84	119 ± 16.7	30.0 ± 4.8
	High-g	41.8 ± 3.4	72.9	123.9 ± 14.5	29.2 ± 3.1
Helianthemum squamatum	Standard-p	44.8 ± 3.0	42.6	3.5 ± 0.4	2.4 ± 0.3
	Low-g	48.8 ± 2.2	42.9	4.4 ± 0.4	1.9 ± 0.2
	Medium-g	46.8 ± 2.4	60	4.1 ± 0.3	2.3 ± 0.2
	High-g	47.4 ± 3.0	78	4.5 ± 0.4	1.8 ± 0.2
Lepidium subulatum	Standard-p	22.6 ± 2.1	25	30.7 ± 11.4	4.9 ± 1.5
	Low-g	15.8 ± 2.3	41.9	10.8 ± 3.2	3.1 ± 0.9
	Medium-g	29.4 ± 3.4	24.4	18.9 ± 10.4	3.4 ± 1.8
	High-g	22.4 ± 2.3	16.7	5.8 ± 1.0	2.9 ± 0.7
Santolina viscosa	Standard-p	41.2 ± 2.6	95.9	15.3 ± 2.5	7.3 ± 1.2
	Low-g	43.8 ± 3.1	97.9	11.4 ± 2.0	5.8 ± 0.8
	Medium-g	60.0 ± 3.7	95.9	13.8 ± 2.3	6.0 ± 0.7
	High-g	56.6 ± 3.0	94.0	11.4 ± 2.3	4.3 ± 0.6
Helianthemum syriacum	Standard-p	78.6 ± 3.1	91.8	5.0 ± 0.1	2.4 ± 0.5
	Low-g	81.8 ± 1.9	80	7.0 ± 0.0	2.3 ± 0.2
	Medium-g	78.0 ± 2.9	91.8	7.1 ± 0.3	2.9 ± 0.3
	High-g	72.4 ± 3.1	82	3.8 ± 0.1	1.2 ± 0.1
Frankenia thymifolia	Standard-p	30.0 ± 3.1	26.2	11.9 ± 3.2	5.9 ± 1.1
	Low-g	47.2 ± 2.6	58.8	7.9 ± 2.2	1.7 ± 0.4
	Medium-g	30.0 ± 2.9	38.6	1.4 ± 0.4	0.5 ± 0.1
	High-g	57.8 ± 2.9	44.9	0.7 ± 0.2	0.3 ± 0.1
Rosmarinus officinalis	Standard-p	51.8 ± 3.2	91.8	32.5 ± 5.3	17.3 ± 1.7
	Low-g	44.0 ± 2.9	100.0	25.1 ± 3.7	15.6 ± 1.8
	Medium-g	38.0 ± 3.3	97.8	26.1 ± 5.4	13.7 ± 1.5
	High-g	50.0 ± 3.9	93.0	21.8 ± 2.4	11.5 ± 0.9
Stipa tenacissima	Standard-p	22.8 ± 2.6	93.2	25.6 ± 3.0	13.8 ± 1.8
	Low-g	15.2 ± 2.0	94.3	27.6 ± 2.6	14.6 ± 1.3
	Medium-g	11.2 ± 2.0	100.0	29.0 ± 3.8	16.0 ± 3.1
	High-g	15.8 ± 2.9	93.3	24.3 ± 1.9	13.3 ± 1.3

Figure 1. Kaplan–Meier survival curves representing species survival over 12 weeks for each treatment. Only the plots for species in which the treatment had significant effect on the survival are shown.

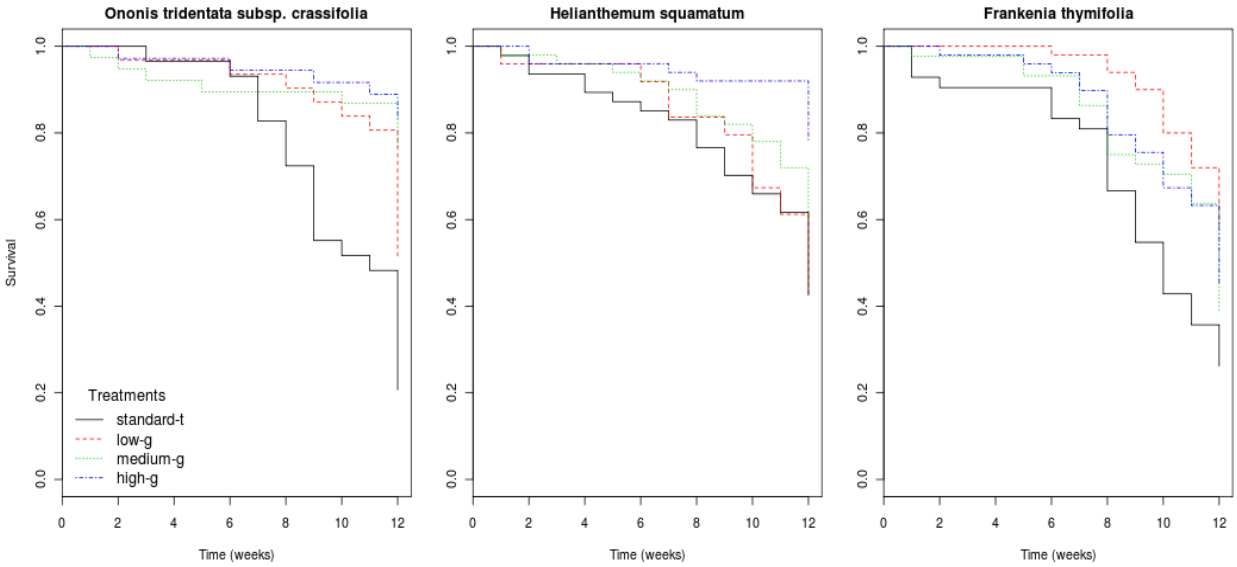

Table 2. Summary of gypsum treatment effects on emergence, survival, shoot growth and root growth by species. Treatments according to weight proportion of gypsum:standard growing medium; H/High-g (50:50), M/Medium-g (25:75), L/Low-g (10:90), standard-p (0:100). Sign of gypsum treatment effect compared to standard-p: (+) positive, (-) negative, (ns) no significant effects, according to GLMs and Cox proportional hazard model (see Appendix A for additional information). ([a]): The number of stages (emergence, survival, growth) favoured by the most beneficial treatment appear in brackets; (*) indicate marginally significant effects.

	Emergence			**Survival**			**Shoot growth**			**Root growth**			**Most beneficial treatment for growing** [a]
	L	**M**	**H**	**L**	**M**	**H**	**L**	**M**	**H**	**L**	**M**	**H**	
O. tridentata c.	+	+	+	+	+	+	+	+	+	+	+	+	**High-g (3)**
H. squamatum	+	+	+	ns	+*	+	+	+	+	ns	ns	ns	**High-g (3)**
***G. struthium* S.**	ns	+	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	**Medium-g (1)**
L. subulatum	-	+	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**Medium-g(1)**
S. viscosa	+*	+	+	ns	ns	ns	ns	ns	-	ns	ns	-	**Medium-g (1)**
H. syriacum	+	ns	-	ns	ns	ns	ns	ns	-	ns	ns	-	**Low-g (1)**
F. thymifolia	+	ns	+	+	+*	+	-	-	-	-	-	-	**Low-g (2)**
R. officinalis	-	-	-*	ns	ns	ns	ns	ns	ns	ns	-	-	**Standard-p (1)**

S. tenacissima	–	–	–	ns	ns	ns	ns	ns	ns	ns	ns	ns	**Standard-p (1)**

4. Discussion

Our results reveal that gypsum treatments had positive effects on seedlings for most of the target species at some of the stages studied (i.e. emergence, survival and/or growth). Gypsum treatments especially favoured the performance of gypsophiles, while the use of standard peat without gypsum benefited only emergence or growth of three gypsovags (Table 2).

We found that emergence was the most affected stage, when gypsum positively influenced most of the species (seven of nine) while the standard treatment favoured only the emergence of two gypsovags. Our results on emergence partially agree with a previous germination study (Cañadas et al., 2014), and the differences could be related to substrate, germination chamber, and type of gypsum treatments (e.g. Boeken et al., 2004; Golle et al., 2010). Regarding survival, we found that gypsum treatments favoured three species while no species benefited by growing in the standard peat. Moreover, gypsum also enhanced growth of two gypsophiles but did not bolster the growth of any gypsovag. Our results are somewhat different to those showed by Boukhris and Lossaint (1975), who stated that gypsophiles grew equally well in soils with high sulphur content and in commercial soils; but it was a different study because sulphur content is just one of the features of gypsum.

Overall, more positive effects of gypsum were found for gypsophiles than for gypsovags, suggesting that effects depend not only on the growing medium properties but also on the ecological strategies of species. In line with our results, different ecological strategies in gypsum species have been linked to plant groups in some studies (i.e. widely distributed gypsophiles, narrowly distributed gypsophiles, and gypsovags; e.g. Palacio et al., 2007; Cañadas et al., 2014; Escudero et al., 2014; Palacio et al., 2014). In particular, Palacio et al., (2014) evidenced plant specialization mechanism to gypsum in gypsophiles, which showed the widespread presence of gypsum and calcium oxalate crystals and the accumulation of sulphates in organic molecules, while gypsovags seem to be stress tolerant plants that tightly regulate the uptake of S and Ca by their roots. These specialization and adaptative mechanism to gypsum could explain a better performance of studied species in gypsum treatments.

However, the functioning of gypsum species and the habitat that they occupy is still not fully understood and further studies are needed in this regard (Escudero et al., 2014).

Certainly, our results revealed that the addition of gypsum to a standard nursery growing medium is advantageous to seedling performance and, therefore, to optimise production of native species for gypsum-habitat restoration. This is an important finding regardless of the specific causes, which could become a relevant theme for a separate study. In seedling production, the harvested seeds can provide greater efficiency if emergence and survival are optimised, which could reduce harvesting costs or problems arising from low availability of seeds. Also other inputs influencing costs of plant production, and therefore of restoration plans, such as space and water could be optimised. In this respect, at least one of the gypsum treatments favoured emergence in seven of the nine species studied as well as the survival in three species, whereas the standard treatment benefited only the emergence of two gypsovag species and did not enhance the survival of any of the species.

In addition, the seedlings of two species (*O. tridentata* and *H. squamatum)* were larger in all of the gypsum treatments than in standard-p. Size is a reliable, easy-to-use indicator of seedling quality (Jacobs et al., 2005; Renou-Wilson et al., 2008; Oliet et al., 2009; Close et al., 2010), and using high-quality seedlings is a key factor in establishing plantations (e.g. Wilson and Jacobs 2006), especially under arid Mediterranean conditions (e.g. Cortina et al., 2006; Oliet et al., 2009; Jiménez et al., 2014). Despite that this issue has not been resolved for gypsophile seedlings in planting, under natural conditions the largest seedlings of *H. squamatum* and *L. subulatum* also showed the highest survival rate (Escudero et al., 1999, 2000). Therefore, the field performance after the planting of species such as *O. tridentata* and *H. squamatum* could be enhanced if seedlings are grown after adding gypsum to the standard peat. However, seedling performance in field also depended on other factors such as shoot-to-root ratio, stem diameter, and physiological condition of seedlings (e.g Ritchie et al., 2010)

Results by species enable us to provide particular suggestions to optimise the production of each species (Table 2), which is feasible because it involves only the addition of gypsum to a standard nursery growing medium in the initial phase. The results are particularly relevant for the two endemic and threatened taxa studied, i.e. *O. tridentata* subsp. *crassifolia* and *S. viscosa*. Gypsum treatments enhanced the emergence of both species, which is especially important for *O. tridentata*, the seeds of which are often difficult to harvest, highly depredated (Ballesteros et al., 2013), and have low germination rates (Cañadas et al., 2014). Furthermore, emerged seedlings of

O. tridentata showed higher survival rates in medium-g and high-g, and all gypsum treatments favoured seedling growth in comparison to standard-p, the high-g treatment being particularly favourable. In addition, emergence, survival, and growth for the gypsophile *H. squamatum* were also benefited by the high-g. This result agrees with Escudero et al. (1999), who found that *H. squamatum* was able to grow in the field on a wide variety of soils, although its survival rate and growth were higher on genuine gypsum soils. We also found that medium-g favoured the emergence of *L. subulatum* and *G. struthium,* while other stages were not significantly influenced by gypsum. Thus, we suggest sowing *O. tridentata* subsp. *crassifolia* and *H. squamatum* using the high-g (because it benefits the three stages studied), and *S. viscosa, G. struthium,* and *L. subulatum* using the medium-g (because it favoured emergence). Regarding the gypsovag group, seedling production of *F. thymifolia* and *H. syriacum* could be also enhanced using the low-g, because it favoured their emergence and *F. thymifolia* survival*.* Conversely, for species such as *R. officinalis* and *S. tenacissima,* we suggest using a non-amended standard growing medium, without adding gypsum, because it yielded the best emergence.

Our study shows the importance of selecting an appropriate growing medium to propagate plants characteristic of special soils such as gypsum soils. (associated to specific substrates, as reported for copper, serpentine or Ballesteros et al., 2012; O'Dell and Claassen, 2009;Whiting et al., 2004).this context, the selection of starting mate-rials determines the success of restoration processes (Bradshaw,2000), and is particularly decisive for the recovery of singular flora

5. Conclusions

Our results reveal that the addition of gypsum to a standard nursery growing medium benefited seedling performance in most of the tested species. This constitutes the first approach to the testing of methods to produce seedlings of gypsum species for restoration purposes. In particular, the gypsum treatments especially benefited emergence as a stage, gypsophiles as a plant group, and *O. tridentata* as *a taxon*. Altogether, seven of nine species benefited from the gypsum treatments to improve emergence and/or survival, implying better use of the available seeds and a reduction in costs associated with seed harvesting, watering or space. Furthermore, larger seedlings of two species resulted after using gypsum, which could favour the establishment in the field of individuals after outplanting. Thus, we suggest applying gypsum treatments to improve efficiency in the propagation of gypsum species, which would cut the costs of gypsum-habitat restoration plans. The results regarding plant performance by species enable us to provide particular suggestions to optimise the cultivation of each species, which are feasible to apply. In general, we recommend using a standard growing medium mixed with 25-50% of gypsum by weight to propagate gypsophiles, while using solely the standard growing medium, or 0-10% of gypsum, to propagate gypsovags. The results may benefit not only the production of widely distributed species commonly affected by gypsum quarrying, but also narrow and threatened endemic species such as *O. tridentata* subsp. *crassifolia*, which require a particularly efficient use of its seeds. Finally, our study shows the importance of using an appropriate growing medium to propagate plants characteristic of special soils such as gypsum soils, which could be also applied to growing plant species to restore other particular habitats.

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