How do cryptogams affect vascular plant establishment?

Ali TAVILI\textsuperscript{a}\textsuperscript{*}, Mohammad JAFARI\textsuperscript{a}, Mohammad Ali Zare CHAHOUKI\textsuperscript{a}, Mohammad SOHRABI\textsuperscript{b}

\textsuperscript{a}Department of Reclamation of Arid & Mountainous Regions, Faculty of Natural Resources, University of Tehran, Karaj, Iran

\textsuperscript{b}Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Iran

Abstract – There are many conflicting reports regarding the effects of cryptogamic biological soil crusts on seed germination, seedling emergence and plant establishment. The current research investigated the effects of cryptogams (mosses and lichens) on seedling emergence and initial establishment of two vascular plants, namely \textit{Stipa barbata} and \textit{S. capensis}, under greenhouse conditions. For this purpose, 28 cylindrical pots were used to carefully transport field soil to the greenhouse, from two adjacent areas with similar conditions with and without cryptogams in the northeast of Golestan province of Iran. For \textit{S. barbata} and \textit{S. capensis} seeds planted in pots, seedling emergence, establishment and performance were evaluated over two months. The rate and percentage were higher for plants, particularly \textit{S. capensis}, on soils with cryptogams than those on soils without cryptogams; such improvements could be related to the effects of cryptogams on moisture, temperature and nutrient increase in soil.

Bryophytes / Lichens / Seedling emergence / \textit{Stipa barbata} / \textit{Stipa capensis} Interactions / Initial growth

INTRODUCTION

Biological soil crust cryptogams consist of mosses, lichens, liverworts, fungi and cyanobacteria. Mosses and lichens form the main (macro)-components of cryptogams and are found in a wide variety of habitats (Seppelt \textit{et al.}, 2010); they are poikilohydric, being able to survive in a dormant state during desiccation, resuming biological activity upon rewetting (Pointing \textit{et al.}, 2015). Despite their sensitivity to biotic and abiotic disturbance, they are of great importance in rangeland ecosystems. Lichens and mosses are not directly grazed by animals, but because of their importance, the effect of livestock grazing on them has been considered in some studies (Memmott \textit{et al.}, 1998).

Cryptogams are potentially important components of the vegetation in different ecosystems and play an important role in the distribution of vascular plants (Zamfir, 2000). Despite the high importance of cryptogams in rangelands, plant research has mostly focused on their vascular plants. A new approach to research on cryptogamic or biological soil crusts (BSCs) has been raised over the past two

\* Corresponding author: atavili@ut.ac.ir

doi/10.7872/cryb/v38.iss3.2017.1
decades. Although initiated in 1950, there have been few studies in this field until the 1990s; since then several researchers, particularly in the USA and Australia, have studied the performance of cryptogams in desert and rangeland ecosystems (Tavili, 2005). The majority conducted on their effects on soil protection against erosion, soil permeability, carbon and nitrogen sequestration (Longton, 1997; Belnap & Lange, 2003; Cornelissen *et al.*, 2007) and the germination and establishment of vascular plants (Sedia & Ehrenfeld, 2003; Hawkes, 2004).

Seed germination is regulated in a concerted manner that involves generating growth potential in the embryo to overcome the mechanical resistance of the endosperm (Rosental *et al.*, 2014) and can be affected by many different factors. One of the factors thought to affect seed germination and seedling emergence of vascular plants is cryptogamic cover, but reports are conflicting; while some show a positive effect (Zamfir, 2000; Belnap & Lange, 2003; Li *et al.*, 2005; Zhang & Nie, 2011), others indicate a negative impact (Sylla 1987; Špačková *et al*. 1998; Li *et al.*, 2005), and a few studies demonstrate no relationship between the presence of cryptogams and vascular plant germination and establishment (Jeffries & Klopatek, 1987). The differences in these results have been considered to be due to various factors, including the composition and structure of cryptogams (Seppelt *et al.*, 2010), their disturbance (Parker, 2001), age and vitality.

Harper and Clair (1985) planted the seeds of two perennial grasses, two perennial forbs, two annual forbs and a shrub species with and without the presence of cryptogams. In the first year after planting, except for two perennial grasses which didn’t show a difference in germination in crusted and non-crusted soils, the other species planted in the cryptogamic soils had germination and establishment averages to 8.2 times that of the non-cryptogamic soils.

In the light of this and the lack of research on the importance of cryptogamic soils in many areas of the world, such as Iran, the present research was conducted to investigate the effect of cryptogams on initial establishment and seedling emergence of two grasses, namely *Stipa capensis* (annual grass) and *S. barbata* (perennial grass) under greenhouse conditions. Based on studies like this, it may be possible to find the relationship between the presence of non-vascular plants and initial stages of succession (Moghaddam, 1998).

**MATERIALS AND METHODS**

**Rate and percentage of seedling emergence**

In order to study the effect of cryptogams on the emergence and initial growth of vascular plant seedlings, rangeland soils with and without cryptogams in pot cultures were used (Zamfir, 2000). For this purpose, cylindrical pots of 20 cm in height and 16 cm in diameter were used; each pot consisted of two detachable parts, pan and cylinder, which made it possible to take a soil sample without disturbing the soil.

After soil sampling from a rangeland located in a semi-arid area in northeast of Iran, cylindrical pots were transferred to the greenhouse. The rangeland was covered by cryptogams including different species of lichen and moss. Those lichen and moss specimens which were transferred within the pots are presented in Table 1. Voucher specimens of lichen collections are deposited in the Iranian Cryptogamic Herbarium (ICH) at the Iranian Research Organization for Science and Technology.
How do cryptogams affect vascular plant establishment?

Table 1. Different species of lichens and mosses included in the study

<table>
<thead>
<tr>
<th>Lichens</th>
<th>Herbarium codes</th>
<th>Mosses</th>
<th>Herbarium codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collema tenax (Sw.) Ach.</td>
<td>Sohrabi 16867 (ICH)</td>
<td>Aloina aloides</td>
<td>Tavili 10/104 (B)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Schultz.) Kindb</td>
<td></td>
</tr>
<tr>
<td>Diploschistes muscorum (Scop.)</td>
<td>Sohrabi 16868 (ICH)</td>
<td>Aloina bifrons</td>
<td>Tavili 10/106 (B)</td>
</tr>
<tr>
<td>R. Sant.</td>
<td></td>
<td>(De Not.) Delgadillo</td>
<td></td>
</tr>
<tr>
<td>Diploschistes diacapsis (Ach.)</td>
<td>Sohrabi 16869 (ICH)</td>
<td>Tortula revolvens</td>
<td>Tavili 10/101 (B)</td>
</tr>
<tr>
<td>Lumbsch</td>
<td></td>
<td>(Schimp.) G. Roth</td>
<td></td>
</tr>
<tr>
<td>Gyalolechia bracteata (Hoffm.) A. Massal.</td>
<td>Sohrabi 16871 (ICH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gyalolechia subbracteata (Nyl.) Sochting, Frödén &amp; Arup</td>
<td>Sohrabi 16872 (ICH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psora decipiens (Hedw.) Hoffm.</td>
<td>Sohrabi 16874 (ICH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toninia sedifolia (Scop.) Timdal</td>
<td>Sohrabi 16875 (ICH)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

and voucher specimens of bryophytes are deposited in the herbarium of the Botanic Garden and Botanical Museum Berlin-Dahlem (B).

In all, 28 pots were used, 14 pots containing soil with cryptogams (lichens and mosses) and 14 pots without cryptogams. During soil sampling, general conditions in terms of factors such as slope, aspect and elevation were considered to be similar, so that only the effects of the target treatments (presence or absence of cryptogams) could be evaluated. For the comparison of the effects on two vascular plants, two species of a single genus *Stipa capensis* (an annual) and *S. barbata* (a perennial), were separately treated. The pots were sub-divided in rows of 4 × 7. Seed collection followed the method adopted in earlier studies (Mollard & Insausti, 2009; Hu et al., 2013).

The primary seed germination test of mentioned species in the germinator showed 85% germination for *Stipa capensis* and 80% for *S. barbata* seeds. Then, seeds were planted in pots under greenhouse conditions (Li et al., 2005), 10 per pot, and irrigated every three days; due to the sensitivity of the bare soil (uncovered by cryptogams) to erosion, irrigation was done with care. Seedling emergence began three days after seed planting. Thereafter accounting, controlling and recording the number of emerged seedlings were conducted every two days after three days. The rate (velocity) of seedling emergence was determined for a period of 50 days using the following equation:

\[
\text{E.V.} = \sum \frac{\text{number of emerged seedlings}}{\text{days of count}}
\]

After the experiment period (50 days) and before removing the samples from the pots, 10 seedlings per group were randomly selected and measured for height.

**Dry matter weight**

After 50 days, all the existing plants in individual pots were removed from the soil, then the roots were cut and aerial parts were placed in the open air to dry for a week (Moghaddam, 1998). Dry weight of the aerial parts related to each pot using the exact SARTORIUS model scale (with an accuracy of 0.001 g) was measured and the average yield of the plants per pot was calculated.
Data analysis

Variable data analysis on rate and percentage of emergence, seedling height, aerial biomass, etc. was carried out factorially with a completely randomized design (CRD). Considering no uniformity of the individual plants in terms of their number in different pots, analysis of covariance was used. Using SPSS software, the Kolmogorov-Smirnov test was performed for normality, and appropriate transformations, such as logarithms, were used in some cases.

RESULTS

Percentage of seedling emergence

Seedling emergence percentage analysis is presented in Table 2. Seedling emergence was almost 3 times as great in crust-covered soil compared to bare soil ($F_{1,29} = 12.17$, $P = ****$, Fig. 1). There were no species effects nor species biocrust interactions. There was greater seedling emergence of Stipa capensis on the crusted soil, but on the bare soil, no differences between the species.

Table 2. Analysis of variance of the emergence percentage of Stipa capensis and S. barbata seedlings in soil with and without cryptogams

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species of Stipa</td>
<td>1</td>
<td>0.273</td>
<td>0.728</td>
<td>ns</td>
</tr>
<tr>
<td>Cryptogams</td>
<td>1</td>
<td>4.570</td>
<td>12.17</td>
<td></td>
</tr>
<tr>
<td>Species × Cryptogams</td>
<td>1</td>
<td>142</td>
<td>0.378</td>
<td>ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.376</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**: Significant difference at the level of 1%, ns: Difference not significant.

Fig. 1. Comparison of the emergence percentage of Stipa barbata (S.ba) and S. capensis (S.ca) in soils with (cr) and without (no-cr) cryptogams (different letters above the bars indicate significant differences at 99% level).
Seedling emergence rate

As seen in Table 3, there is no significant difference in the seedling emergence rate between the two *Stipa* species. However, the seedling emergence rate on cryptogamic v. non-cryptogamic soils is significantly different (P < 0.01). Seedling emergence rate comparison for *S. barbata* and *S. capensis* in crusted and no-crusted pots are presented in Figure 2. In both *Stipa* species, seedling emergence rate was significantly more in cryptogamic pots compared to non-cryptogamic ones. Comparing mentioned proportion in cryptogamic pots reveals that *S. capensis* seedling emergence rate is twice that of *S. barbata* (0.4 v. 0.2). In addition, seedlings of *S. capensis* seem to have an emergence rate far bigger in cryptogamic pots compared to non-cryptogamic ones (0.4 v. 0.2 for cryptogamic and non-cryptogamic pots, respectively). The results of this part of the experiment show that the annual *Stipa capensis* has been benefitted significantly more than the perennial *Stipa barbata* by the presence of the cryptogamic crust.

Table 3. Analysis of variance of the emergence rate of *Stipa capensis* and *S. barbata* seedlings in soil with and without cryptogams

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species of <em>Stipa</em></td>
<td>1</td>
<td>0.064</td>
<td>3.747</td>
<td>ns</td>
</tr>
<tr>
<td>Cryptogams</td>
<td>1</td>
<td>0.227</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Species × Cryptogams</td>
<td>1</td>
<td>0.094</td>
<td>5.477</td>
<td>*</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.017</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**: Significant difference at the level of 1%, *: Significant difference at the level of 5%, ns: Difference not significant.

![Fig. 2. Comparison of the seedling emergence rate in soils with (cr) and without (no-cr) cryptogams (different letters above the bars indicate significant differences at 95% level).](image-url)
Height of *Stipa barbata* and *S. capensis* seedlings

Table 4 shows the results of analysis of variance for seedling height with and without cryptogamic soils. For plant height, there was little difference in final height between species on the crusted soil. On bare soil, however, there was greater emergence of *Stipa barbata* than *Stipa capensis* (Fig. 3).

Table 4. Analysis of variance of the height of *Stipa capensis* and *S. barbata* seedlings in soil with and without cryptogams

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species of Stipa</td>
<td>1</td>
<td>18.090</td>
<td>3.280</td>
<td>ns</td>
</tr>
<tr>
<td>Cryptogams</td>
<td>1</td>
<td>148.610</td>
<td>27.019</td>
<td>**</td>
</tr>
<tr>
<td>Species × Cryptogams</td>
<td>1</td>
<td>57.360</td>
<td>10.429</td>
<td>**</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>5.500</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**: Significant difference at the level of 1%, ns: Difference not significant.

Fig. 3. Comparison of the *Stipa barbata* (S.ba) and *S. capensis* (S.ca) seedling heights growing in soil with and without cryptogams (different letters above the bars indicate significant differences at 99% level).
Dry matter of aerial parts of *Stipa barbata* and *S. capensis*

As described above, dry matter of aerial parts was measured by weighing all aerial parts of *Stipa* seedlings at the end of the experiment period. Due to the non-uniformity of the number of seedlings per pots and the drying up of some of the seedlings during the study period, in order to eliminate the effect of the number of seedlings on the dry weight of aerial parts in each pot, an analysis of covariance was used. As seen in Table 5, the covariate is statistically significant at the 5% level, and there is also a significant difference at $P \leq .05$ between species and between treatments, including soils with and without cryptogams for the dry weight of aerial parts. Figure 4 shows the comparison of the aerial parts dry weight based on the species growing in soil with and without cryptogams. The dry weight was slightly higher for *S. capensis* (0.4 g) compared to *S. barbata* (0.3 g) grown in pots including cryptogams. The highest (0.4 g) and lowest (0.15 g) dry weights were for *S. capensis* in cryptogamic crust soils and in non-cryptogamic crust soils respectively.

Table 5. Analysis of variance of the aerial parts dry weight of aerial parts of *Stipa capensis* and *S. barbata* seedlings in soil with and without cryptogams

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>df</th>
<th>$MS$</th>
<th>$F$</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covariate</td>
<td>1</td>
<td>0.1430</td>
<td>7.586</td>
<td>*</td>
</tr>
<tr>
<td>Species of <em>Stipa</em></td>
<td>1</td>
<td>0.0809</td>
<td>4.282</td>
<td>*</td>
</tr>
<tr>
<td>Cryptogams</td>
<td>1</td>
<td>0.0839</td>
<td>4.442</td>
<td>*</td>
</tr>
<tr>
<td>Species × Cryptogams</td>
<td>1</td>
<td>0.0064</td>
<td>0.344</td>
<td>ns</td>
</tr>
<tr>
<td>Error</td>
<td>23</td>
<td>0.0189</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*: Significant difference at the level of 5%, ns: Difference not significant.

Fig. 4. Comparison of the aerial parts dry weight (gr) of *Stipa barbata* (S.ba) and *S. capensis* (S.ca) in soil with and without cryptogams (different letters above the bars indicate significant differences at 99% level).
DISCUSSION

The effects of cryptogams on seed germination and seedling emergence of vascular plants could be investigated in many different aspects. The results of the current study showed their positive effect on the studied characteristics of *Stipa barbata* and *S. capensis* in their initial development stage. Seed germination is influenced by several environmental factors such as temperature (Thompson & Grime, 1983; Sánchez *et al.*, 2014), moisture (Finch *et al.*, 2013), oxygen, nitrogen and light (Chachalis & Reddy, 2000; Koger *et al.* 2004), of which temperature and humidity are the main factors. Semi-arid areas are typically covered with a biological soil crust (Büdel, 2002) that creates a micro-topography on the soil surface that affects the fluctuations of temperature and moisture (Belnap & Lange, 2003). Cryptogams play a role in the intensity of the environmental factors that affect the plant seed germination and consequently seedling emergence and initial growth. The presence of cryptogams according to their usually dark colour, can raise the soil temperature compared to other soils without this biological crust. In this study an increase of 4°C was recorded between crusted and non-crusted soils. The warm temperature is required for embryo growth and root emergence of some species (Baskin *et al.*, 2008). Therefore, a cryptogam presence leads to an increase in seed germination and seedling emergence, plant growth and nutrient uptake ratio by the plant in some species (Gold & Bliss, 1995). This is more important, particularly when the humidity is favourable but due to low temperature, seed germination and subsequent initial growth could not occur (Soudzilovskaia *et al.*, 2011).

Adequate moisture in the soil is another factor which is very important for germination (Mollard & Insausti, 2009), emergence and subsequent growth of the vascular plants. Water scarcity always reduces germination (Sánchez *et al.*, 2014). Some of these cryptogam species not only increase soil infiltration and prevent runoff, but also hold water up to several times their volume in the subsoil for several days after rainfall (Rivera-Aguilar *et al.*, 2005). Mücher *et al.* (1988) reported that Australian shrub plant community seeds in the presence of a biological crust had a higher germination compared to the adjacent area where the soil had no biological crust. They mentioned that high germination and acceptable seedling establishment is related to the presence of moisture that can result from the presence of biological crusts. Serpe *et al.* (2006) emphasized the cryptogam role, especially in providing essential moisture for seed germination. Other studies showed the positive effect of cryptogam presence on seed germination and establishment of such species as *Arenaria serpyllifolia*, *Festuca ovina*, *Filipendula vulgaris* and *Veronica spicata* (Zamfir, 2000), and *Mimosa luisana* and *Myrtillocactus geometrizans* (Rivera-Aguilar *et al.*, 2005). Parker (2001) also reported that an undisturbed cryptogam layer facilitates *Cytisus scoparius* establishment.

The role of cryptogams in sequestering and increasing nitrogen in soil has been demonstrated (Belnap, 2002; Li *et al.*, 2005). On the other hand, it has been shown that adding nitrogen to the soil can stimulate and accelerate seed germination (Baskin & Baskin, 1998). Moghaddam (2005) emphasizes that moisture, temperature, oxygen, light and nitrate are the most important environmental factors for seed germination and seedling emergence. In addition to the desirable emergence of seedlings in the presence of cryptogams, seedling height and dry weight was more beneficial than non-cryptogamic soils. Harper and Belnap (2001) and Zhang and Nie (2011) note that a soil covered by cryptogams not only affects soil temperature and humidity conditions, but also affects soil fertility in terms of optimum amounts of nutrients such as N, K, Ca, Mg and P, which leads to better seed germination,
seedling emergence and initial growth. By providing nutrients, cryptogamic crusts enhance seed germination and seedling growth (Luna & Moreno, 2009; Bravo et al., 2014). Furthermore, several studies have reported nutrient resorption and translocation in cryptogam tissues (Kytöviita & Crittenden, 2007; Bench et al., 2002; Lang et al., 2014). Due to their special anatomical structures (Sanei Shariatpanahi, 1996), lichens and mosses have a high ability to absorb elements from the soil, water and air, and because of their metabolic processes, the amount and concentration of elements absorbed is more than the element concentration around their environment (Szczepanika & Biziuk, 2003). Therefore, because of wetting or other factors, elements will return to the soil in higher concentrations. In a study in southern Arizona on the performance of four species of vascular plants, McIlvaine (1942) reported that vascular plant growth properties in soils with cryptogams had three times better condition compared with the planted seeds in barren soil. Pendleton and Warren (1995) reported that the weight of stems and total weight of the aerial parts of Bromus tectorum, Gaillardia pulchella, Sitanion hystrix, Sphaeralcea munroana and Coleogyne ramosissima in cryptogamic soil showed a significant increase compared to adjacent area with soils without cryptogams, and that this resulted from an appropriate moisture content and more nutrients available in the soil in the presence of a biological crust.

Although conducted under greenhouse condition, the results of the present study supported the positive role of cryptogams in the initial growth stage of vascular plants. Germination, emergence and establishment of plants were facilitated, especially for an annual plant (Stipa capensis in this study), indicating that the effects are species-specific. In addition, someone could conclude that lichens and mosses play a significant role in plant community formation such as the early stages of succession, land-plant evolution (Pratt et al., 1978) and their impact on the improvement of soil conditions. Crusts enhanced germination production and growth rate, but that the effects are species-specific. Mosses and lichens have a significant role in the early stages of succession (Moghaddam, 2005), part of which is related to their impact on seed germination and seedling establishment.

REFERENCES


HARPER K.T. & St. CLAIR L.L., 1985 — *Cryptogamic soil crusts on arid and semi-arid rangelands* in Utah: effects on seedling establishment and soil stability. Department of Botany and Range Science. Brigham Young University. Provo, Utah. USA.


MOLLARD F.P. & INSAUSTI P., 2009 — Soil moisture conditions affect the sensitivity of *Bromus catharticus* dormant seed to light and the emergence pattern of seedlings. *Seed science research* 19: 81-89.


How do cryptogams affect vascular plant establishment?


SYLLA D., 1987 — Effect of microphytic crust on emergence of rangeland grasses. MSc. thesis. School of Renewable Natural Resources, University of Arizona.


TAVILI A., 2005 — *Study of some moss and lichen species effects on range soil and plants*. Ph.D. Dissertation. Faculty of Natural Resources, University of Tehran.


