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A Technology for Rapid Reconstruction of Moss-Dominated Soil Crusts

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Abstract

Reconstruction of moss-dominated soil crusts is crucial for ecological restoration. A desert moss *Tortula desertorum* (Broth.), the main component of biological soil crusts in the Gurbantunggut desert, was used to build a procedure for construction of moss-dominated crust. Micropropagation of desert mosses is a core technology to build biological soil crusts. Although the micropropagation of mosses has been extensively studied, little is known about that of desert mosses. Effects of media, explants, and culture temperature on regeneration potential of *T. desertorum* were investigated respectively in this study. The factors such as relative humidity, light intensity, and photoperiod, which might take an important role in the regeneration and development of the desert moss *T. desertorum*, were optimized through an orthogonal test design. The life cycle of the desert moss *T. desertorum* in cultivation was also investigated according to several factors affecting asexual reproduction. The results showed the explants cultivated with *in situ* soil had produced more protonema and shoots than those cultivated with other media. Detached leaves as explants yielded more biomass per gametophyte. It was found that a slightly higher temperature facilitated shoot growth. It should be pointed out that the favorable period for *T. desertorum* asexual reproduction occurred mainly in early summer, which contributes to the efficient protection of biological soil crusts. Based on the life cycle of *T. desertorum* in cultivation, a procedure for artificial reconstruction of moss-dominated soil crusts was established and assessed. A large number of protonema were induced to grow by breeding detached green leaves in agar-solid Knop medium after 1 month as day/night temperature and humidity were set at 20/10°C and 60–85%, respectively. Moss-dominated crusts formed through transplanting protonema into sand supplemented with liquid Knop medium under day/night temperatures of 25/15°C after another month.

Key words: ecological restoration; moss dominated soil crusts; *Tortula desertorum* Broth

Introduction

BIOLOGICAL SOIL CRUSTS (BSCs) are widely distributed in arid and semiarid lands (Belnap, 1995). The integrated BSCs are susceptible disturbance, but if intact, appear to play a role in providing nutrients, especially nitrogen, to higher plants in arid regions (Belnap, 1995, 2003; Gretarsdottir *et al*., 2004). A reduction in BSC-mediated ecosystem function under anthropogenic disturbance is both a component and accelerator of desertification (Belnap *et al*., 1994; Buttars *et al*., 1998). BSCs are typically composed of cyanobacteria, fungi, bacteria, lichens, and mosses (Belnap, 2002; Hawkes, 2003). Moss-dominated crusts represent the successional climax community of biological crusts, and are often richer in diversity than the other crusts. Such crusts take decades to develop their component species and community functions. Therefore, the growth information and micropropagation methods of desert mosses and other microbes are valuable for the restoration of BSCs.

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The potential utility of mosses for developmental and tissue culture protocols has been studied for more than 50 years (Duckett *et al*., 2004; Hohe and Reski, 2005). However, there are few literature reports on desert mosses. Lab cultivation methods for desert mosses and moss-dominated BSCs are also sparse. The artificial cultivation of desert moss has encountered an obstacle in exploring mechanisms of resistance to abiotic stresses or reconstruction of moss dominated crusts. Tian *et al* (2005) investigated desert moss regeneration characteristics of different explants in soil crust using *Bryum* and *Didymodon*. Bowker *et al*. (2000) and Stark *et al*. (2004) have assessed the relationships between sex-specific rates of leaf regeneration potential through cultivation of *Syntrichia* caninervis and surveyed the effects of abiotic stresses such as heat or desiccation on leaf regeneration (Stark *et al*., 2005a, 2005b; Stark and McLetchie, 2006).

The purpose for the reconstruction of BSCs is to rapidly propagate the desert moss. Some studies have been published on the cultivation of the desert moss *T. desertorum*. Nie *et al*. (2005) utilized the fragments of *T. desertorum* gametophytes to study the effect of different sand burial depths on regeneration, and results showed that maximal ratios of new shoots to primary gametophytes were less than 45%. Stark and McLetchie (2006) pointed out that each juvenile leaf of *S. caninervis* regenerated more than three new shoots. Our former work has shown that a whole gametophyte of *T. desertorum* taken as explant produced about 1.5 shoots per gametophyte when cultivated in soil from farm land (Xuan *et al*., 2004a). Asexual methods of gametophyte reproduction are important in population development and maintenance for mosses, especially desert mosses, that is, *T. desertorum* (Stark *et al*., 2004). It is necessary to select optical explants to promote shoot number and biomass output of the moss. The growth time of *T. desertorum* is focused on the period from spring to summer. Two to 3°C temperature alterations will induce significant changes of biomass (Xuan *et al*., 2004a). Which period is the most important stage: late spring or early summer? It is significant to know the preferred growth time for reasonable ecological restoration. Which minerals are crucial in the desert moss cultivation, and do the desert mosses have the same cultivation protocol as others? The objective of this study was to answer the above questions, and the desert moss *Tortula desertorum* (Mitt.) Broth. (also known as *Syntrichia caninervis*) is chosen to explore a protocol for rebuilding moss-dominated soil crusts. The effects of different media, explants, and environmental factors on shoots growth were investigated. Furthermore, the life cycle of *T. desertorum* in lab cultivation was studied. Based on the cultivation of *T. desertorum*, methods for artificial reconstruction of BSCs were developed.

Materials and Methods

Methods

For micropropagating the desert moss *T. desertorum*, the main factors to be taken into account included nutrition sources, explants, relative humidity (RH), photoperiod, light intensity, sterilization methods, hormones, and temperatures; these were tested to optimize cultivation conditions (Duckett *et al*., 2004). In contrast to the majority of seed plants *in vitro* culture systems, *in vitro* cultivation of bryophytes usually grow photoautotrophically. Hence, physiology and development of bryophytes *in vitro* are probably quite similar with their natural conditions such as light intensity and growth temperature (Bopp, 1983). Nutrition sources, growth temperature, and explants are the main factors. Here, their effects on asexual reproduction of the desert moss T. *desertorum* were studied, respectively. In addition, the effects of RH, light intensity, and photoperiod on cultivation were investigated by an orthogonal test design.

For construction of moss-dominated crusts, sand (diameter <0.5 mm) was used as substrate. The sand collected from Huangpu River was sieved through a 0.5-mm mesh and washed three times by diluted hydrochloric acid of 10% m/v and sodium hydroxide 10% m/v, respectively, to remove the absorptive substances. The adsorptive organic matter and inorganic salts would influence the medium's components, so these should be washed away. Then the sand was autoclaved for 120 min at 180°C and loaded in Petri dishes as the substrate of biological soil crusts.

Nutrient sources

Six kinds of inorganic media, Benecke's medium (Be), Nitsch medium (Ni), Murashige and Skoog's medium (MS), Modified MS-Medium (MM), Knop medium (Kn), and Kofler medium (Ko) were applied in moss tissue culture after being sterilized. The main inorganic salts of those media are shown in Table 2 (Bopp, 1983; Cove, 1983). Soil collected near the metapopulation (called *In situ* soil), was used in moss cultivation. Organic matter (OM) used in the nutrient sources was measured by the potassium dichromate oxidation method. N (total N, TN; alkaline hydrolysis N, AN) contents of soil crust were determined by the Kjeldahl digestion respectively (KJELTEC 2300 FOSS, Inc. Denmark) (Keeney, 1982; Nelson and Sommers, 1982). Available P (AP) was measured by the Olsen method (Olsen *et al*., 1954) and total P content colorimetrically after digesting soil with perchloric acid. Total K (TK), water-soluble K (AK), and soil-exchangeable Ca were determined by inductively coupled plasma mass spectroscopy (Iris Advangtage 1000, Thermo Elemental Corp., Billerica, MA).

Table 1. The Content of Inorganic Salts in Different Media

	Inorganic salts (mg L^{-1})				
Media	*$NO3^-$*	*$NH4^+$*	*$PO4^{3-}$*	*K^+*	*Ca^{2+}*
Be	155	45	70	28	36
Ni	1096	162	48	386	45
MS	244	37	12	5	15
MM		570	1	5	1
Kn	680		176	115	170
Ko	139	2	171	170	42

Murashige and Skoog's medium (MS), Nistch medium (Ni), Beneche medium (Be), Knop medium (Kn), modified MS medium (MM), and Kofler media (Ko) were always used in moss tissue culture.

Table 2. The Nutrient Contents of Moss Dominated Crusts and Algae Crusts Collected from Gurbantunggut Desert

Type of biological crust	*Organic matter (%)*	*Total content (%)*			*Available content (mg kg^{-1})*			*Soil exchangeable Ca*
		TN	*TP*	*TK*	*AN*	*AP*	*AK*	
Algae crust	0.115 ± 0.009	0.007 ± 0.0008	0.023 ± 0.0010	1.50 ± 0.117	28.43 ± 1.974	5.74 ± 0.453	152 ± 6.8	219 ± 17.1
Moss crust	0.231 ± 0.018*	0.010 ± 0.0003	0.0235 ± 0.0024	1.55 ± 0.046	32.56 ± 0.997	6.57 ± 0.231	118 ± 8.7	240 ± 14.5

The layers of moss and algae were removed from two types' crusts by scoop, and then the residual of biological crusts were used for determining the nutrient contents. Means (± SE, $n = 3$) followed by asterisks represent the significant difference between two types of crust.

Culture temperature

The influences of low temperature between 5 and 10°C in culture on *T. desertorum* have been studied in our former work (Xuan *et al*., 2004a). The temperature range in experiments depends on the natural requirement of the particular species (Glime and Acton, 1979). Therefore, to investigate the effects of slightly higher temperatures on reproduction, the desert moss *T. desertorum* was exposed to two temperature regimes: 20/10°C and 25/15°C, which are similar to late spring and early summer temperatures.

Explants

Tortula desertorum

Dominated crusts were collected from Gurbantunggut desert of Xinjiang, China, in September, 2005. These crust patches were defined as a series of more or less contiguous clumps. *T. desertorum* gametophytes were washed by double distilled water and dried in a ventilation chamber. The dried gametophytes stored at room temperature in black polyethylene bags. The leaves of *T. desertorum* were differentiated as juvenile, green, yellow-green, and yellow. Leaf age was calculated by assuming an annual growth interval of 0.25 mm after subtracting the measured distance from the point of attachment to the shoot apex as described by Stark *et al*. (2004). Various tissues including apical shoots, apical cut gametophytes, and detached leaves at each age were used to select the optimal reproduction materials.

Observations

After being cultured in Petri dishes, all tested materials were placed into growth chambers (Jiangnan, China) and the chamber settings were changed according to different intentions. From the fifth day after cultivation, each treated explant was examined under a dissecting microscope (Jiangnan XTB-01, China), noting protonema presence and shoot number every 3 days through 2 months. The areas of protonema extension were measured using image analysis software (Image Pro Plus 5.0, Media Cybernetics, Inc., Bethesda, MD). The biomass in each test was weighed at the end of culture. The ultrastructure of moss dominated soil crust was investigated by Scanning electron microscopy (FEI Sirion 200, U.K.).

Statistics

Viability, protonema occurrence, days for protonema emergence, protonema extension, and shoot number was

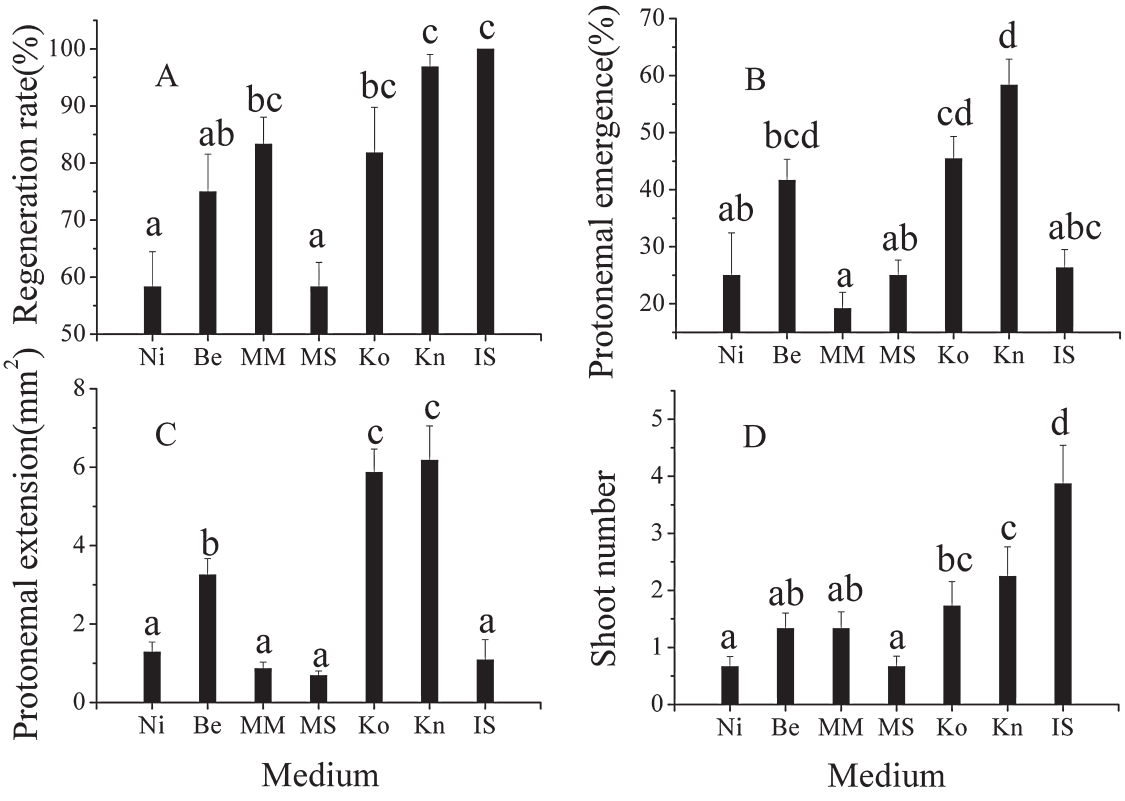

FIG. 1. Regeneration rate (**A**), protonema emergence rate (**B**), mean protonema extension (**C**), and shoot number (**D**) per gametophyte were set as indexes to assess different minerals salts effect on regeneration potential of the desert moss *Tortula desertorum*. Thirty of gametophytes were cultivated in three Petri dishes containing each kind of medium and used to calculate the viability, locations of new shoots, protonema emergence time, and extension shoot number. *In situ* soil (IS) was directly used as the substrate and nutrient source, and the others used sand as substrate supplemented with liquid media. All data were determined after 2 months cultivation. Error bars represent standard deviation ($n = 3$). Different small letters within rows are significantly different ($p < 0.05$) according to Duncan's multiple range test

TABLE 3. EXPLANTS EFFECT ON REGENERATION POTENTIAL OF *TORTULA DESERTORUM* AFTER 1 MONTH CULTIVATION

Explants	*Viability (%)*	*Locations of regeneration*	*Days to protonema emergence*	*Mean protonema extension (mm^2)*	*Shoot number*
Shoot tip	100 b	Apical and basal	5 ± 0.6 a	7.56 ± 1.80 d	2.6 ± 1.0 b
Tip cut segment	87 b	Medial	10 ± 2.2 bc	4.66 ± 2.63 c	3.5 ± 1.2 c
Juvenile leaf	100 b	Medial and basal	6 ± 0.8 ab	2.47 ± 0.88 b	0.7 ± 0.4 a
Green leaf	97 b	Apical and basal	9 ± 1.5 ab	4.29 ± 1.75 c	0.2 ± 0.1 a
Green-yellow leaf	93 b	Medial	11 ± 1.2 c	2.26 ± 1.38 b	0 a
Brow leaf	*37 a	Apical	23 ± 5.6 d	0.44 ± 0.21 a	0 a
†Fragment of Gametophyte	—	—	3	—	0.41

All explants were cultured with *italic* soil under temperature 20/10°C (day/night). Thirty of each kind of explants regenerated in three Petri dishes is used to calculate viability, locations of new shoots, protonema emergence time and extension, shoot number; *data was determined after 2 months cultivation; †data were collected from previous report (Nie *et al*., 2005). Means (± SE, $n = 3$) followed by different letters within rows are significantly different ($p < 0.05$) according to Duncan's multiple range test.

each analyzed separately be means of a one-way ANOVA. Viability was expressed by protonema or bud emergence. There were 30 explants cultivated in three Petri dishes for each tissue culture condition. The orthogonal test design was set at two replicates and analyzed by range analysis. Data were analyzed for significance using the Student's *t*-test and Duncan's multiple range test ($p \leq 0.05$).

Results and Discussion

Effect of different media on regeneration potential

The effects of nutrient source on moss regeneration were investigated through cultivating the gametophytes on sand with different liquid media. *In situ* soil was used as a consult. The main inorganic salts of several media and *in situ* soil are shown in Table 1 and Table 2, respectively. Each medium had different ratios of the main inorganic salts (Table 1). *In situ* soil from different types of crust had low content of organic matter and high content in TP, TN, TK, and AK (Table 2). There were no significant differences in several nutrient contents between two types of soil crusts, except for the content of organic matter. The effects of several media on moss reproduction potential are shown in Fig. 1. *T. desertorum* cultivated in sand with Kn media and *in situ* soil had higher viability than these cultivated with the other media (Fig. 1A). The gametophytes in Kn medium tended to produce protonemata and yielded larger area of protonema than those in the other media (Fig. 1B and C). The gametophytes cultivated with *in situ* soil produced the most shoots (Fig. 1D), and Kn took second place. The results showed that both Kn and *in situ* soil were suitable for micropropagation of *T. desertorum* and that different inorganic salts had various effects on asexual reproduction. The content of exchangeable Ca was higher in sand with Kn medium and *in situ* soil than in the other media. There was a positive linear correlation between Ca content and shoot number ($R^2 = 0.84$) (Tables 2 and 3, and Fig. 1). This suggested exchangeable Ca had a role in enhancing shoot differentiation (Zhang

FIG. 2. The detached green leaves cultured with *in situ* soil under day night temperature at 20/10°C (**A,** original magnification ×60) and 25/15°C (**B,** original magnification ×60) after 4 weeks. Protonema (an arrow in **A**), original leaf and shoot (arrows in **B**) emerged during cultivation

Table 4. Orthogonal Test Design and Visualized Analysis of *Tortula desertorum* for Relative Humidity (A), Light Intensity (B), and Photoperiod (C) Optimization

	Factors			Results	
				Protonema emergence area (mm^2)	
No.	A (Relative humidity) (%)	B (Light intensity) ($\mu mol\ m^{-2}\ s^{-1}$)	C (Photoperiod) (h)	I	II
1	1 (< 30)	1 (30)	1 (12)	0.61	0.75
2	1	2 (60)	2 (14)	1.45	1.42
3	1	3 (120)	3 (16)	2.85	2.25
4	2 (65–80)	1	2	8.20	10.4
5	2	2	3	16.76	15.8
6	2	3	1	12.25	10.26
7	3 (90)	1	3	10.80	6.57
8	3	2	1	4.57	5.26
9	3	3	2	8.55	6.80
T_1	1.56 a	6.22 a	5.62 a		
T_2	12.28 c	7.54 a	6.14 a		
T_3	7.09 b	7.16 a	9.17 b		
R	10.72	1.32	3.35		

The areas of protonema emergence were measured after 1 month cultivation and there were two replicates (I and II) for the statistics. Different small letters within rows are significantly different ($p < 0.05$) according to Duncan's multiple range test.

et al., 1995). Duckett *et al*. (2004) pointed out different nitrogen compounds in chemical speciation had diverse influences on moss development. However, there were no significant differences between nitric nitrogen and ammonia nitrogen in cultivation of desert moss *T. desertorum* (seen in Fig. 1). The chelating agent EDTA did enhance the bud formation of moss *Bartramidula bartramioides* (Rahbar and Chopra, 1983); however, it seldom took a role in that of the desert moss *T. desertorum* in the MS and MM media (Fig. 1D). This phenomenon suggested there were dramatic differences among various mosses in choosing suitable compounds to induce buds (Duckett *et al*., 2004; Hohe and Reski, 2005).

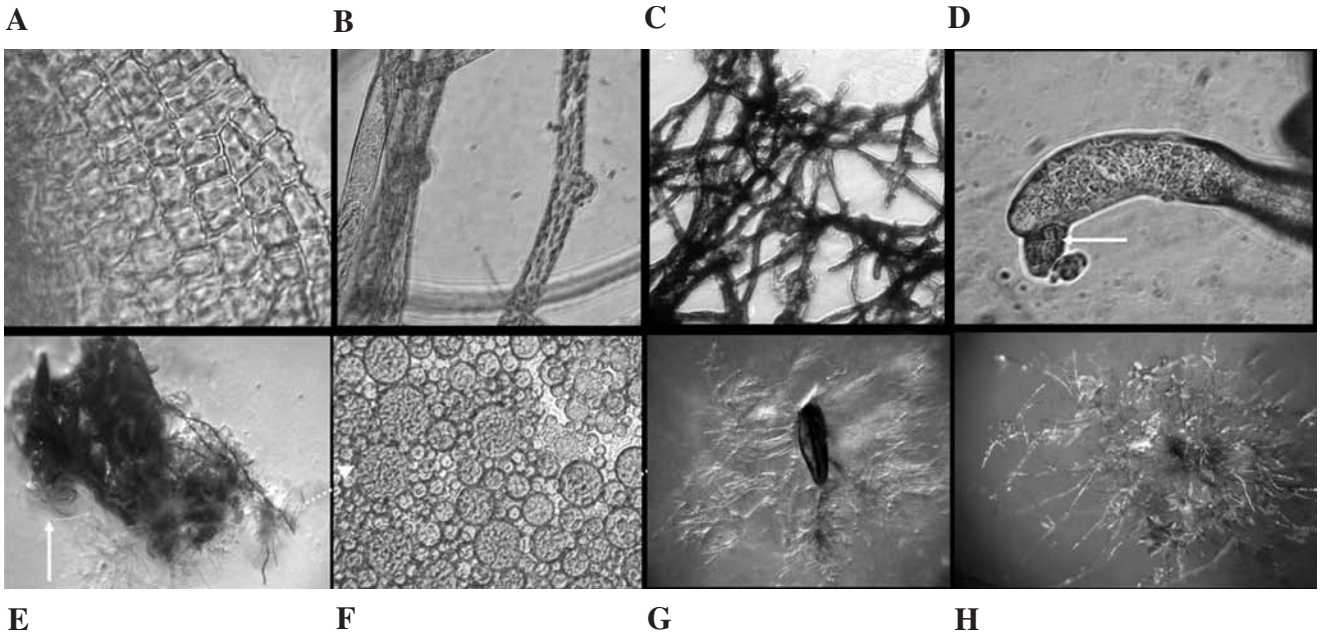

FIG. 3. The simplified life cycle of the desert moss *T. desertorum* in tissue culture by using apical shoot (**A–F**) or detached green leaves (**G, H**) as explants after cultured in agar-solid Kn. (**A**) Photograph of the leaf recovery from desert condition after 3 days (original magnification ×400); (**B**) Photograph of chloronema emerges after 1 week culture (original magnification ×400); (**C**) Photograph of plenty of caulonema after 2 weeks (original magnification ×100); (**D**) Photograph of magnification the part of caulonema, an arrow indicates a gamma (original magnification ×1000); (**E**) Photograph of the apical shoots of gametophyte after being cultured 4 weeks. A real arrow indicates new bud (original magnification ×15); (**F**) Photograph of alga contamination, a dash arrow indicates the magnification of part from E (original magnification ×400); (**G**) Photograph of protonemata emerged aside the detached leaf after 4 weeks (original magnification ×75); (**H**) Photograph of protonemata and shoots around the detached leaf after 2 months (original magnification ×75).

Effect of different explants on regeneration potential

The effect of different explants on moss regeneration potential was assessed by taking *in situ* soil as substrate and nutrient source. Regeneration of *T. desertorum* was significantly affected by explants (Table 3). Shoot tips and juvenile leaves had 100% viability, slightly higher than other parts of gametophytes. The locations of shoot regeneration ranged from the apex to the base of the explants. Protonema emerged earliest in apical shoots and the average area of protonema extension was 7 mm^2 after 1 month cultivation. The detached green leaf also produced 4.29 mm^2 of protonema. The viability of brown leaves was significantly lower than the other explants and their recovery needed more than 20 days. There were no significant differences in shoot number among detached leaves at all ages but the situation was exactly the opposite in protonema extension area. Using the detached leaves as explants, each gametophyte produced more 100 shoots after 2 months cultivation, which was 10 times the rate of shoot production of the sum of shoot tip and tip-cut gametophytes and 200 times the rate of shoot production by fragments.

Effect of culture temperature on leaf regeneration

The temperatures between 5 and 25°C always fit moss growth (Duckett *et al*., 2004). The assessment of temperature effects on the leaf regeneration potential was carried out by cultivating detached green leaves using *in situ* soil as substrate at different day/night temperatures (Fig. 2). The detached leaf bred larger area of protonemata at 20/10°C (day/night) (Fig. 2A) than at 25/15°C (Fig. 2B) but yielded fewer shoots than in the latter. The results showed that temperature had a significant influence on bud formation for desert moss *T. desertorum*. This difference might originate from the induction of meristems changing to bud. On the one hand, low temperature improved the extension of protonema, whereas slightly higher temperatures promoted shoot formation in soil culture. The optimal developmental temperature may be up to 25°C tropical mosses (Chopra and Rawat, 1973). This finding demonstrated the thermotolerance of *T. desertorum*, similar with a previous study (Stark and McLetchie, 2006). On the other hand, the results revealed that the suitable time period for reproduction in the desert moss *T. desertorum*, especially for shoot development, is in early summer, which is the best season for restoring BSCs.

Effect of other environmental factors on leaf regeneration

RH, light intensity, and photoperiod were optimized through cultivation of the detached green leaves with *in situ* soil. All leaves for cultivation were not sterilized. The area of protonemal emergence was set as a parameter to assess the interaction among these three environmental factors (Table 4). There was no significant difference between the results of two parallel groups ($p > 0.05$). The influence on protonemal emergence area of the different factors decreased in the order: A (relative humidity) > B (Photoperiod) > C (light intensity) according to the range (R) values. The optimization combination of the three environmental factors for protonemal development was A2B2C3 (shown in Table 4). That is to say, the detached leaves could produce a maximal amount of protonema with RH of 60–85%, light intensity of 60 μmol m^{-2} s^{-1} and photoperiod of 16 h. Duncan's multiple range tests showed that different levels of two factors A (RH) and B (photoperiod) significantly affected the protonema growth. The results suggested that the humidity was the most important inhibitor for the desert moss development in natural conditions and it, either exorbitant (>90) or too low (<30), would impact the moss reproduction. The 16 h of light period was suitable for the growth of protonema and light intensity did not significantly influence the cultivation. Those findings indicate a broad range in light adaptability of the desert moss *T. desertorum*.

The effects of hormone on moss development have been extensively studied by several research groups (Bopp, 1983; Schumaker and Dietrich, 1998; Duckett *et al*., 2004). Influence of 2, 4-dichlorophenoxyacetic acid (2, 4-D), naphthaleneacetic acid (NAA) and gibberellic acid (GA3) on the development of *T. desertorum* had been investigated in our former studies (Xuan *et al*., 2004b). Maximal number of shoots emerged in

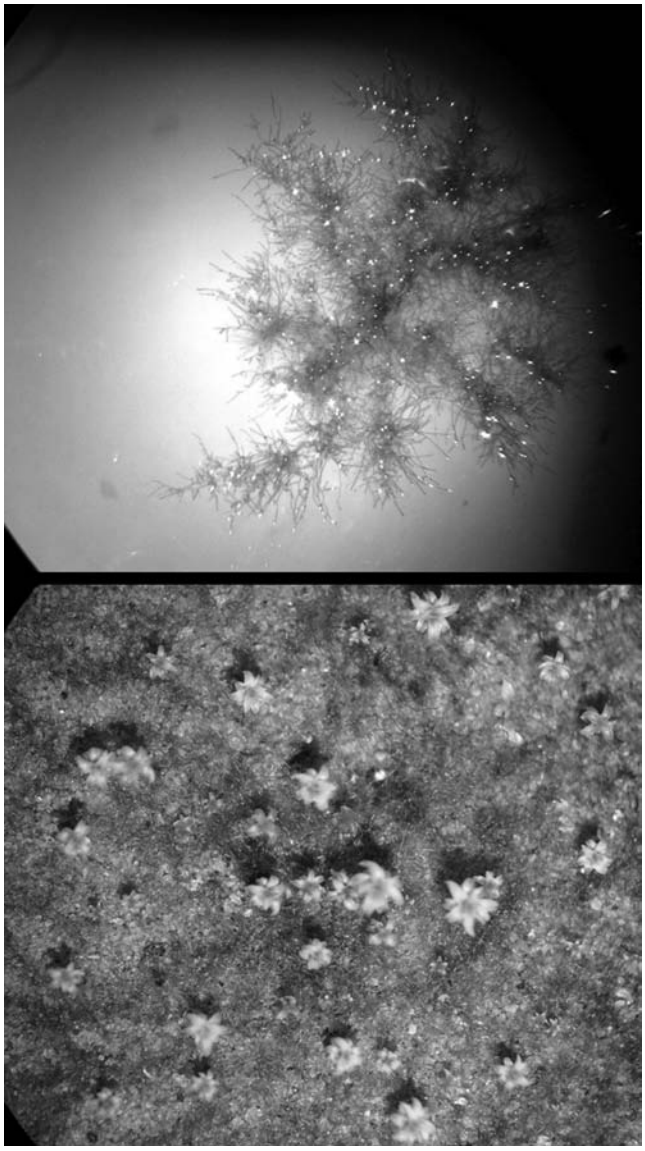

FIG. 4. Photograph of lots of protonema emerged around of a green leaf after 1 month cultivation (**A,** original magnification ×60). Protonema detached from one green leaf were cultured in sand at day/night temperature 25/15°C after 1 month cultivation and a piece of biological soil crust formed (**B,** original magnification ×60).

soil collected from farm land containing 2, 4-D. However, a majority of media containing sucrose or any hormone were contaminated by plenty of fungi, and the sterilized leaves produced little protonemata after 2 months of cultivation using agar-solid Kn in our preparative tests (data not published). There were large areas of fungi emergence in Petri dishes when leaves were sterilized. So one should be cautious in using the hormone in cultivation of *T. desertorum*.

Life cycle of T. desertorum *in cultivation*

Before large scale breeding of new tissues, the life cycle of the desert moss *T. desertorum* in culture was investigated by using apical shoots and detached green leaves cultivated in agar-solid Kn. The culture conditions were set as: day/night temperature at 25/15°C, photoperiod at 16 h, light intensity from 85 to 120 μmol m^{-2} s^{-1} and humidity at 65–80%. The profile of *T. desertorum* development in Kn is shown in Figure 3. The moss leaves resumed from the desiccation condition and become greener (Fig. 3A) after 3 days of cultivation and chloronema emerged after another 3 days (Fig. 3B). Caulonema extended over the Petri dishes (Fig. 3C) and several protonemal gemmae were magnified in Fig. 3D. However, these gemmae failed to form gametophytes by the end of cultivation. Some new shoots grew out from basal leaves (undetached from stem) (Fig. 3E) and patches of desert alga emerged in Kn (Fig. 3F) after 1 month cultivation. The area of algae extension was three times more than that of protonema extension, suggesting desert algae grew well in agar-solid Kn. There were lots of protonema (Fig. 3G) and more than 25 new shoots appeared in Kn using detached leaves as reproduction materials (Fig. 3H). These results mean that the detached leaves and undetached leaves had two different modes to produce shoots in Kn, that is, the former depending on protonemata, the latter on leaf meristems, but the two reproductive procedures were influenced by different media (Fig. 2B, Fig. 3H). The above work illustrates that the asexual reproduction of the desert moss *T. desertorum* probably depends on protonemal gemmae developing into gametophytes.

Construction of moss-dominated soil crusts

The detached green leaves took the place of spores to cultivate the protonema because of the infrequency spores in natural conditions (Stark *et al*., 2004) or in lab cultivation (shown in Fig. 3). Temperature and photoperiod in the growth chamber were set at 20/10°C and 16 h, respectively. After 1-month cultivation, about 6.5 cm^2 per leaf of protonemata emerged in agar-solid Kn (Fig. 4A). The protonemata then were transferred onto other Petri dishes supplemented with sand as a substrate and liquid Kn as a nutrient source. The day/night temperature shifted to 25/10°C. More than 40 shoots emerged after another month of cultivation and a patch of incipient moss-dominated crust of more than 15 cm^2 formed (Fig. 4B). A piece of integrated moss dominated crust needs more than 10 years to develop according to the published reports (Zhang *et al*., 2002; Stark *et al*., 2004). The technology for construction of incipient moss dominated crusts in the present study only requires 2 months. This method developed herein indicated a moss-dominated incipient crust population could be obtained in the lab in 2 months of cultivation. However, whether it was feasible to use such incipient crusts in field reclamation studies of the crusts requires further research.

The comparison of artificial crust and wild crusts in cover and ultrastructure is shown in Fig. 5. The gametophytes seemed slightly fine in artificial crust and the intervals among them larger than that in wild type (Fig. 5A and B). The rhizoids originated from protonema closely twined with sand (Fig. 5C), similar with the phenomenon in wild-type crust (Fig. 5D). How to successfully transplant the incipient BSCs from lab to desert was an obstacle for ecological restoration, and several schemes have been chosen for further studies. The function of artificial crust at sand-fixing and capability of antiwind erosion would be monitored and modified following the transplantation.

In conclusion, the procedure for construction of moss dominated crusts was divided into two steps. First, the detached leaves were cultivated in agar-solid Kn for 1 month

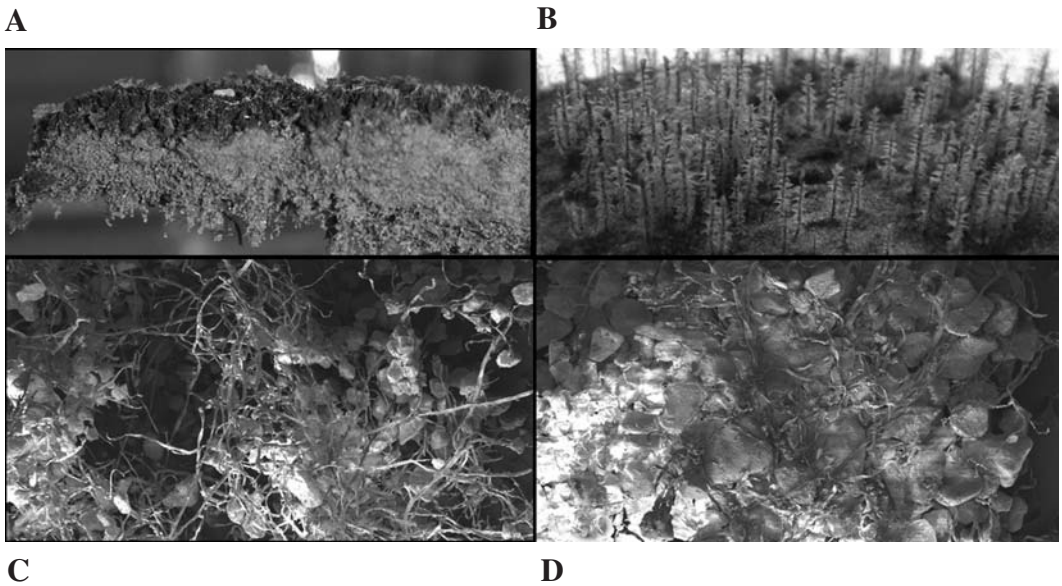

FIG. 5. Photographs of both wild-type (**A,** original magnification ×5.6; **C,** original magnification ×100) and artificial moss dominated (**B,** × 6; **D,** ×170) crusts by light microscopy and scanning electron microscopy.

to induce protonemata. Day/night temperature, light intensity, light period, and RH were set at 20/10°C, 120 μmol m^{-2} s^{-1}, 16 h and 65–80%, respectively. Then, those protonemata were transferred onto sand supplemented with liquid Kn for another month of cultivation to construct moss dominated soil crusts with the day/night regime changed to 25/15°C. This protocol not only provided enough materials to study functional genes and proteomics of the desert moss *T. desertorum*, but also established a foundation of reconstructing moss dominated soil crusts in arid regions.

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

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