Effects of storage temperature on the physiological characteristics and vegetative propagation of desiccation-tolerant mosses

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Abstract. Mosses, as major components of later successional biological soil crusts (biocrusts), play many critical roles in arid and semi-arid ecosystems. Recently, some species of desiccation-tolerant mosses have been artificially cultured with the aim of acceleratingto speed up the recovery of biocrusts. Revealing the factors that influence the vegetative propagation of mosses, which is an important reproductive mode of mosses in dry habitats, will benefit the restoration of moss crusts, which is an important reproductive mode of mosses in arid and semi-arid regions. In this study, three air-dried desiccation-tolerant mosses (*Barbula unguiculata*, *Didymodon vinealis* and *Didymodon tectorum*) were hermetically sealed and stored, air-dried and hermetically sealed at five temperature levels (0 ℃, 4 ℃, 17 ℃, 25 ℃ and 30 ℃) for 40 days. Then, the vegetative propagation and physiological characteristics of the three mosses were investigated to determine the influence of storage temperature on the vegetative propagation of desiccation-tolerant mosses and its the mechanism. The results showed that the vegetative propagation of the three mosses varied with temperature, and the. The most significant variation change in vegetative propagation among storage temperatures was observed in *D. tectorum*, followed by the variation observed in *B. unguiculata*after storage at different temperatures. ConverselyIn contrast, no significant difference in propagation among temperatures was found in *D. vinealis*. Only the germination percentage of *B. unguiculata* was not significantly different at all storage temperatures. The enhancement in regenerative capacity of the three mosses was accompanied by anincreased with increasinged temperature from 0 ℃ to 17 ℃, accompanied by a decrease in malondialdehyde (MDA) content, and decreased thereafter and a decrease beyond that. The malondialdehyde (MDA). As the temperature increased, the chlorophyll and soluble protein contents increased in *B. unguiculata* but decreased in *D. vinealis* and *D. tectorum*. As to storage, the MDA and soluble sugar contents have increased after storage. The MDA content of the three mosses increased to each of the investigated temperatures by more than 50% at all of the investigated temperatures; meanwhile from the initial values;; and the soluble sugar content increasedbecame higher than before in the three mosses. However, a decreasing trend was observed in the MDA content from 0 to 17 ℃. As the temperature increased, the contents of chlorophyll and soluble protein in *B. unguiculata* increased, while those in *D. vinealis* and *D. tectorum* decreased. The integrity of cells and their cell membranes is probably likely the most important factor influencing the vegetative propagation of desiccation-tolerant mosses. Although a A 40-day storage period caused cell injury.,, ourOur results suggested that the storage temperature could can enhance or suppress such injury and change the vegetative propagation

regenerative capacity of the three mosses. ~~It could be concluded~~The data indicate that the suitable storage temperature ~~of~~ is 4 ℃ for *B. unguiculata* ~~was 4 ℃,~~ and 17 ℃ ~~the optimal temperature~~ for both *D. vinealis* and *D. tectorum* ~~was 17 ℃~~.

1 Introduction

Biological soil crusts (biocrusts) are composed of microscopic (cyanobacteria, algae, fungi, and bacteria) and macroscopic (lichens, mosses) poikilohydric organisms ~~that occur on or within the top few centimeters of the soil surface~~ (Belnap et al., 2016). ~~They~~ Biocrusts are widely distributed ~~and play important roles~~ in ~~many~~ arid and semi-arid ecosystems and play important roles ~~, such as~~ in soil surface stabilization, soil fertility enhancement and soil hydrology regulation (Belnap and Lange, 2003). As major components of later successional biocrusts, mosses exert~~ed~~ much stronger ecological functions than do cyanobacteria (Seppelt et al., 2016; Gao et al., 2017; Lan et al., 2012). Thus, some researchers suggest artificially culturing moss biocrusts on degraded soil surfaces ~~so as~~ to ~~speed up~~accelerate the recovery of degraded arid and semi-arid ecosystems (Belnap and Eldridge, 2003; Zhao et al., 2016). Recently, some mosses have been investigated by culturing gametophytes (Jones and Rosentreter, 2006; Xiao et al., 2015). However, cultivation research on moss crusts remains tentative, potentially due to the lack of knowledge regarding the vegetative propagation of mosses~~little is known about the vegetative propagation of such mosses, which may be an important reason why the cultivation research of moss crusts is still tentative~~.

Vegetative propagation is an important reproduction mode of bryophytes (hornworts, liverworts and mosses) in dry habitats, and gametophyte fragments may serve as the dominant inoculum in mosses (Mishler, 1988; Tian et al., 2005). ~~So far~~To date, ~~a number of~~several moss cultivation experiments have been conducted in which~~that used~~ gametophyte fragments ~~have been conducted~~are used to establish new colonies in the laboratory and field (Cleavitt, 2002; Jones and Rosentreter, 2006; Xiao et al., 2015). All ~~such studies~~ of these experiments have demonstrated that artificial cultivation ~~could speed up~~can accelerate the succession process of moss crusts. For example, Antoninka et al. (2016) found that the coverage and biomass of mosses on an ~~the~~ artificially inoculated soil surface increased ~~faster~~ more rapidly than ~~that~~ they did on uninoculated soil. ~~Furthermore, s~~Some researchers have suggested that ~~the~~ inoculation material should be mass-produced by vegetative regeneration with rapid development (Jones and Rosentreter, 2006; Mishler, 1988) because of the need for moss biocrusts to inoculate large areas. ~~Although the~~ The factors that influence~~d~~ the tissue cultivation of mosses have been investigated for ~~a long time~~many years (Duckett et al., 2004; Hoffman, 1966; Sabovljevic et al., 2003)~~;~~, however, the mechanism of moss regeneration ~~still needs to be further studied~~remains unclear.

After mosses regenerate protonema and gametophytes suffer desiccation stress, desiccation ~~-~~tolerance (DT) has a critical influence on their survival and restoration abilities (Proctor et al., 2007). Adult gametophytes of some species can ~~even~~ recover physiological activities and generate new shoots after being stored for more than ten years in a desiccated state (Stark et al., 2017; Keever, 1957)~~, because the metabolism of .~~ ~~d~~Desiccation-tolerant mosses ~~is suspended under water stress, and cell integrity can be maintained~~ can suspend metabolism and maintain cell integrity during ~~the~~ dry periods (Mansour and Hallet, 1981; Platt et al., 1994)~~. Then the~~; then, within a few minutes to a few hours after

being rehydrated, they can resume cellular activity ~~of mosses will resume~~ and return to a normal, hydrated state ~~within a few minutes to a few hours after being rehydrated~~ (Platt et al., 1994; Pressel et al., 2006). However, the decline and disappearance of the regenerative capacity of *Syntrichia ruralis* showed that long-term desiccation ~~would~~ can cause irreversible damage, despite viability differences among individuals (Stark et al., 2017). It ~~was still~~remains unclear why the potential ~~of~~ for vegetative propagation ~~of~~ in mosses ~~was~~ can be altered by storage~~, or the~~ and why recovery ~~abilities between~~ ability following drought-induced dormancy varies among moss species ~~were different after drought dormancy, which would~~. The lack of knowledge in these areas has impeded the study of moss cultivation.

~~In general, DT investigations concentrate more~~Investigations of DT in mosses have primarily focused on the mechanism and evolutionary history (Proctor et al., 2007; Oliver et al., 2000), with fewer investigations addressing DT in ~~and less on~~ artificial cultivation. ~~There are lots of theoretical~~ However, many studies that DT research can help improve artificial cultivation methods~~support further utilization~~. For ~~instance~~example, the impact of desiccation stress on moss regeneration varies with drying time and storage temperature (Keever, 1957; Burch, 2003), and an understanding of these relationships may guide research on the regenerative mechanism of mosses upon desiccation and their asexual propagation~~artificial cultivation of mosses~~. Furthermore, DT plays essential roles in moss regeneration in dry habitats, ~~which reminds us to investigate~~highlighting the potential value of investigating the ~~link~~ relationships between the physiological characteristics of mosses and their vegetative propagation ~~of the mosses~~. Based on the ~~study results mentioned~~ above observations, it can be hypothesized that (1) dry storage ~~may~~ impacts the vegetative propagation of desiccation-tolerant mosses, (2) ~~the~~ changes ~~of~~ in vegetative propagation after storage ~~may relate to~~involve the influences of storage on the physiological characteristics of mosses, and (3) the degree to which storage affects ~~the~~ vegetative propagation and physiological characteristics is related to the storage temperature.

~~Consequently, i~~In this study, three desiccation-tolerant mosses, ~~including~~ *Barbula unguiculata*, *Didymodon vinealis* and *Didymodon tectorum*, which ~~were~~ are the dominant mosses in biocrust~~s~~ communities in the Loess Plateau region, were ~~selected and~~ stored at five temperature~~s~~ ~~levels~~ (0 ℃, 4 ℃, 17 ℃, 25 ℃ and 30 ℃) for 40 days. Then, (1) the effect of storage temperature~~s~~ on the vegetative propagation of ~~the three~~each mosse~~s~~ and (2) the changes in~~of~~ physiological indices from before to after storage, including the contents of chlorophyll, soluble sugar, soluble protein and malondialdehyde (MDA), were investigated ~~so as~~to reveal the influences of storage temperature on the vegetative propagation of mosses and ~~its~~ the mechanism.

2 Materials and methods

2.1 Study site and moss species

The study was conducted in Ansai Country, Shaanxi Provence, China (36 ℃1'**N,** 109 ℃19'-E~~, 36 ℃1' N~~), which is located in the central part of the Loess Plateau. The elevation of the sampling plot varies from 1,068 to 1,309 m. The plot has a typical semi-arid continental climate, with an average annual temperature of 8.8 ℃, and its average temperature~~s~~ in January and July ~~are~~ is -7.2 and 22.8 ℃,

respectively. The average annual precipitation is 500 mm, with 60% or more of the precipitation falling between June and September (Zhang et al., 2011). ~~In fact, the average monthly precipitation was 11.98 mm when the moss crusts were collected in November 2016, and the average monthly temperature ranged from 9.88 to −3.64 °C~~For the month of November, when the moss crusts were collected, the average monthly precipitation was 11.98 mm, and the average monthly temperature was 9.88 ℃ (high) to -3.64 ℃ (low) (Chinese Central Meteorological Station, 2017). Cyanobacteria and mosses dominate~~d~~ the biocrust communities in this region, and the coverage of moss-dominated biocrusts ~~might even~~can reach ~~around~~ approximately 80% on north-facing slopes in the study region (Zhao et al., 2014).

The moss taxa used in the study were *Barbula unguiculata*, *Didymodon vinealis* and *Didymodon tectorum*, which dominated the moss crusts in ~~different~~ the plot~~s~~. ~~Lots of~~ *B. unguiculata* dominated in woodland areas and was~~were~~ found in shadowed areas and under vegetation coverage~~, which dominated in the woodland~~. *D. vinealis* was widely distributed in the study site ~~under~~ among different water and light environments~~. Samples of~~ and the species were collected from croplands that had been abandoned ~~croplands~~ for more than ten years. The ~~dominated~~ dominant vegetation of the ~~plot~~ croplands was grasses; thus, most of ~~moss crusts~~ *D. vinealis* were exposed to sunlight in the winter. *D. tectorum* grew on side slopes and ~~sometimes~~ were occasionally collected from under the shade of vascular plants.

2.2 Experimental design

~~Each~~Some of the three moss crusts were used to measure initial values of physiological indices (chlorophyll content, soluble sugar content, soluble protein content and MDA content) and germination parameters (gametophyte germination, gametophyte increment and gametophyte vigor index) ~~was separated into two parts as soon as they were~~immediately following their transport~~ed~~ to the laboratory. ~~One part was used to measure initial physiological indices (chlorophyll content, soluble sugar content, soluble protein content and MDA content) and germination parameters (gametophyte germination, gametophyte increment and gametophyte vigor index).~~ The ~~other was~~ rest of moss crusts were stored at one of five temperature levels, i.e., 0 ℃, 4 ℃, 17 ℃, 25 ℃ and 30 ℃. ~~All the~~ Each temperature~~s were~~ was controlled within ± 1 ℃ around the target. On the 41st day of storage, ~~Then,~~ the ~~mosses~~ moss crusts were ~~taken out on the 41st day of storage~~removed, and the physiological indices and germination parameters ~~mentioned~~ described above were measured.

2.3 Moss crusts storage and mosses collection ~~and storage~~

The crusts of t~~T~~hree species of mosses ~~crusts~~ were collected from many colonies and then air-dried in the shade for 24-48 hours ~~after being collected from many colonies, although~~ ; most of ~~mosses~~ crust samples were dried in the field. Then, the samples were transported to the laboratory of the State Key Laboratory of Soil Erosion and Dry-land Farming on the Loess Plateau ~~which is~~ in Yangling, Shaanxi Province. Samples were stored in one of two refrigerators (at 0 ℃ ~~and~~ or 4 ℃) ~~and~~ or one of three growth chambers (at 17 ℃, 25 ℃ and 30 ℃). Before storage, ~~Thus, the~~the moss crusts ~~were packed~~ had been placed in ~~Ziploc baggies~~re-sealable plastic bags to prevent changes in ~~the~~ water content

before being stored, and then they were kept . The samples were stored in the dark under a light-blocking fabric. The measurement of the water contents measurements of the moss gametophytes were all less than 10%, and the equilibrating relative humidity during storage was 55% during storage. After the 40-day dry period, some subsamples of desiccated gametophytes were collected as subsamples to measure the physiological indicesex and germination parameters.

2.4 Measurement of the physiological indicesex and germination parameters

2.4.1 Physiological indicesex

The lLiving mature gametophytes of *B. unguiculata*, *D. vinealis* and *D. tectorum* were collected from the moss crusts. to measureShortly after being rehydrated and washed with deionized water, the gametophytes were measured for the contents of chlorophyll, soluble sugar, soluble protein and MDA shortly after being rehydrated and washed with deionized water. Approximately 0.1 g fresh mass of the gametophytes was used to measure the contents of soluble sugar, soluble protein and MDA in every each replicate, while whereas the measurements of the chlorophyll content used approximately 0.05 g fresh mass as a per replicate. The four indicators were measured by using the following protocols with three replications.

The chlorophyll was extracted by 95% (v/v) ethanol, and then the solution was boiled at 85 ℃ for 5 min. After being centrifuged at 4,000 rpm for 10 min, the chlorophyll in the supernatant was measured at absorbances of 665 and 649 nm with a spectrophotometer (UV-2300, *Techcomp*, Shanghai, China) (Wellburn and Lichtenthaler, 1984).

After the soluble protein was extracted into an ice-cold 50 mmol L^{-1} phosphate buffer (pH 7.8), the suspension was centrifuged at 8,000 rpm for 30 min at 4 ℃, and the supernatant was collected after being centrifuged at 8000 rpm for 30 min at 4 ℃. The soluble protein was stained with Coomassie brilliant blue G-250, and the absorbance was read at 595 nm (Bradford, 1976).

The MDA as well as theand soluble protein was were extracted and centrifuged. Then, the supernatant was homogenized with 0.6% (W/V) thiobarbituric acid dissolved by 1 mol L^{-1} NaOH and 10% (W/V) trichloroacetic acid. The mixed solution was heated at 100 ℃ for 20 min, and then the absorbance was read at 450, 523 and 600 nm (Hodges et al., 1999). The *Techcomp* UV-2300 spectrophotometer was also used to measure the absorbance of the MDA and soluble protein.

The sSoluble sugar was extracted by distilled water at 100 ℃ for 30 min. After being filtered and diluted, the extract was added to an anthrone–sulfuric acid solution. The mixed solution was used to measure the absorbance at 620 nm with the a spectrophotometer (UV-1601, *Shimadzu*, Kyoto, Japan) (Morris, 1948).

The fresh weight of gametophytes was measured shortly after rehydration, and then their dry weight was measured after oven drying to a constant weight at 70 ℃ (Schonfeld et al., 1988). Both of themThe fresh and dry weights were used to calculate the four physiological indices on a dry basis.

2.4.2 Germination parameters

At the same time as the physiological indices was measured, some gametophytes of each of the three moss species were collected to test measure the germination parameters. The loessial soil (uniform soil

texture of Calciustepts) collected from the study region was used to culture the mosses. The soil was sieved through a 0.25-mm mesh and placed in each pore of a 6-well plate~~, whose~~; each pore had a diameter ~~was~~ of 35 mm and a depth ~~was~~ of 12 mm. Then, the soil water content was adjusted ~~by deionized water~~ to 23% (W/W) (the field water holding capacity of the soil) by adding deionized water, and the surface was flattened before inoculation. Five inocula, ~~which were~~representing the top 2 mm of living mature gametophytes of the mosses, were cut, rehydrated, washed and placed ~~separately~~ in ~~in one~~each well. ~~30~~ Thirty inocula were placed in each 6-well plate as one replication. Three 6-well plates were ~~set~~ established for each moss species. In total, 90 experimental inoculations were ~~set up~~ established for the measurement of germination parameters before and after ~~being stored~~storage at each of the five temperature levels for each moss species. Meanwhile, three 6-well plates without inoculated mosses were set up as experimental controls ~~in the experiment~~ to ~~eliminate~~ control for the effect of other propagules, ~~like~~ such as spores, in the experimental soil ~~used~~. The 6-well plates were wrapped tightly with transparent plastic film~~s~~ to ~~hold~~ retain the soil moisture. ~~After that~~Next, they were ~~put~~ placed into a growth chamber (AGC-D003N, *Qiushi*, Hangzhou, China) to incubate. The parameters of the growth chamber were set ~~as~~ to a 12-h photoperiod (4,500-5,500 Lux), a constant temperature of 17 ℃ (± 1 ℃) and a relative humidity of 60-70%. During the incubation period ~~of incubation~~, deionized water was supplied ~~so as~~ to ~~keep~~ maintain the soil moisture at 23%. The new gametophytes were counted every five days beginning on the day they were found. ~~There were f~~ Five observations ~~altogether during~~ were made over the ~~next~~ subsequent 25 days. ~~The~~ This paper report~~sed~~ the results of cultivation ~~under~~ at the fifth observation. ~~It was noteworthy that no~~No new gametophyte~~s was~~ were found in the blank 6-well plates during ~~all of~~ the entire incubation ~~in the study~~period.~~;~~ ~~however, it~~It was difficult to distinguish protonemal germination between the underside of original inocula and the soil substrate~~;~~. therefore, protonemal growth was not quantified.

By analogy with seed germination, the vegetative propagation of moss gametophytes was described by three germination parameters~~, including:~~ gametophyte germination, ~~the~~ gametophyte increment and the gametophyte vigor index. In this paper, gametophyte germination ~~means~~ is defined as the percent of moss inocula that germinated. ~~The g~~Gametophyte increment ~~means~~ is the average number of new gametophytes ~~in a~~ per 6-well plate. The gametophyte vigor index ~~refers~~ is analogous to the seed vigor index, which ~~was~~ is calculated by multiplying the seed germination percentage by the length of the hypocotyl (Abdul-baki and Anderson, 1973). Here, ~~The~~ the seed germination percentage ~~of seeds~~ and the length of hypocotyl were replaced by the gametophyte germination and gametophyte increment, respectively, ~~in~~ and used to calculate the gametophyte vigor index. ~~Then~~Thus, the germination parameters were calculated by Eq. (1) - (3):

$$\text{gametophyte germination} = \frac{\text{number of germinated inocula}}{\text{number of total inocula}} \times 100\% \quad (1)$$

$$\text{gametophyte increment} = \frac{\text{number of new gametophyte}}{\text{number of total inocula}} \quad (2)$$

$$\text{gametophyte vigor index} = \text{gametophyte germination} \times \text{gametophyte increment} \quad (3)$$

According to Eq. (1) - (3), the gametophyte vigor index ~~could summarily describe~~summarizes the vegetative propagation of the mosses.

2.5 Statistical analyses

The differences in physiological indices and germination parameters among treatments and mosses were tested using ~~a~~ one-way analysis of variance (ANOVA) with Fisher's least significant difference post hoc test (LSD) at $P < 0.05$. The relationships between the physiological indices and germination parameters of the three moss species were quantified by calculating ~~the~~ Pearson correlation coefficients. These statistical analyses were completed using SPSS 22.0.

The effects of physiological characteristics on vegetative propagation ~~was~~ were analyzed by a gray incidence analysis in Microsoft Excel 2010 (Deng, 1984; Lin et al., 2009). The gray incidence degree between each of the reference sequences (physiological indices) and the compared sequence (gametophyte vigor index) was calculated by Eq. (4) - (6):

$$\Delta_i(k) = |y(k) - x_i(k)|, k = 1,2,\dots\dots,n; i = 1,2,3,4 \quad (4)$$

$$\xi_i(X_i,\ Y) = \frac{\min_i \min_k \Delta_i(k) + \rho \max_i \max_k \Delta_i(k)}{\Delta_i(k) + \rho \max_i \max_k \Delta_i(k)}, k = 1,2,\dots\dots,n; i = 1,2,3,4 \quad (5)$$

$$r_i = \frac{1}{n}\sum_{k=1}^{n} \xi_i(k), k = 1,2,\dots\dots,n;\ i = 1,2,3,4 \quad (6)$$

where $\Delta_i(k)$ and $\xi_i(X_i,\ Y)$ are the absolute difference and the gray relational coefficient, respectively, between X_i (physiological ~~indices~~index *i*) and Y (gametophyte vigor index) at point k. The gray relational coefficient (r_i) is between the i_{th} physiological index and its gametophyte vigor index when the distinguishing coefficient (ρ) is 0.5.

The gray incidence degree is the~~a~~ sum of the gray relational coefficients.

3 Results

3.1 The initial ~~state~~ measurement values of the mosses

Table 1 ~~The i~~Initial values of physiological indices~~ex~~ and germination parameters in the three mosses

Index	*B. unguiculata*	*D. vinealis*	*D. tectorum*
chlorophyll content (mg g^{-1})	1.53 ±0.13 a	3.33 ±0.18 b	2.19 ±0.44 a
soluble sugar content (mg g^{-1})	30.02 ±3.67 a	44.13 ±3.41 b	14.19 ±1.77 c
soluble protein content (mg g^{-1})	6.28 ±1.40 a	12.24 ±0.26 b	7.92 ±0.46 a
MDA content (μmol g^{-1})	24.02 ±0.47 a	35.07 ±3.12 b	23.68 ±0.50 a
gametophyte germination (%)	82.93 ±10.00 a	100.00 ±0.00 a	98.33 ±2.36 a
gametophyte increment	1.54 ±0.18 a	1.82 ±0.40 ab	2.37 ±0.05 b
gametophyte vigor index	1.28 ±0.15 a	1.82 ±0.40 ab	2.33 ±0.05 b

Data are average ±1 SE, and different letters indicate significant differences ($P < 0.05$) among the three species.

The three moss species began to germinate new gametophytes from the original inocula at different times, ~~while~~ whereas no gametophyte germinated in the control groups ~~in~~ as of the ~~last~~ final ~~measurement~~ (fifth) ~~(fifth~~ observation~~)~~. *B. unguiculata* germinated on the eleventh day of inoculation, ~~so that~~ and the entire length of its cultivation ~~time~~ period was 35 days. *D. vinealis* and *D. tectorum* each germinated on the sixth day, with a 30-day cultivation period. The initial values of the physiological indices and germination parameters of the three mosses ~~was~~ are shown in Table 1. It can be seen that the four physiological indices and gametophyte germination of *D. vinealis* were significantly higher than those of the other two species. The ~~biggest~~ largest values of gametophyte increment and gametophyte vigor index were found in *D. tectorum*, and the ~~smallest~~ lowest germination parameter~~s~~

values were found in *B. unguiculata*. However, no significant differences in the contents of chlorophyll, soluble protein and MDA between *D. tectorum* and *B. unguiculata* were found.

3.2 Effect of storage temperature on the vegetative propagation of mosses

The germination times of each of the three mosses ~~and controls~~ after storage at each temperature did not differ significantly from~~were the same as~~ the initial ~~state~~values, whereas controls still had no gametophyte. ~~In~~ At the fifth observation, the gametophyte germination of ~~all~~ each of the three species had changed from the initial value by no more than 20% (Fig. 1a; Table 1). The highest gametophyte germination of *B. unguiculata* was 94.44% at 17 ℃. No significant difference was found between the maximum value and minimum value (75.56%, at 0 ℃). In *D. vinealis*, gametophyte germination did not~~there was no~~ significantly differ~~ent gametophyte germination~~ among ~~all~~ the storage temperatures~~, which ranged~~ and ranged from 95.56% (0 ℃) to 98.89% (17 ℃). The only significant difference in gametophyte germination was observed in *D. tectorum* and was between 81.92% and 100% after storage at 0 ℃ and 25 ℃, respectively~~, in the gametophyte germination of *D. tectorum* after being stored~~.

The changes ~~of~~ in gametophyte increment were all more than 20% after ~~being stored,~~ storage except in *D. tectorum* at 30 ℃, for which ~~for~~ a slight decrease of 6.57% was observed ~~in *D. tectorum* at 30 ℃~~ (Fig. 1b; Table 1). After storage, the largest gametophyte increment of *B. unguiculata* was 1.11 at 4 ℃, ~~while~~ whereas the smallest gametophyte increment was 0.81 at 25 ℃. Except for ~~the~~ a significant difference between 4 ℃ and 25 ℃, no significant~~ly difference int~~ difference in gametophyte increment was found among the storage temperatures in *B. unguiculata*~~ after being stored~~. Similarly, no significant~~ly different~~ difference in the gametophyte increment of *D. vinealis* was observed among ~~all~~ the storage temperatures. The maximum and minimum gametophyte increments after storage were 1.03 and 1.23 at 0 ℃ and 17 ℃, respectively, *D. vinealis*. ~~A bigger variation of~~Larger differences in gametophyte increment among the storage temperatures ~~was presented~~were observed in *D. tectorum*~~ at all storage temperatures,~~ except for the difference in gametophyte increment between 0 ℃ and 4 ℃. The maximum gametophyte increment of *D. tectorum* was 3.74 at 17 ℃ after ~~being stored~~storage, and the minimum value was 1.32 at 0 ℃.

The gametophyte vigor index of the three moss species showed significant changes ~~in a~~ over the 40-day storage period (Table 2). The largest change~~s of~~ in gametophyte vigor index after ~~being stored~~ storage ~~was displayed~~were observed in *D. tectorum*, with the index ~~a range~~ranging from a 53.36% decrease (0 ℃) from the initial value to a 57.32% increase (17 ℃). No significant ~~change~~ difference ~~was found~~ in the gametophyte vigor index~~ of *D. vinealis*~~ among the five temperatures was observed in *D. vinealis*. However, ~~these~~ the index values~~gametophyte vigor indices~~ were all significantly lower than ~~that~~ the initial value (before storage), representing ~~and decreased by~~decreases of 32.86% (17 ℃) to 45.65% (0 ℃). After ~~being stored~~storage, the gametophyte vigor index values~~ices~~ of *B. unguiculata* decreased the least by 18.81% at 4 ℃ and the most by 49.20% at 25 ℃, ~~which indicated the change of *B. unguiculata*~~representing changes intermediate ~~was~~ between those of *D. vinealis* and *D. tectorum*.

After the 40-day storage at the five temperatures, the highest gametophyte germination percentages of *B. unguiculata* and *D. vinealis* were at 17 ℃, ~~while~~ whereas the ~~peak~~ highest percentage

in *D. tectorum* was at 25 ℃. The highest gametophyte increment of *B. unguiculata* was at 4 ℃~~, and the~~. ~~peak~~ The highest gametophyte increment values in *D. vinealis* and *D. tectorum* were both at 17 ℃, ~~which was the same for~~ as observed for the gametophyte vigor index values of these two species.

5

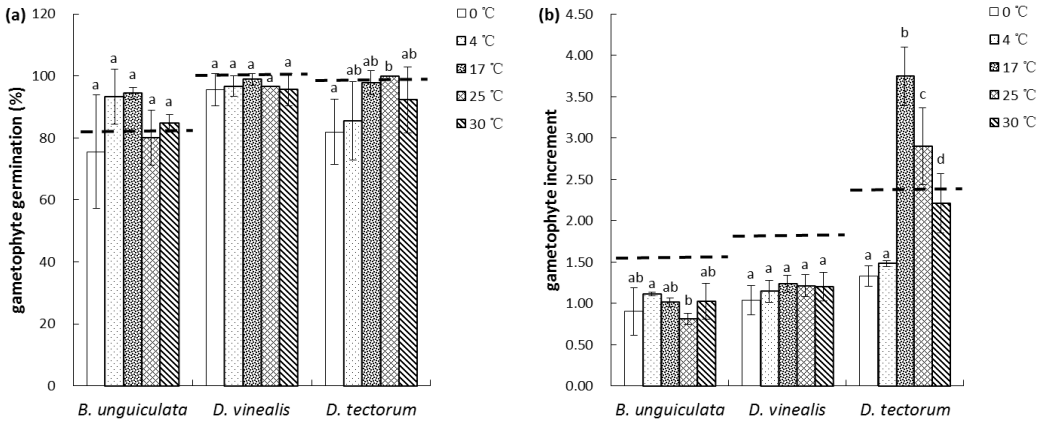

Fig. 1. Data (average ± 1 SE) of the three moss species ~~in~~ on **(a)** gametophyte germination and **(b)** gametophyte increment after the 40-day storage period at each of the five temperatures. Different letters indicate significant differences ($P < 0.05$) among the five temperatures within the same species. Dotted lines represent the approximate values of the ~~three species in~~ two germination parameters before storage for each species (the true values are shown in Table 1).

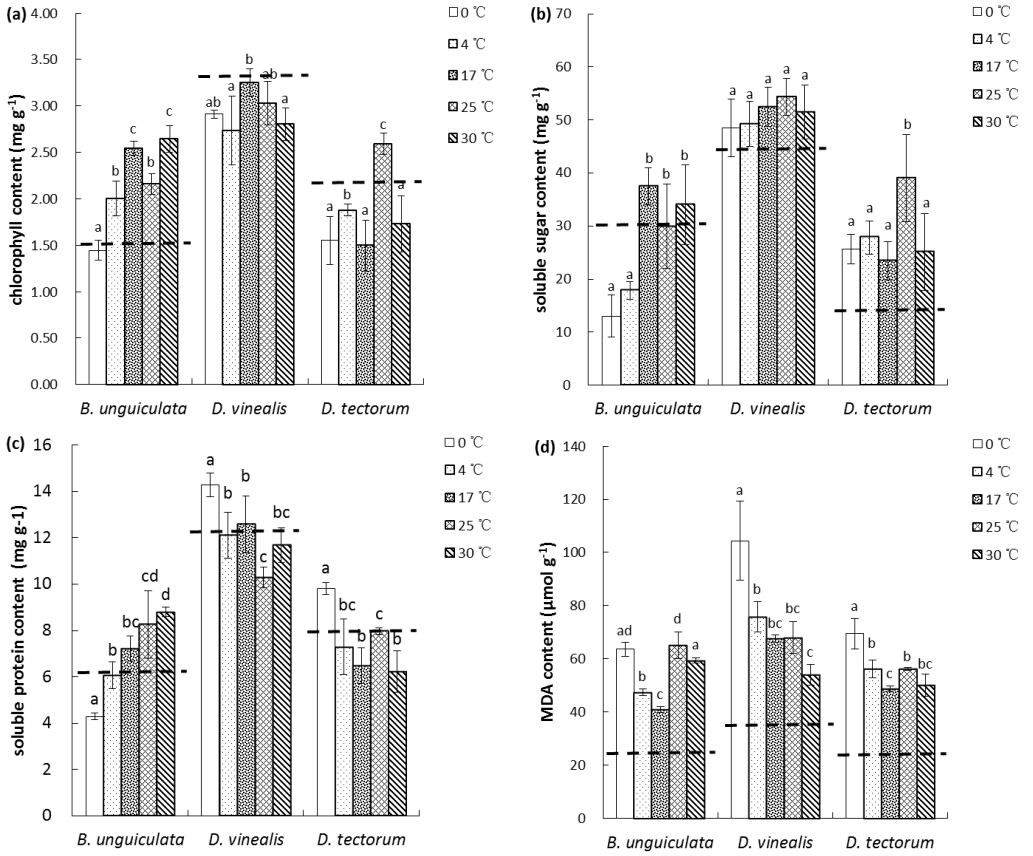

Fig. 2. (a-d) Data (average ± 1 SE) of the three moss species ~~in~~ on **(a)** chlorophyll content, **(b)** soluble sugar content, **(c)** soluble protein content and **(d)** MDA content after the 40-day storage period at each of the five temperatures. Different letters indicate significant differences ($P < 0.05$) among the five temperatures within the same species. Dotted lines represent the approximate values of the ~~three species in~~ two germination parameters before storage for each species (the true values are shown in Table 1).

Table 2 Gametophyte vigor index of the three mosses ~~at different~~ under treatments

Treatment	*B. unguiculata*	*D. vinealis*	*D. tectorum*
initial value	1.28 ±0.15 a	1.82 ±0.40 a	2.33 ±0.05 a
0 ℃	0.68 ±0.22 b	0.99 ±0.17 b	1.09 ±0.10 b

4 ℃	1.04 ±0.02 ac	1.11 ±0.13 b	1.26 ±0.03 b
17 ℃	0.95 ±0.05 c	1.22 ±0.10 b	3.66 ±0.35 c
25 ℃	0.65 ±0.06 b	1.17 ±0.13 b	2.90 ±0.46 a
30 ℃	0.86 ±0.18 bc	1.15 ±0.17 b	2.04 ±0.33 a

Data are average ±1 SE, and different letters indicate significant differences ($P < 0.05$) among treatments within the same species.

Table 3 Correlation coefficients ~~among~~ between physiological indices and germination parameters across all mosses and treatments~~of the mosses in different treatments~~

Variables	chlorophyll	sugar	protein	MDA	germination	increment
sugar	.762**					
protein	.747**	.781**				
MDA	.220	.402**	.510**			
germination	.473**	.414**	.313*	-.022		
increment	-.239	-.187	-.249	-.344*	.388**	
vigor index	-.158	-.122	-.191	-.328*	.441**	.995**

chlorophyll: chlorophyll content; sugar: soluble sugar content; protein: soluble protein content; MDA: MDA content~~.~~; germination: gametophyte germination; increment: gametophyte increment; vigor index: gametophyte vigor index~~.~~

* indicates a significant correlation at $P < 0.05$; ** indicates a significant correlation at $P < 0.01$.

Table 4 Gray incidence degree between physiological indices and the gametophyte vigor index across all treatments~~of the three mosses in different treatments~~

Reference sequences	*B. unguiculata*	*D. vinealis*	*D. tectorum*
chlorophyll content (X1)	0.60 ±0.20	0.55 ±0.27	0.66 ±0.21
soluble sugar content (X2)	0.57 ±0.20	0.62 ±0.23	0.62 ±0.17
soluble protein content (X3)	0.57 ±0.22	0.74 ±0.28	0.70 ±0.25
MDA content (X4)	0.77 ±0.20	0.73 ±0.22	0.76 ±0.27

3.3 Effects of storage temperature on the physiological indices~~ex~~ of mosses

As shown in Table 1 and Fig. 2a, the chlorophyll content of *B. unguiculata* increased after ~~being stored~~ storage at four of the five temperatures, i.e., all but ~~with the exception of~~ 0 ℃. The chlorophyll content of *B. unguiculata* showed an increasing trend ~~along~~ with ~~the~~ increasing storage temperature, ~~and a~~ with the maximum increase of 73.08% ~~occurred~~ observed at 30 ℃. The smallest change in ~~the~~ chlorophyll content was ~~found~~ observed in *D. vinealis*, ~~with~~ which showed a maximum decrease of 17.89% at 4 ℃ and a minimum decrease of 2.39% at 17 ℃. The chlorophyll content of *D. tectorum* after storage was decreased by 31.51% at 17 ℃ and increased by 18.50% at 25 ℃, ~~which were~~ yielding the highest and lowest content~~s~~ values, respectively.

A similar increasing trend ~~as the~~with temperature was ~~rose also~~ found ~~in the~~ for soluble sugar content (Fig. 2b). The soluble sugar content was consistently higher after storage than before ~~storage,~~ except ~~for the content of~~in *B. unguiculata*, ~~which~~ where sugar content was decreased by 56.52% and 40.47% at 0 ℃ and 4 ℃, respectively (Fig. 2b; Table 1) The soluble sugar content of *D. vinealis* showed less variation than did the other species. No significant difference was found between the

minimum and maximum increases, which were 9.92% at 0 ℃ and 23.14% at 25 ℃, respectively. The greatest changes in ~~the~~ soluble sugar content, with greater than 65% increases at all storage temperatures, occurred in *D. tectorum*~~, which increased by more than 65% under all storage temperatures~~.

~~The~~ MDA content showed greater variation than did sugar content~~had a more significant variation~~, increasing by more than 50% in all stored gametophytes (Fig. 2d; Table 1). The MDA content of both *B. unguiculata* and *D. tectorum* ~~both~~ decreased as the temperature ~~rose~~ increased from 0 to 17 ℃~~ and~~; ~~then increased. Both *B. unguiculata* and *D. tectorum* had a~~ the minimum ~~MDA content at 17 ℃~~ value of MDA content (at 17 ℃) was 1.70 times and 2.06 times the initial value, respectively. However, the MDA content of *D. vinealis* was 1.54 to 2.98 times the initial value after storage and continuously decreased with increasing temperature~~decreased to a minimum at 30 ℃~~.

Some temperatures caused the soluble protein content to change significantly (Fig. 2c; Table 1). The soluble protein content of *B. unguiculata* ~~decreased~~increased abruptly from ~~the value~~ a 31.79% decrease from the initial value to a 40.06% ~~above to the value 31.79% below~~ increase with ~~the~~ increasing temperature~~ decrease. On the contrary,~~. In contrast, soluble protein showed the opposite trend in *D. vinealis* and *D. tectorum*. Both species presented ~~the~~ a maximum increase at 0 ℃, which was 16.64% in *D. vinealis* and 23.65% in *D. tectorum*. The lowest soluble protein content of *D. vinealis* and *D. tectorum* ~~showed~~ represented a decrease of 16.00% at 25 ℃ and a decrease of 21.38% at 30 ℃, respectively.

Our results indicated that the ~~fastest~~ sharpest changes in ~~the~~ chlorophyll content and soluble protein content with increasing temperature were observed in *B. unguiculata*~~ as the temperature rose, and the contents of~~; furthermore, soluble sugar content and MDA content changed more rapidly with increasing temperature in this species than ~~that of~~in *D. vinealis* and *D. tectorum*, respectively (Fig. 2a-d; Table 1). *D. vinealis* showed slower changes of chlorophyll, soluble sugar and soluble protein contents with increasing temperature than did the other two species. ~~The~~ MDA content, however, varied ~~rapidly~~widely with temperature. The ~~biggest change that~~ largest increases in soluble sugar content and MDA ~~increase was~~ content after 40 days of storage were observed in *D. tectorum*~~ after the 40-day storage. Finally, in~~. In all three moss species, the ~~maximum index of~~greatest changes were observed in ~~was~~ MDA content, ~~and the second largest change was in~~followed by soluble sugar content (Fig. 2b, 2d; Table 1).

3.4 Relationships between physiological characteristics and the vegetative propagation of mosses

After analyzing the correlations ~~among~~ between the physiological indices and germination parameters of the desiccation-tolerant mosses, a significant correlation ($P < 0.01$) was found between each physiological index ~~among the four physiological indices and the three germination parameters,~~ except for ~~the~~ chlorophyll content and MDA content (Table 3). ~~The~~ gGametophyte germination ~~showed a~~was significantly ~~correlation~~ correlated ($P < 0.05$) with ~~the~~ soluble protein content and ~~a~~ highly significantly ~~correlation~~ correlated ($P < 0.01$) with ~~the~~ both chlorophyll content and soluble sugar content. ~~Only the~~ MDA content was ~~found to have~~ significantly negatively correlated~~ion~~ ($P < 0.05$) with both ~~the~~ gametophyte increment and gametophyte vigor index.

~~When the~~At a distinguishing coefficient ~~was~~ of 0.5, the gray incidence degree between the physiological indices (X1: chlorophyll content; X2: soluble sugar content; X3: soluble protein content; X4: MDA content) and the gametophyte vigor index ~~of~~ in the three moss species were (1) X4 > X1 > X2 = X3 in *B. unguiculata*, (2) X3 > X4 > X2 > X1 in *D. vinealis* and (3) X4 > X3 > X1 > X2 in *D. tectorum* (Table 4).

4 Discussion

4.1 Effects of storage temperature on the vegetative propagation of mosses

For more than a century, researchers have studied many aspects of mosses, such as inocula, pre-treatment (e.g., storage and sterilization), culture methods and culture conditions (Duckett et al., 2004; Hoffman, 1966). Some of these studies have implied that the physiological characteristics of moss gametophytes ~~were~~ are closely related to the success of artificial cultivation~~,~~; for ~~instance~~ example, pretreatment with sucrose and/or abscisic acid ~~could facilitate~~ can improve the viability of mosses by increasing DT (Burch and Wilkinson, 2002). In line with previous studies, this study ~~also indicated different results of~~found that gametophyte regeneration within the same species after desiccation ~~at~~ varied among different temperatures (Fig. 1a, 1b; Table 2), which ~~was probably~~is likely related to species-specific DT. ~~The gametophyte vigor index of *D. tectorum* was the most sensitive to the change of storage temperature, while it was the least changed and was not significantly different under storage temperature levels in *D. vinealis*.~~ The ~~vegetative propagation~~ regenerative capacity of mosses ~~could~~ can be summarily described by the gametophyte vigor index~~,~~ on the basis of Eq. (1) - (3) and Table 3. The gametophyte vigor index most sensitive to storage temperature was that of *D. tectorum*, whereas that of *D. vinealis* varied little with storage temperature, with no significant differences among temperatures (Table 2). Thus, the effect of storage temperature on ~~the vegetative propagation of *D. tectorum*~~ regenerative capacity was ~~the largest, in contrast to~~strongest in *D. tectorum* and weakest in *D. vinealis*.

~~Although the~~The 40-day storage period adversely affected regeneration in most ~~moss inocula~~gametophytes (Fig. 1a, 1b; Table 1)~~;~~, however, some ~~inocula~~gametophytes of *D. tectorum* stored at 17 ℃ and 25 ℃ produced more new ~~individuals~~ shoots than before. It ~~was~~ is not clear whether ~~the enhancement of~~this enhanced regeneration was ~~correlated~~ associated with the low-temperature tolerance of *D. tectorum*. ~~In other words,~~ *D. tectorum* possibly suffered low-temperature stress in early winter. ~~Meanwhile~~Furthermore, higher temperatures (~~like~~ e.g., 30 ℃) ~~also~~ injured ~~inocula~~ the gametophytes of *D. tectorum*, as did the lower temperatures of 0 ℃ and 4 ℃~~,~~ ~~which implied~~ . These findings suggest that extreme temperatures ~~were~~ are unsuitable for storing this moss species. ~~It is assumed that a further hypothesis could be made about~~Further studies are warranted on the impact of the storage environment on desiccation-tolerant mosses. For example, Burch (2003) found that the survival and regeneration of ~~dehydration~~dehydrated protonemata were reduced after cryopreservation~~, which was related~~ due to damage caused by intra-cellular ice crystals. The desiccation time ~~could~~can also affect the restorability of vegetative propagation ~~and physiological characteristics~~ in desiccation-tolerant mosses and their physiological characteristics (Keever, 1957;

Proctor, 2001). ~~Some~~ Environmental changes ~~caused by the environment and/or time that occurred in dormant cells could yield different~~ or variation in the dormancy period of cells might influence the restoration results after rehydration.

4.2 Effects of storage temperature on the physiological characteristics of mosses

MDA, an important product of membrane lipid peroxidation, increased in all mosses~~, which showed~~ over the storage period. This finding indicated that the 40-day storage period caused cell damage (Fig. 2d; Table 1). ~~Hence~~Accordingly, the soluble sugar content increased ~~correspondingly in order~~ to protect the membranes and proteins in the dried gametophytes (Fig. 2b; Table 1) ~~since s~~. Sugars are the main substance used to stabilize protein structures ~~below 0.3 (g H_2O) (g dry weight)$^{-1}$~~ in desiccation-tolerant cells (Hoekstra et al., 2001). ~~Conversely~~However, the soluble sugar content of *B. unguiculata* stored at 0 ℃ and 4 ℃ was decreased ~~after being stored~~relative to the initial value. ~~The reason could be that~~This result might have been due to the low temperatures ~~prevented~~preventing the conversion from starch to soluble sugar (Pressel et al., 2006). When mosses suffered oxidative damage, the increases in ~~the~~ chlorophyll content and soluble protein content in some gametophytes ~~was~~ were related to the recovery ability of desiccation-tolerant cells (Fig. 2a, 2c; Table 1). ~~Researchers found that~~In previous studies, the chlorophyll content of mosses increased during desiccation, and their photosynthetic capacity recovered rapidly after rewetting (Alpert, 1988; Csintalan et al., 1999). Similarly, ~~as did~~ protein synthesis recovered after rehydration (Oliver, 1991)~~,~~ since cellular recovery is an important part of DT (Proctor et al., 2007).

The recovery of photosynthesis and protein synthesis in *B. unguiculata* was facilitated by higher temperatures (not more than 30 ℃) (Fig. 2a, 2c)~~, which offered the opposite illusion that the~~ . This finding is inconsistent with the pattern in other mosses, in which viability ~~of other mosses tended~~tends to be ~~weaker with~~ lower at increased temperatures (Hearnshaw and Proctor, 1982). However, the increasing trend ~~in the~~of MDA content from 17 to 30 ℃ ~~implied~~ suggests that more extensive membrane damage may be caused by storage temperatures above 30 ℃ (Fig. 2d). The adverse effects of the ~~relatively~~ higher temperatures in *D. vinealis* and *D. tectorum* were clearly reflected by the slower recovery of photosynthesis and protein synthesis (Fig. 2a, 2c). ~~Although the~~ The changes in the MDA content in *D. vinealis* ~~showed a faster~~suggested more rapid repair of cell membrane ~~as the temperatures rose~~with increasing temperature; however, the ~~moss~~ species possibly had stronger tolerance under the protection of abundant sugars when the recovery of photosynthesis and protein synthesis was slower (Fig. 2a-d).

The responses of the physiological characteristics of the three species to temperature~~s~~ reflected ~~different restoration abilities in~~species variation in restoration ability over a short rehydration time. ~~If~~Because the rewetting periods were longer than 30 days in the cultivation, the ~~result of~~ vegetative propagation ~~could~~ results can be ~~defined~~ considered as reflecting the long-term recovery of mosses. Thus, the long-term effect of cell recovery during short-term rehydration ~~could~~ can be explained by the relationships between the physiological characteristics and vegetative propagation of desiccation-tolerant mosses.

4.3 Relationships between physiological characteristics and the vegetative propagation of mosses

Before ~~the~~ storage, the four physiological indices of gametophytes showed significant differences between *D. vinealis* and *D. tectorum*. However, no significant differences between the two species were observed in regard to the three germination parameters (Table 1). ~~It could be seen that similar fertility between mosses was accompanied by significantly different physiological characteristics. Then, species specific~~Mosses of similar fertility showed significant differences in physiological characteristics. Species differences in DT led to larger differences in~~made the~~ vegetative propagation among species ~~present bigger differences~~ than before, ~~which were shown~~as evidenced by the values of the gametophyte vigor indices ~~under~~ within the same treatment (Table 1; Table 2). Therefore, the recovery ability of dried mosses with respect to development and regeneration ~~of dried mosses~~ might ~~play a more~~ be more ~~beneficial role to~~ informative for screening suitable inocula than ~~in~~is using fresh mosses in dry habitats. ~~Although many~~Many studies have indicated that desiccation-tolerant mosses ~~could~~ can recover from drying ~~when~~ once they are rehydrated (Csintalan et al., 1999; Pressel et al., 2006)~~, the overlong~~. However, long periods of desiccation would impede the reuse of moss specimens and the restoration of dried biocrusts. This study ~~also~~ showed that cells were subjected to oxidative damage after the 40-day desiccation period (Fig. 2d; Table 1). ~~At the same time~~Over this period, the regenerative capacity of the three species declined (Table 2), which ~~implied~~ suggested that membrane integrity and/or other factors ~~had an effect on~~affected the vegetative propagation of the desiccation-tolerant mosses.

Based on the correlation coefficients among the physiological indices and germination parameters of desiccation-tolerant mosses (Table 3), ~~the~~ gametophyte germination ~~revealed a~~was significantly and positively correlated ~~correlation~~ with ~~the~~ chlorophyll content, soluble sugar content and soluble protein content. ~~On the contrary~~In addition, ~~the~~ gametophyte increment and gametophyte vigor index were ~~only~~ significantly and negatively correlated with ~~the~~ MDA content. ~~It was a possible reminder~~These findings are in accordance with the observations that metabolic repair ~~was~~ is favorable to the germination of new gametophytes~~,~~ and ~~the result of~~ that long-term recovery is ~~depended~~ more dependent on cell integrity than metabolic repair. Therefore, to quantitatively compare the effects of the four physiological indices on vegetative propagation, the gray incidence degree between the physiological indices and the gametophyte vigor index ~~of~~ for each of the three moss species was calculated by Eq. (4)-(6). As shown in Table 4, the effect of ~~the~~ MDA content on the gametophyte vigor index was the ~~greatest~~ strongest in *B. unguiculata* and *D. tectorum*, and the incidence degree of MDA (0.73) in *D. vinealis* was ~~quite~~ similar to the maximum (~~the former was 0.73 and the latter was~~ 0.74). ~~The~~ In all three mosses, MDA content ~~of the three mosses~~ increased as ~~the~~ storage temperature decreased from 17 to 0 ℃~~, when the smaller~~. Smaller gametophyte vigor index values were observed for ~~of~~ *D. vinealis* and *D. tectorum* ~~presented~~ at 0 ℃ and 4 ℃ ~~rather~~ than at 25 ℃ and 30 ℃ (Fig. 2d; Table 2). This result indicated that the greater membrane damage incurred at low temperatures caused the decline in regenerative capacity. In addition, the higher gametophyte vigor ~~indices~~index values of *D. tectorum* at 17 ℃ and 25 ℃ than before storage were possibly related to the ~~reduction of~~reduced formation of intra-cellular ice crystals at these temperatures during the storage period (Burch, 2003), which facilitated ~~faster~~ more rapid recovery upon rehydration ~~than fresh gametophytes~~ (Table 2). However, the number of negative effects on physiological characteristics increased~~there were an~~

~~increasing number of negative influences~~ with increasing temperature~~ that presented in the physiological characteristics~~ (Fig. 2a-c). The~~se~~ high temperatures were unfavorable to the recovery of the mosses (Hearnshaw and Proctor, 1982). When cells suffered damage under desiccation and temperature stress, the protection ~~of more~~provided by additional sugars was ~~particularly~~ important ~~to~~ for maintaining cell integrity in the~~a~~ dry state (Fig. 2d; Table 1). ~~The possible reason for this is that~~ *D. vinealis* showed no significant difference in ~~the~~ regenerative capacity among temperatures, potentially because the level cellular protection was equivalent ~~despite~~ among the different temperatures.

Researchers have summarized the recovery mechanisms of mosses upon rehydration, such as the rapid recovery of photosynthesis, respiration and protein synthesis within minutes to hours (Proctor et al., 2007). However, ~~the~~ recovery of the carbon balance, cell cycle and the cytoskeleton require~~d~~ more than 24 hours (Alpert and Oechel, 1985; Mansour and Hallet, 1981; Pressel et al., 2006). Based on these results, it has been speculated that cell integrity ~~was supposed to be~~is more difficult to recover than are physiological reactions and that cell integrity ~~had a~~ great~~l~~y limit ~~on~~ the recovery and regenerative capacity of desiccation-tolerant mosses. ~~During~~ Over long-term desiccation, the cumulative damage affect~~sed~~ cell function and integrity ~~over time~~ (Proctor, 2001)~~, which was influenced by~~; different temperatures ~~that~~ might enhance or suppress such cell damage~~ according to the research. This result means the~~. Thus, the effects of temperature on the ecology of DT in bryophytes ~~are worth paying attention~~warrant investigation, especially during the dry season in semiarid and arid areas. The ~~more sensitive response~~ greater sensitivity of *D. tectorum* observed here might provide insight into~~implied one of reasons~~ why ~~it was not~~this species is not a widely ~~-~~distributed species~~, like~~ such as *D. vinealis*, in the ~~site~~study region. Furthermore, the ecological niche requirements of different mosses ~~both~~ in both dry ~~time~~ and wet ~~time~~ periods will influence the choice of moss inocula ~~on~~ for artificial cultivation and biocrust restoration. ~~Despite the distinction between the results in between laboratory conditions and the field, it might influence to match the ecological requirements, and furthermore~~Field studies are needed to better understand the ecological requirements of dried mosses. Furthermore, a precise description of microclimates and the application of quantitative methods would be helpful~~ to the issue~~.

5 Conclusions

The conducted experiment explored the effect of storage temperature on the vegetative propagation of desiccation-tolerant mosses and ~~critical~~ influencing factors. The results indicated that the decline ~~of the~~ in regenerative capacity in mosses observed following storage was related to cell damage caused by ~~dehydrated~~dehydration during storage. The storage temperature~~s~~ during dehydration ~~also~~ influenced the vegetative propagation of mosses ~~because of temperature induced~~through changes in moss cell activity. Further analysis showed that the factor with the strongest effect ~~of membrane damage~~ on vegetative propagation was ~~the greatest. Meanwhile,~~membrane damage. During storage, soluble sugars increased ~~in order~~ to protect the cells, highlighting the important role of cell integrity ~~played~~ in influencing the physiological characteristics and vegetative propagation of desiccation-tolerant mosses. In this study, the optimal storage temperature of *D. vinealis* and *D. tectorum* was 17 ℃, ~~while~~ whereas the ~~suitable~~ optimal temperature for *B. unguiculata* was 4 ℃~~ for B. unguiculata~~. Different responses to ~~the~~

~~temperatures in~~ temperature among the three moss species were ~~linked~~ associated with species~~-specific~~ differences in DT~~, which could~~. These findings can potentially guide future research ~~to study~~on suitable storage methods of inoculation material ~~on~~ to improve the artificial cultivation of moss biocrusts.

In general, the properties of inoculation material are key factors affecting the development and recovery of moss biocrusts, such as species, physiological ~~feathers~~ features and/or other factors. The results ~~helped to partly explain~~provide insight into the factors that influence the vegetative propagation of desiccation-tolerant mosses~~,~~ and ~~furthermore, to offer a new view of the fast~~highlight the potential applicability of a rapid experimental approach ~~to~~ for screening suitable inocula.

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