Answer to Reviewers

Answer to reviewer #1

The authors present a very nice paper looking at the effects of dry storage temperature of three biocrust moss species on viability of the mosses in terms of regeneration and some physiological attributes. They demonstrate that optimal storage temperature varies by species, and can have an impact on the heath and gametophyte reproduction. This is a nice addition to the literature, as mosses contribute great ecosystem benefit to drylands, and can be used in restoration. Dry storage is essential to this endeavor. It also speaks to how much more there is to learn about the ecology of these dessication tolerant species. I highlight below a few issues where more information, clarification or interpretation need to be addressed. A careful edit for proper English grammar could be benefit, but overall the manuscript is well written.

Thank you for your highly praise and helpful comments on our manuscript. We will modify English in the revised manuscript and address each issue below:

Methods Line 23, page 3: "average accumulated temperature...3733 and 3283C" is an odd way to share the temperature range. Instead, please present mean annual temp, mean annual high and mean annual low.

Agreed. We will add the three temperatures in the revised manuscript.

How were mosses collected? How were moss storage temperatures maintained? Were they in incubation chambers? I'm unclear on your sampling/splitting design. Did you collect from one colony and split this many ways (for initial, and then the 5 temperature gradients)? You say you have 3 duplicates or subsamples. Does this mean you originally collected from 3 colonies per species, or you split the one colony into "replicates" for each temperature level?

In fact, we stored samples at 0 ℃ and 4 ℃ in two refrigerators, respectively. Other samples were stored at 17 ℃, 25 ℃ and 30 ℃ in three growth chambers, respectively. In our sampling design, we collected moss crusts of a given species from many colonies, and then moss crusts were packed in 3 ziplock baggies. After stored, we collected some gametophytes from moss crusts as subsamples. Thus, we will revise the sentence.

Germination parameters: what do you mean by "5 inocula" (line 39 page 4). Does this mean 5 stems?

Yes. The "5 inocula" means five 2-mm stems of living mature gametophytes.

Results For the physiological parameters, it might be more helpful to say the change from the initial condition, rather than the total. In this way, we can look at positive or negative effects of storage more easily.

We also consider that readers can look at effects of storage from changes of physiological parameters more easily. However, we found bigger standard errors than true value appearing in figures of change percentages. It might cause misunderstanding. Therefore we described percentages of parameter changes in words. In addition, Reviewer #3 suggested adding the initial values (depicted by horizontal lines) into Fig. 2, which may be easier to comprehend.

The grey incidence analysis is over-interpreted. Most of the values overlap and thus, cannot be interpreted as greater or lesser than one another.

We tried to quantify the impact of physiological parameters on vegetative propagation by using grey incidence analysis, which can help to determine if there were different impacts between physiological parameters. We think the method is more suitable the analysis of moss vegetative propagation with few information. Bigger incidence degree meant relatively more impact on vegetative propagation, and even little difference might be meaningful in a grey system. Nevertheless, more precise method or model will be required to quantify the impact of physiological parameters in further studies.

Answer to reviewer #2

This paper examines the influence of dry storage temperature on regeneration and physiology in

three DT mosses from the Loess Plateau. It is a relatively simple study, but does provide important information for moss cultivation in a restoration context, though because of species-specific responses, pre-treatment environmental effects, and RH considerations during drying and dry periods, may not be widely generalizable.

Thank you for carefully reading and many helpful comments. We will consider and response every comments below:

Abstract: L14. I think you mean temperature "levels"

Yes. It will be changed in the revised manuscript.

L28. cell injury seems vague here. Perhaps mention when you discuss what MDA is above

The decrease of soluble sugar may cause cellular protein denaturation upon desiccation. Thus, the phrase “cell injury” actually included not only membrane damage showed by MDA, but also protein injury showed by soluble sugar.

Introduction In general, you miss out on some key background research by Stark and Greenwood, who have been examining desiccation and rehydration in Syntrichia for years.

Thank you for providing important information about DT. They will be added to the revised manuscript.

L36. "soil fertility accumulation" is an odd phrase

Agreed. It will be revised.

P2 L3. "culturing artificially" perhaps should be transposed?

Agreed. It will be revised.

L6. What is this "theory"? Is this necessary to say?

No. There is little theory research on vegetative propagation of mosses compare with sexual reproduction. We will delete the word.

Paragraph starting with "Desiccation tolerance.." is hard to follow. There seems to be too many ideas in it, and the info on Grimmia seems oddly specific

Agreed. The information about DT should be simplified. In recently years, some researchers (e.g. Stark et al. 2005) studied DT by culturing shoots, which guided us to consider impact of DT on vegetative propagation. However, we have some logistic problems and they will be revised.

L35. Omit sentence beginning with "Actually.."

Agreed. It will be revised.

Methods Collection: Were they all growing together when they were collected? Were the different species in different microclimates?

The three species were collected from different plots. Unfortunately, we do not have any data about the microclimates, though we collected a given species in same plot.

P3L25. How long did it take moss to dry? Was it different for each species? What was the RH? These are crucial points that relate to regeneration.

We dried all three species for 24-48 hours. Unfortunately, we do not have the data on RH during drying. Nevertheless, most of gametophytes were dry (e.g. Figure 1) when we collected moss crust. We believed there was little effect caused by RH during drying.

Figure 1 *D. vinealis* before collected

L37. What was the equilibrating RH during storage? Also, I am unclear on the actual function of the ziploc baggies here.

The equilibrating RH was 55% and will be add to the revised manuscript. On account of we stored mosses in refrigerators or growth chambers (the detail can also read in Answer to Reviewer #1) with different RH, the ziplock baggies were used for preventing water from air.

P5L10. Was 25 days the entire length of the regeneration study then?

After new gametophytes germinated, we continued culturing for 25 days. Thus, the entire lengths of the regeneration study were 30 days in *D. vinealis* and *D. tectorum*, and 35 days in *B. unguiculata*.

L11. Save for results.

It will be revised.

L12. Anaogy with seed germination is an interesting idea, but I think you're missing out on key life stages that are missing in angiosperms, like protonema. Was protonemal presence / extent quantified? What about gemmae?

In fact, we ever tried to measure the timing of protonemal production and protonemal growth rate in trial tests. Nevertheless, mosses protonema germinated lately made it difficult to differentiate from soil. Furthermore, there were not gemmae in three species except *Didymodon tectorum*.

Results Fig.1A is hard to interpret. Are the bars totals after the 25 day regeneration period?

Fig. 1 shows results of fifth observation. Thus, the bars are totals after full regeneration period. We will revise description of the figure.

Table 1 and Fig 2 kind of go together, and I wished to be able to compare them more easily. Is there a way to incorporate the initial values into Fig. 2 or at least place the table closer to it?

Reviewer #3 suggested adding the initial values (depicted by horizontal lines) into Fig. 2, which may be easier to comprehend.

Table 4: Why not label the columns with the physiological indexes?

Agreed. It will be revised.

Discussion Careful with over-use of adverbs (Contrarily, Particularly) that don't improve sentences. Overall, while the separate sections are nice, the organization within them is a bit challenging. For example, L35 I don't think a conclusion is appropriate here.

Also, in section 4.3 and others I'm noticing less time is spent discussing the current work, and more is spent bringing in related work. It begins to get cumbersome, and the reader loses sight of the key results. A general reframing to focus on key results would be helpful.
Thank you for your comments in language and organization. We will make effort to improve English and revise the organization in the revised manuscript.

Discussion L6-7. I don't understand what the point of this sentence is.
After read again, we find this sentence should be deleted.

Notes on select specific BG criteria: The paper presents some novel data, but the scope is limited. Much of the scientific methods are valid and outlined well, although the authors miss out on specific drying and storage conditions that could have influenced results more than temperature. Language could be more fluent and precise in numerous places.
Thank you for pointing out mistakes and providing many advices! We will revise the manuscript as your suggestion.

Answer to reviewer #3

Thank you for helpful comments and constructive suggestions on the manuscript. We will consider and response every comments below:

When I read title and abstract of the paper, my first impression was that the mosses were stored in a field-wet state. In became clear just in the M&M section that the mosses were stored air-dried and hermetically sealed. I suggest to mention that point in the abstract.
Agreed. We will add the storage state in the abstract.

I think it would also be a great idea to get an impression of the relative humidity during storage.
We agree with you and Reviewer #2. It will be revised.

The success of incubation experiments often depends on how well experimental conditions match the niche requirements of the target organisms, in particular those with narrow ecological amplitudes. For example, low gametophyte increment, germination rate and delayed initial germination of Barbula unguiculata does not necessarily mean that this species generally is outperformed by the Dedymodon species. It may also indicate that the experimental conditions better matched the ecological requirements of the latter, and that other experimental conditions may show a different picture. Hence, I miss in the paper some discussion of the ecological niche requirements of the particular species investigated. For example, Barbula unguiculata Hedw. and Didymodon vinealis (Brid.) Zander var. vinealis differ with their requirements to light: While both of these species prefer open lands, Barbula unguiculata Hedw. may grow in shadowed areas with down to 30% of relative light intensity, whereas Didymodon vinealis (Brid.) Zander var. vinealis does not develop at relative light intensities below 50% (ISBN-13: 978-3825281045). As the samples were taken at north facing slopes, which possibly receive shadow, I recommend to consult the botanical literature and to consider ecological niche requirements in the discussion of implications for the practice. Further, a more precise description of the sampling procedure and sampling spots might be helpful.
Thank you for insightful comment on ecological niche requirements of mosses. We will add a list of moss species including ecological information and more precise sampling design to the revised manuscript. We believe that different niche requirement (e.g. species-specific DT) will influence the choice of moss inocula on artificial cultivation and biocrust restoration, thus three species were compared in the paper. However, it seems be unclear to readers. We will revise the manuscript.

Minor remarks
M&M
p. 4 l. 5 ff.: The weights of 100 and 50 mg of sample for sugar and chlorophyll measurement seem little to ascertain representative sampling. How many replicates were analysed?
Three replicates were analysed and had 100 mg in every replicate for sugar measurement.

Similarly, there was 50 mg in a replicate for chlorophyll measurement.

p. 5 l. 17.: Please check the correct usage of the terms "seed" and "hypocotyl" in conjunction with mosses. Again, I recommend to consult the botanical literature to be more precise.

It will be revised.

Results

Figure 2: I needed to switch between Table 1 and Figure 2 to compare initial values with the temperature effect. I would find Figure 2 easier to comprehend if the initial values could be somehow depicted there (as horizontal lines?).

Good idea! We will revise figures as you said.

The marked-up manuscript

Effects of storage temperature on 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 to speed up the recovery of biocrusts. Revealing the factors that ~~influencing factors on~~influence the vegetative propagation of mosses will benefit the restoration of moss crusts, which is an important reproductive mode of mosses in arid and semi-arid ~~region~~regions. In this study, three desiccation-tolerant mosses (*Barbula unguiculata*, *Didymodon vinealis* and *Didymodon tectorum*) were stored, air-dried and hermetically sealed at five temperature ~~gradients~~ 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 mechanism. The results showed that the vegetative propagation of the three mosses varied with temperature, and the most significant change was observed in *D. tectorum* after storage at different temperatures. Conversely, no significant difference 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 an increased temperature from 0 ℃ to 17 ℃ and a decrease beyond that. ~~Malondialdehyde~~ The malondialdehyde (MDA) content of the three mosses ~~was~~ increased by more than 50% at all of the investigated temperatures~~,~~; meanwhile, the soluble sugar content increased in the three mosses. However, a ~~decrease~~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 ~~decreased~~ those in *D. vinealis* and *D. tectorum* decreased. The integrity of ~~cell~~ cells and ~~its membrane probably~~ their membranes is probably the most important ~~influencing~~ factor ~~on~~ influencing the vegetative propagation of desiccation-tolerant mosses. Although a 40-day storage period caused cell injury, our results suggested that the storage temperature could enhance or suppress such injury and change the vegetative propagation capacity of the three mosses. It could be concluded that the suitable storage temperature of *B. unguiculata* was 4 ℃, and the optimal temperature ~~was 17 ℃~~ for *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 are widely distributed and play important roles in many arid and semi-arid ecosystems, such as soil surface stabilization, soil fertility enhancement ~~accumulation~~ and soil hydrology ~~regulating~~regulation (Belnap and Lange, 2003). As major components of later successional biocrusts, mosses exerted much stronger ecological functions than cyanobacteria (Seppelt et al., 2016; Gao et al., 2017; Lan et al., 2012). Thus, some researchers suggest artificially culturing ~~artificially~~ moss biocrusts on ~~the~~ degraded soil ~~surfaces~~surfaces so as to speed up 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~~using the theory of vegetative reproduction~~ (Jones and Rosentreter, 2006; Xiao et al., 2015). However, little is known about the vegetative propagation of such mosses~~. It~~, 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, a number of moss cultivation experiments that used gametophyte fragments have been conducted to establish new colonies in the laboratory and field (Cleavitt, 2002; Jones and Rosentreter, 2006; Xiao et al., 2015). All ~~of~~ such studies demonstrated that artificial cultivation could speed up the succession process of moss crusts. For example, Antoninka et al. (2016) found that the coverage and biomass of mosses on the artificially inoculated soil surface increased faster than that ~~of~~on uninoculated soil. Furthermore, some researchers suggested that the inoculation material should be mass-produced by vegetative regeneration with rapid development (Jones and Rosentreter, 2006; Mishler, 1988) because of the ~~necessity when~~ need for moss biocrusts ~~would~~ to inoculate ~~in~~ large areas. Although ~~influencing~~ the factors ~~of~~that influenced the tissue cultivation of mosses ~~were~~have been investigated for a long time (Duckett et al., 2004; Hoffman, 1966; Sabovljevic et al., 2003), the mechanism of ~~mosses~~moss regeneration still needs to be further studied.

~~Desiccation tolerance (DT) is a remarkable feature of biocrust mosses. The feature makes mosses can lose virtually all of the free intra cellular water and recover normal function after rehydration (Proctor et al., 2007). Because~~ 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 desiccation-tolerant mosses is suspended under water stress, and ~~the~~ cell integrity can be maintained during the dry periods (Mansour and Hallet, 1981; Platt et al., 1994). Then, the cellular activity of mosses will resume and return to ~~the~~a normal hydrated state within a few minutes to a few hours after being rehydrated (Platt et al., 1994; Pressel et al., 2006). ~~Therefore, mosses could regenerate even after more than a decade of dry storage (Bristol, 1916; Keever, 1957).~~ However, the decline and disappearance ~~in~~of the regenerative capacity of ~~*Grimmia laevigata* exerted~~*Syntrichia ruralis* showed that ~~mosses might suffer irreversible damage in a~~ long-term

desiccation would cause irreversible damage despite viability differences among individuals (Stark et al., 2017~~Keever, 1957~~). It was still unclear why the potential of vegetative propagation of mosses was altered by storage, or the recovery abilities between moss species were different after drought dormancy, which would impede the study of moss cultivation.

In general, DT investigations concentrate more on ~~its~~ mechanism and evolutionary history (Proctor et al., 2007; Oliver et al., 2000)~~,~~ and less on artificial cultivation. ~~Actually, t~~There are lots of theoretical studies that can support further utilization. For instance, the impact of desiccation stress on moss regeneration varies with drying time and storage temperature (Keever, 1957; Burch, 2003) and may guide research on the regenerative mechanism of mosses upon desiccation and the artificial cultivation of mosses. Furthermore, DT plays essential roles in ~~mosses~~moss regeneration in dry habitats, which ~~reminder~~reminds us to investigate the link between physiological characteristics and vegetative propagation of the mosses. Based on the study results mentioned above, it can be hypothesized that (1) dry storage may impact the vegetative propagation of desiccation-tolerant mosses, (2) the change of vegetative propagation after storage may relate to the influence of storage on the physiological characteristics of mosses, and (3) the ~~effect~~ degree ~~of~~to which storage ~~on~~ affects the vegetative propagation and physiological characteristics is related to the storage temperature.

Consequently, in this study, three desiccation-tolerant mosses, including *Barbula unguiculata*, *Didymodon vinealis* and *Didymodon tectorum*, which were the ~~dominated~~dominant mosses in biocrusts communities in the Loess Plateau region, were selected and stored at five temperature ~~gradients~~ levels (0 ℃, 4 ℃, 17 ℃, 25 ℃ and 30 ℃) for 40 days. Then, (1) the effect of storage temperatures on the vegetative propagation of the three mosses and (2) the change of physiological ~~indexes~~indices, including the contents of chlorophyll, soluble sugar, soluble protein and malondialdehyde (MDA), were investigated so as to reveal the influence of storage temperature on the vegetative propagation of mosses and its mechanism.

2 Materials and methods

2.1 Study site and moss species ~~Moss species and their collection~~

~~Moss taxa used in the study were *Barbula unguiculata*, *Didymodon vinealis* and *Didymodon tectorum*. The mosses were collected from moss-dominated biocrusts with coverage around 80% on north-facing slopes in the study region.~~ The study was conducted in Ansai Country, Shaanxi Provence, China (109 °19' E, 36 °51' **N**), which is located in the central part of the Loess Plateau. ~~Elevation~~ The elevation of the sampling plot varies from 1,068 to 1,309 m. The plot has a typical semi-arid continental climate, with ~~the~~ an average annual temperature of 8.8 ℃, and its average temperatures in January and July are -7.2 and 22.8 ℃, respectively. ~~the range of monthly average temperature is from 22 ℃ to -7 ℃ in July and January, respectively. The average annual accumulated temperature above 0 and 10 ℃ are 3733 and 3283 ℃, respectively.~~ The average annual precipitation is 500 mm, with 60% or more 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 (Chinese Central Meteorological Station, 2017). Cyanobacteria and

mosses dominated the biocrust communities, and the coverage of moss-dominated biocrusts might even reach around 80% on north-facing slopes in the study region (Zhao et al., 2014). ~~The moss crusts were air-dried (all of the water content of mosses less than 10%) in the shade as soon as they were collected. Then, they were transported to the laboratory of State Key Laboratory of Soil Erosion and Dry-land Farming on the Loess Plateau which is in Yangling, Shaanxi Province.~~

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

2.2 Experimental design

Each of the three moss crusts was separated into two parts as soon as they were transported to the laboratory. One was used to measure initial physiological ~~indexes~~ indices (chlorophyll content, soluble sugar content, soluble protein content and MDA content) and germination parameters (gametophyte germination, gametophyte increment and gametophyte ~~vigour~~ vigor index). The other was stored at five temperature ~~gradients~~levels, i.e., 0 ℃, 4 ℃, 17 ℃, 25 ℃ and 30 ℃. All the temperatures were controlled within ± 1 ℃ around the target. ~~Three separated subsamples (duplicates) of each moss species were stored at each temperature gradient. Before being storing, the moss samples were packed in ziplock baggies to block the change of water content, and then they were kept in the dark under light blocking fabric.~~ Then, the mosses were taken out on the 41st day of storage, and the physiological ~~indexes~~ indices and germination parameters ~~as~~ mentioned above were measured.

2.3 Moss collection and storage

Three species of moss crusts were air-dried in the shade for 24-48 hours after being collected from many colonies, although most of mosses 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 two refrigerators (0 ℃ and 4 ℃) and three growth chambers (17 ℃, 25 ℃ and 30 ℃). Thus, the moss crusts were packed in Ziploc baggies to prevent change in the water content before being stored, and then they were kept in the dark under a light-blocking fabric. The measurement of the water contents of moss gametophyte were less than 10%, and the equilibrating relative humidity was 55% during storage. After the 40-day dry period, some desiccated gametophytes were collected as subsamples to measure the physiological index and germination parameters.

2.~~3~~ 4 Measurement of physiological index and germination parameters

2.~~3~~4.1 Physiological index

The living mature gametophytes of *B. unguiculata*, *D. vinealis* and *D. tectorum* were collected from

moss crusts ~~for measuring~~ to measure 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 replicate,~~;~~ while ~~approximately 0.05 g fresh mass was used for measurement~~the measurements of the chlorophyll content used approximately 0.05 g fresh mass as a replicate. The four indicators were measured by the following protocols with three replications.

The ~~chlorophylls were~~ chlorophyll was extracted by 95% (v/v) ethanol and then ~~boiled~~ the solution was boiled at 85 ℃ for 5 min. After being ~~centrifugation~~ centrifuged at 4000 rpm for 10 min, ~~chlorophylls~~the chlorophyll in the supernatant ~~were~~was measured ~~the absorbance~~ at absorbances of 665 and 649 nm with ~~the~~a spectrophotometer (UV-2300, *Techcomp*, 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 supernatant was collected after being ~~centrifugation~~centrifuged at 8000 rpm for 30 min at 4 ℃. The soluble protein was stained with Coomassie brilliant blue G-250 and ~~read~~ the absorbance was read at 595 nm (Bradford, 1976).

The MDA as well as the soluble protein was 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 ~~read~~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 MDA and soluble protein.

The soluble 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 spectrophotometer (UV-1601, *Shimadzu*, 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 them were used to calculate the four physiological ~~indexes~~ indices on a dry basis.

2.~~3~~4.2 Germination parameters

At the same time ~~of measuring~~as the physiological ~~indexes~~indices was measured, some gametophytes of the three moss species were collected to test the germination parameters. The loessial soil (uniform soil texture of *Calciustepts*) collected from the study region was used ~~for culturing~~ 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 diameter ~~is~~was 35 mm and depth ~~is~~was 12 mm. Then soil ~~was adjusted~~ water content was adjusted by deionized water to 23% (W/W) (the field water holding capacity of the soil), and the surface was flattened before inoculation. Five inocula, which were the~~The~~ top 2 mm of living mature gametophytes of the mosses, were cut, rehydrated, washed and placed separately in one well. ~~inoculated on the flatted surface of the soil. Five inocula were placed separately in one well. Thus,~~ 30 inocula were ~~inoculated~~ placed in each 6-well plate as one replication. Three 6-well plates were set for each moss species. ~~Totally~~In total, 90 experimental inoculations were set up for the measurement of germination

parameters before and after being stored at the five temperature ~~gradients~~levels for each moss species. Meanwhile, three 6-well plates without inoculated mosses were set up as controls in the experiment ~~in order~~ to eliminate the effect of other propagules, like spores in the soil used. The 6-well plates were wrapped tightly with transparent plastic films ~~for holding~~ to hold the soil moisture. After that, they were put into a growth chamber (AGC-D003N, China) to incubate. ~~Parameters~~The parameters of the growth chamber were set as a 12-h photoperiod (4500-5500 Lux), a constant temperature of 17 ℃ (± 1 ℃) and a relative humidity of 60-70%. During the period of incubation, deionized water was supplied so as to keep the soil moisture at 23%. The new gametophytes were counted every five days ~~since the first day when~~ beginning on the day they were found. There were five observations altogether during the next 25 days. The paper reported the results of cultivation under the fifth observation. It was noteworthy that no new gametophyte was found in the blank 6-well plates during all of the incubation in the study; however, it was difficult to distinguish protonemal germination between the underside of original inocula and soil substrate.

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

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

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

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

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

2.~~4~~ 5 Statistical analyses

The differences ~~of~~in physiological ~~indexes~~ indices and germination parameters 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 physiological ~~indexes~~ indices and germination parameters of the three moss species were quantified by calculating the Pearson correlation coefficient. These statistical analyses were completed using SPSS 22.0.

The effect of physiological characteristics on vegetative propagation was analyzed by ~~grey~~a gray incidence analysis in Microsoft Excel 2010 (Deng, 1984; Lin et al., 2009). The ~~grey~~gray incidence degree between the reference sequences (physiological ~~indexes~~indices) and the compared sequence

(gametophyte ~~vigour~~vigor index) ~~were~~ was calculated by ~~Eqs~~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 ~~grey~~ gray relational coefficient, respectively, between X_i (physiological ~~indexes~~indices) and Y (gametophyte ~~vigour~~vigor index) at point k. The ~~grey~~gray relational coefficient (r_i) is between the i_{th} physiological index and its gametophyte ~~vigour~~vigor index when the distinguishing coefficient (ρ) is 0.5.

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

3 Results

3.1 The initial state of the mosses

~~The initial physiological indexes and germination parameters of the three mosses were shown in Table 1. It could be seen that the four physiological indexes and gametophyte germination of *D. vinealis* were significantly higher than the other two species. The biggest gametophyte increment and gametophyte vigour index were found in *D. tectorum* and the smallest germination parameters were found in *B. unguiculata*. However, no significant difference in the contents of chlorophyll, soluble protein and MDA between *D. tectorum* and *B. unguiculata* was found.~~

Table 1 The initial value of physiological index and germination parameters in the three mosses

~~index~~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 ~~vigour~~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 from original inocula at different times, while no gametophyte germinated in the control groups in the last measurement (fifth observation). *B. unguiculata* germinated on the eleventh day of inoculation, so that the entire length of its cultivation time was 35 days. *D. vinealis* and *D. tectorum* germinated on the sixth day with a 30-day cultivation. The initial physiological indices and germination parameters of the three mosses was 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 gametophyte increment and gametophyte vigor index were found in *D. tectorum*, and the smallest germination parameters 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 three moss species began to germinate at different time. *B. unguiculata* germinated on the eleventh day of inoculation. *D. vinealis* and *D. tectorum* germinated on the sixth day.~~

The ~~gametophyte~~ germination times of ~~all~~ the three mosses and controls after storage were the same as the initial state. In the fifth observation, the gametophyte germination of all three species changed 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*, there was no significantly different gametophyte germination among all storage temperatures, which ranged from 95.56% (0 ℃) to 98.89% (17 ℃). The only significant difference was between 81.92% and 100% at 0 ℃ and 25 ℃, respectively, in the gametophyte germination of *D. tectorum* after being stored.

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

The gametophyte ~~vigour~~vigor index of the three moss species showed significant changes in a 40-day storage period (Table 2). The largest change of gametophyte ~~vigour~~vigor index after being stored was displayed in *D. tectorum* with a range from 53.36% decrease (0 ℃) to 57.32% increase (17 ℃). No significant change was found in the gametophyte ~~vigour~~vigor index of *D. vinealis* among the five temperatures. However, these gametophyte ~~vigour indexes~~vigor indices were all significantly lower than that before storage and decreased by 32.86% (17 ℃) to 45.65% (0 ℃). After being stored, the gametophyte ~~vigour indexes~~vigor indices 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* was between *D. vinealis* and *D. tectorum*.

After the 40-day storage at the five temperatures, the highest gametophyte germination of *B. unguiculata* and *D. vinealis* were ~~presented~~ at 17 ℃, while the peak in *D. tectorum* was ~~presented~~ at 25 ℃. The highest gametophyte increment of *B. unguiculata* was at 4 ℃, and the peak in *D. vinealis* and *D. tectorum* were both at 17 ℃ ~~as well as~~, which was the same for the gametophyte ~~vigour~~vigor index.

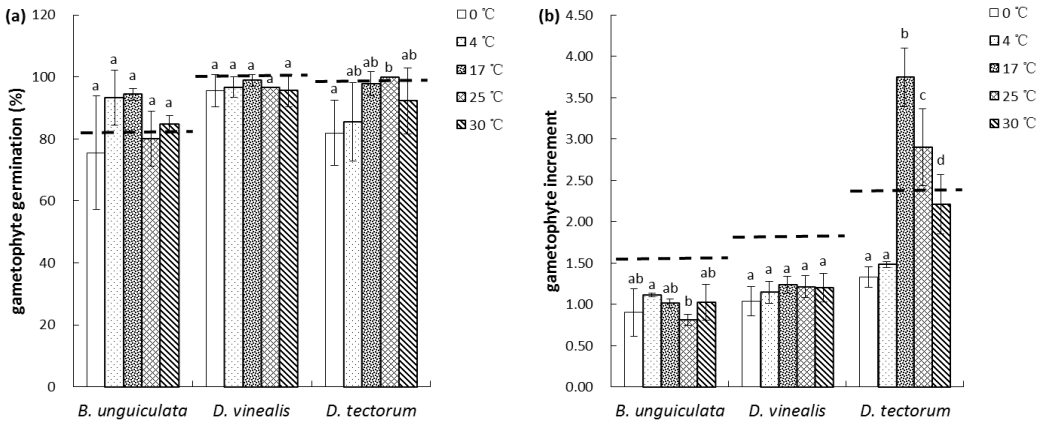

Fig. 1. Data (average ± 1 SE) of the three moss species in **(a)** gametophyte germination and **(b)** gametophyte increment after the 40-day storage at five temperatures. Different letters indicate significant differences ($P < 0.05$) among the five temperatures within the same species. Dotted lines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

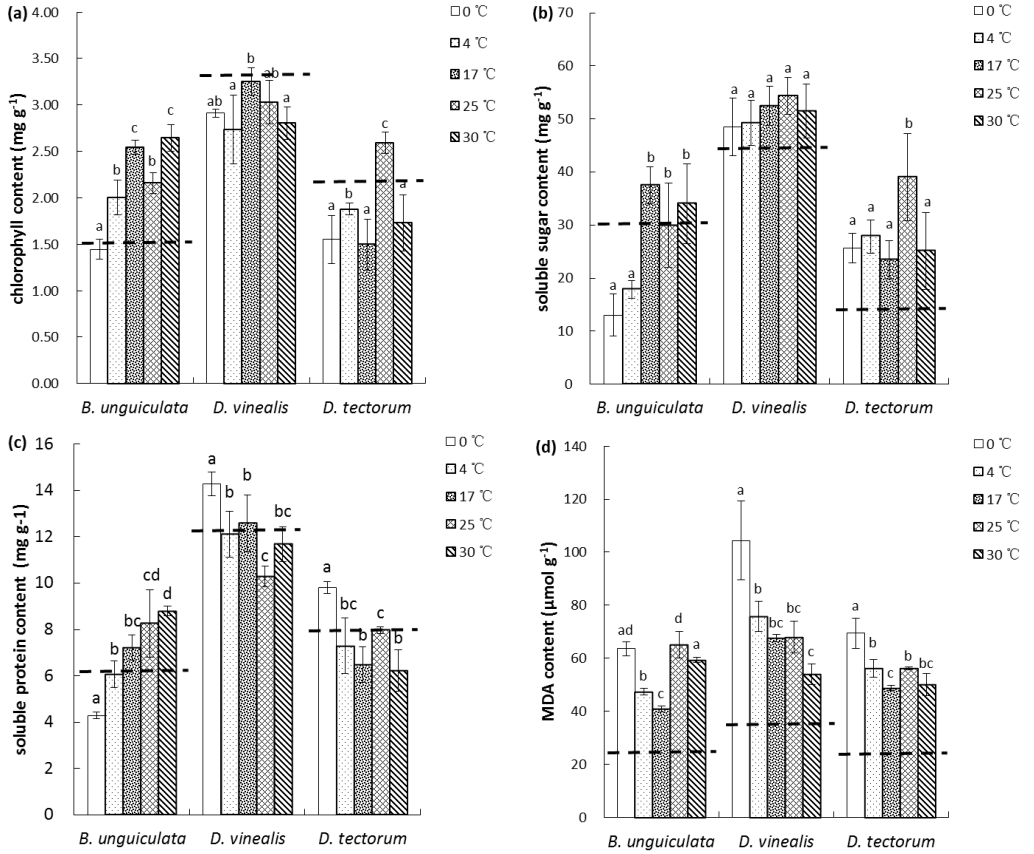

Fig. 2. (a-d) Data (average ± 1 SE) of the three moss species in **(a)** chlorophyll content, **(b)** soluble sugar content, **(c)** soluble protein content and **(d)** MDA content after the 40-day storage at five temperatures. Different letters indicate significant differences ($P < 0.05$) among the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

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

~~treatment~~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 physiological ~~indexes~~ indices and germination parameters 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**	
~~vigour~~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; ~~vigour~~vigor index: gametophyte ~~vigour~~vigor index.

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

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

	~~X1~~	~~X2~~	~~X3~~	~~X4~~
~~*B. unguiculata*~~	~~0.60±0.20~~	~~0.57±0.20~~	~~0.57±0.22~~	~~0.77±0.20~~
~~*D. vinealis*~~	~~0.55±0.27~~	~~0.62±0.23~~	~~0.74±0.28~~	~~0.73±0.22~~
~~*D. tectorum*~~	~~0.66±0.21~~	~~0.62±0.17~~	~~0.70±0.25~~	~~0.76±0.27~~

~~X1: chlorophyll content; X2: soluble sugar content; X3: soluble protein content; X4: MDA content.~~

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 Effect of storage temperature on the physiological index of mosses

As shown in Table 1 and Fig. 2a, the chlorophyll content of *B. unguiculata* ~~was~~ increased after being stored at four of the five temperatures ~~except~~, with the exception of 0 ℃. ~~Chlorophyll~~The chlorophyll content of *B. unguiculata* showed an ~~increase~~ increasing trend along with the storage temperature, and a maximum increase of 73.08% ~~was presented~~ occurred at 30 ℃. The smallest change ~~of~~in the chlorophyll content was found in *D. vinealis*, with ~~the maximal~~ a maximum decrease ~~was~~ of 17.89% at

4 ℃ and ~~the minimal decreases was~~ a minimum decrease of 2.39% at 17 ℃. ~~Chlorophyll~~The chlorophyll content of *D. tectorum* after storage ~~was reduced~~ decreased by 31.51% at 17 ℃ and increased by 18.50% at 25 ℃, which were the highest ~~content~~ and lowest ~~content~~contents, respectively.

~~The~~A similar increasing trend ~~to increase~~ as the temperature was rose also found in the soluble sugar content (Fig. 2b). The soluble sugar content was higher than ~~any~~ before storage, except for the content of *B. unguiculata*, which decreased by 56.52% and 40.47% at 0 ℃ and 4 ℃, respectively (Fig. 2b; Table 1) The soluble sugar content of *D. vinealis* ~~was observed~~ showed less variation than the other species. No significant difference was found between the ~~maximal and minimal increase~~minimum and maximum increases, which were 9.92% at 0 ℃ and 23.14% at 25 ℃, respectively. The greatest change ~~of~~ in the soluble sugar content ~~was presented~~ occurred in *D. tectorum*, which increased by more than 65% under all storage temperatures.

The MDA content had a more significant ~~variance with~~variation, increasing by more than 50% in all stored gametophytes (Fig. 2d; Table 1). The MDA content of *B. unguiculata* and *D. tectorum* both decreased as the temperature ~~rise~~ rose from 0 to 17 ℃ and then increased. Both *B. unguiculata* and *D. tectorum* had a ~~minimal~~minimum MDA content at 17 ℃. However, the MDA content of *D. vinealis* continuously decreased to ~~the~~a minimum at 30 ℃.

Some ~~temperature could cause~~temperatures caused the soluble protein content to change significantly ~~changed~~ (Fig. 2c; Table 1). The soluble protein content of *B. unguiculata* decreased abruptly from the value 40.06% above to the value 31.79% below with the temperature decrease. On the country, soluble protein showed ~~an~~the opposite trend ~~of change~~ in *D. vinealis* and *D. tectorum*. Both ~~of the two~~ species ~~were~~ presented the ~~maximal~~maximum increase at 0 ℃, which ~~were~~ was 16.64% in *D. vinealis* and 23.65% in *D. tectorum*. The ~~least~~ lowest soluble protein content of *D. vinealis* and *D. tectorum* ~~were reported~~ showed a decrease ~~by~~of 16.00% at 25 ℃ and a decrease ~~by~~ of 21.38% at 30 ℃, respectively.

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

3.4 Relationship between physiological characteristics and vegetative propagation of mosses

After analyzing the correlation among physiological ~~indexes~~ indices and germination parameters of desiccation-tolerant mosses, a significant correlation ($P < 0.01$) was found among the four physiological ~~indexes~~ indices and the three germination parameters, except for the chlorophyll content and MDA content (Table 3). The gametophyte germination showed a significant correlation ($P < 0.05$) with the soluble protein content and ~~more~~a highly significant correlation ($P < 0.01$) with the chlorophyll content and soluble sugar content. Only the MDA content was found ~~a~~to have significant

negative correlation ($P < 0.05$) with the gametophyte increment and gametophyte ~~vigour~~vigor index.

When the distinguishing coefficient was 0.5, ~~grey~~the gray incidence degree between physiological ~~indexes~~ indices (X1: chlorophyll content; X2: soluble sugar content; X3: soluble protein content; X4: MDA content) and the gametophyte ~~vigour~~vigor index of 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 Effect of storage temperature on 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 physiological characteristics of moss gametophytes were closely related to the success of artificial cultivation, for instance pretreatment with sucrose and/or abscisic acid could facilitate the viability of mosses by increasing DT (Burch and Wilkinson, 2002). ~~In fact, DT is not a constant feature of mosses, like seasonal variation in the desiccation responses (Dilks and Proctor, 1976).~~ In line with previous studies, this study also indicated different results of gametophyte regeneration within the same species after desiccation at different temperatures (Fig. 1a, 1b; Table 2), which was probably related to species-specific DT. The gametophyte ~~vigour~~vigor index of *D. tectorum* was the most sensitive to the change of storage temperature~~. Contrarily~~, while it ~~the gametophyte vigour index of *D. vinealis*~~ was the least changed and was not significantly different under storage temperature levels in *D. vinealis*. The vegetative propagation of mosses could be summarily described by the gametophyte ~~vigour~~vigor index, on the basis of ~~Eqs~~Eq. (1) - (3) and Table 3. Thus, the effect of storage temperature on the vegetative propagation of *D. tectorum* was the ~~biggest~~ largest, in contrast to *D. vinealis*.

~~Particularly, a~~Although the 40-day storage adversely affected regeneration in most moss inocula (Fig. 1a, 1b; Table 1), some inocula of *D. tectorum* stored at 17 ℃ and 25 ℃ produced more new individuals than before. It was not clear whether the enhancement of regeneration was correlated with the low-temperature tolerance of *D. tectorum*. In other words, *D. tectorum* possibly suffered low-temperature stress in early winter. Meanwhile, higher ~~temperature~~temperatures (like 30 ℃) also injured inocula of *D. tectorum*, which implied extreme temperatures were unsuitable for storing moss. It is assumed that a further hypothesis could be made about the impact of the storage environment ~~to~~on desiccation-tolerant mosses. For example, Burch (2003) found that the survival and regeneration of dehydration protonemata were reduced after cryopreservation, which was related to damage caused by intra-cellular ice ~~crystal. Desiccation~~crystals. The desiccation time could also affect the restorability of vegetative propagation and physiological characteristics in desiccation-tolerant mosses (Keever, 1957; Proctor, 2001). ~~In conclusion, some~~ Some changes caused by the environment and/or time ~~occurring~~ that occurred in dormant cells could yield different restoration results after rehydration.

4.2 Effect of storage temperature on physiological characteristics of mosses

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

The recovery of photosynthesis and protein synthesis in *B. unguiculata* ~~on photosynthesis and protein synthesis~~ was facilitated by higher temperatures (not more than 30 ℃) (Fig. 2a, 2c), which offered ~~an~~ the opposite illusion that the viability of other mosses tended to be weaker with increased temperature (Hearnshaw and Proctor, 1982). However, the ~~increase~~ increasing trend ~~of~~ in the MDA content from 17 to 30 ℃ implied that more membrane damage may be caused by storage temperature above 30 ℃ (Fig. 2d). The adverse effects of the relatively high ~~temperature~~ temperatures in *D. vinealis* and *D. tectorum* were clearly reflected by the slower recovery of photosynthesis and protein synthesis (Fig. 2a, 2c). Although the change ~~of~~in the MDA content in *D. vinealis* showed a faster repair of cell membrane as the temperatures rose, 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 response of the physiological characteristics of the three species to temperatures ~~on physiological characteristics~~ reflected different ~~restorability~~restoration abilities in a short rehydration time. If rewetting periods were longer than 30 days in the cultivation, the result of vegetative propagation could be defined as ~~a~~the long-term recovery of mosses. Thus, the long-term effect of cell recovery during short-term rehydration could be explained by the relationship between physiological characteristics and vegetative propagation of desiccation-tolerant mosses.

4.3 Relationship between physiological characteristics and vegetative propagation of mosses

Before the storage, the four physiological ~~indexes~~indices of gametophytes showed significant ~~difference~~ differences between *D. vinealis* and *D. tectorum*. However, no significant ~~difference~~differences between the two species ~~was~~ 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 DT made the vegetative propagation among species present bigger ~~difference~~ differences than before, which ~~was~~ were shown by the gametophyte ~~vigour indexes~~ vigor indices under the same treatment (Table 1; Table 2).

Therefore, the recovery ability of development and regeneration of dried mosses might play a more beneficial role to screen suitable inocula than in fresh ~~ones~~mosses. Although many ~~researches~~ studies have indicated that desiccation-tolerant mosses could recover from drying when they are rehydrated (Csintalan et al., 1999; Pressel et al., 2006), the overlong desiccation would impede the reuse of moss specimens and the restoration of dried biocrusts~~ made mosses fail to germinate (Keever, 1957)~~. This study also showed that ~~cell was~~cells were subjected to oxidative damage after the 40-day desiccation (Fig. 2d; Table 1). At the same time, the regenerative capacity of the three species ~~was~~ declined (Table 2), which implied that membrane integrity and/or other factors had an effect on the vegetative propagation of desiccation-tolerant mosses.

Based on the correlation coefficients among the physiological ~~indexes~~indices and germination parameters of desiccation-tolerant mosses (Table 3), the gametophyte germination revealed a significant positive correlation with the chlorophyll content, soluble sugar content and soluble protein content. On the country, the gametophyte increment and gametophyte ~~vigour~~vigor index were only ~~significant negative correlation~~significantly negatively correlated with the MDA content. It was a possible reminder that metabolic repair was favorable to the germination of new ~~gametophyte~~ gametophytes, and the result of long-term recovery depended more on cell integrity. Therefore, ~~in order~~ to quantitatively compare the effects of the four physiological ~~indexes~~indices on vegetative propagation, the ~~grey~~gray incidence degree between physiological ~~indexes~~indices and the gametophyte ~~vigour~~vigor index of the three moss species ~~were~~ was calculated by ~~Eqs~~Eq. (4)-(6). As shown in Table 4, the effect of the MDA content on the gametophyte ~~vigour~~vigor index was the greatest in *B. unguiculata* and *D. tectorum*, and the incidence degree of MDA in *D. vinealis* was quite similar to the maximum (the former was 0.73 and the latter was 0.74). The MDA content of the three mosses increased as the storage temperature ~~decrease~~ decreased from 17 to 0 ℃, when the smaller gametophyte ~~vigour~~vigor index of *D. vinealis* and *D. tectorum* presented at 0 ℃ and 4 ℃ rather than 25 ℃ and 30 ℃ (Fig. 2d; Table 2). ~~It could be indicated that more~~ This result indicated that greater membrane damage at low ~~temperature~~ temperatures caused the decline in regenerative capacity~~ decline~~. In addition, the higher gametophyte ~~vigour indexes~~vigor indices of *D. tectorum* at 17 ℃ and 25 ℃ than before ~~was~~ were possibly related to the reduction of intra-cellular ice ~~crystal~~ crystals during the storage period (Burch, 2003), which facilitated faster recovery upon rehydration than fresh gametophytes (Table 2). However, there were an increasing number of negative influences with increasing temperature that presented in the physiological characteristics (Fig. 2a-c). These high temperatures were unfavorable to the recovery of mosses (Hearnshaw and Proctor, 1982). When ~~cell~~ cells suffered damage under desiccation and temperature stress, the protection of more sugars was particularly important to maintain cell integrity in a dry state (Fig. 2d; Table 1). The possible reason for this is that *D. vinealis* showed no significant difference in the regenerative capacity ~~as~~ because the cellular protection was equivalent despite different temperatures.

Researchers have summarized the recovery mechanism 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 required more than 24 hours (Alpert and Oechel, 1985; Mansour and Hallet, 1981; Pressel et al., 2006). Based on

these results, cell integrity was supposed to be more difficult to ~~recovery~~recover than physiological reaction and had a great limit on recovery and regenerative capacity of desiccation-tolerant mosses. During long-term desiccation, cumulative damage affected cell function and integrity over time (Proctor, 2001), which ~~might result in different regenerative capacity of mosses with varied storage time (Keever, 1957). The~~ process was influenced by temperatures that might enhance or suppress cell damage according to the research. It means effects of temperature on ecology of DT in bryophytes are worth paying attention, especially during dry season in the semiarid and arid areas. More sensitive response of *D. tectorum* implied one of reasons why it was not a widely-distributed species like *D. vinealis* in the site. Furthermore, ecological niche requirements of different mosses both in dry time and wet time will influence the choice of moss inocula on artificial cultivation and biocrust restoration. Despite the distinction between the results in laboratorial conditions and the field, it might influence to match the ecological requirements, and furthermore precise description of microclimates and quantitative methods would be helpful to the issue.

~~According to the above analysis, cell integrity may be a critical influencing factor on the vegetative propagation of mosses.~~

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 regenerative capacity in mosses was related to cell damage caused by dehydrated storage. The storage temperatures during dehydration also influenced vegetative propagation of mosses because of temperature-induced changes in moss cell activity. A further analysis showed the effect of membrane damage on vegetative propagation was the maximal. Meanwhile, soluble sugars increased for protecting cells highlighting the important role cell integrity played in 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 the suitable temperature was 4 ℃ for *B. unguiculata*. Different responses to the temperatures in the three moss species were linked with species-specific DT, which could guide future research to study some suitable storage methods of inoculation material on the artificial cultivation of moss biocrusts.

In general, properties of inoculation material are key factors effecting the development and recovery of moss biocrusts, such as species, physiological feathers and/or other factors. The results helped to partly explain influencing factors on vegetative propagation of desiccation-tolerant mosses and furthermore to offer a new view about fast experimental approach to screen suitable inocula.

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