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COMPARISON OF DESICCATION TOLERANCE OF DESERT MOSS *SYNTRICHIA CANINERVIS* IN TISSUE CULTURE AND SAND CULTURE

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Abstract

Syntrichia caninervis is a stress-tolerant moss that has become an important experimental model for researches on desiccation tolerance and ecological restoration. However, its desiccation tolerance of *In vitro* culture remains poorly characterized. Here, we compared the morphological and physiological traits related to desiccation tolerance of regenerated mosses from Knop medium culture and sand substrate culture. In tissue culture, the explants first formed protonemata and then developed into gametophytes, whereas, the shoots grown on sand produced both protonemata and new gametophytes simultaneously. These results suggest the two regeneration patterns of *S. caninervis* have different phenotypic plasticity. Furthermore, under desiccation tolerance, the sand-cultured moss exhibits verrucose and spinous leaf surface, with higher abscisic acid (ABA) and trehalose contents than the tissue-cultured moss. However, the water responses during the processes of desiccation and rehydration were the same between the two cultured mosses, indicating that the two regenerated mosses possess the same desiccation-tolerant characteristics as wild mosses. By describing the desiccation tolerance of the two *In vitro* regenerated mosses, the findings of this study will help with efforts to provide sustainable and consistent materials for ongoing studies.

Key words: *Syntrichia caninervis*, Desiccation tolerance, Regeneration, *In vitro*, Tissue culture.

Introduction

Desiccation is caused by severe water loss, in which the cell loses a lot of the 'free' or 'bulk' water, and survives only on the 'bound water' in the cell-matrix (Ramanjulu & Bartels, 2002). When an organism survives desiccation by maintaining the equilibrium of the cell's water potential and can grow and metabolize upon rehydration, the phenomenon is called desiccation tolerance (Oliver & Payton, 2007). Desiccation tolerance dates back to the early land plants where it evolved in their vegetative tissues (Cushman & Oliver, 2011). For survival, most angiosperms require more than 60% of the relative cellular water content, but seeds of angiosperms and some resurrection plants do exhibit desiccation tolerance (Giarola *et al*., 2017).

The non-reducing sugar trehalose commonly found in several organisms including microorganisms, plants, and insects, protects them against various environmental stresses (Elbein *et al*., 2003). Presence of trehalose in plants negates the adverse effects of abiotic stresses, such as drought, salinity, and temperature (Koshland & Tapia, 2019). The ABA, produced in the vegetative tissues during drought stress, plays an important role in abiotic stress tolerance and signal transduction (Chen *et al*., 2019). Bryophytes, one of the earliest diverging lineages of the surviving land plants, inhabit ecological niches having high temperature, high light intensities, and desiccation and rehydration cycles that are unpredictable (Liu *et al*., 2020). Because Bryophytes represent an ancient lineage it was thought that although many of the functions of trehalose and ABA had been well documented in plants that it might have specific functions in mosses that were not well documented in angiosperms.

Syntrichia caninervis, a dominant moss found in Gurbantunggut Desert in China (Zhang *et al*., 2007), survives in the arid environment with numerous but limited water resources (Pan *et al*., 2016; Zhang & Zhang, 2020). This extremely desiccation-tolerant species can not only survive water loss, but can regain full turgor within some seconds upon rehydration (Coe *et al*., 2020; Stark *et al*., 2012). *S. caninervis* experiences physiological stress from constant exposure to extreme environmental conditions, such as elevated temperature, high light intensities, and hydration and dehydration cycles (Li *et al*., 2010).

In recent years, *S. caninervis* has often been studied as a desiccation-tolerant model species, including studies addressing its rapid resurrection speed (Wu *et al*., 2012), the physiological regulation of microhabitats (Yin & Zhang, 2016), the coupled multiscaled water collection structures (Pan *et al*., 2016), desiccation-tolerant genome(Silva *et al*., 2021), abscisic acid (ABA) signaling factor (Zhang *et al*., 2018), early light-induced protein (Liu *et al*., 2020), and *AP2/EREBP* and *ScALDH* gene families (Li *et al*., 2019a; Yang *et al*., 2019). Therefore, *S. caninervis* is more suitable for understanding desiccation-tolerance mechanisms and also for enhancing desiccation tolerance by genetic engineering in crops growing in arid regions. Meanwhile, *S. caninervis*, found in biological soil crusts, plays an important role in arid ecosystems, contributing to nitrogen cycling, carbon storage, and soil stability and fertility, and providing beneficial microhabitats for organisms (Li *et al*., 2019b). Therefore, *S. caninervis* possesses a more powerful tool to explore the molecular mechanism and conservation of the ecosystem.

S. caninervis is a dominant plant that grows in susceptible environments; once the moss is disturbed, its recovery period will be extremely long, typically around 10 years or more (Xu *et al*., 2008). Therefore, for ecological restoration and scientific research, it is important to develop moss-dominated soil crusts. Previous studies indicated that the leaf of *S. caninervis* could be cultured on Knop medium and the protonemata could generate large amounts of gametophytes (Zheng *et al*., 2011). Other reports pointed out that the protonemata of *S. caninervis* can be reproduced directly on the sand, and the explants that were cultivated with *in situ* soils produced more protonemata and shoots (Xu *et al*., 2008). Also, differential fragment regeneration of *S. caninervis* was compared on the surface of moistened sands, which implicated clonal growth as the main reproductive pattern in the desert (Zhuo *et al*., 2018). Yet, those studies had focused on the regeneration system of *S. caninervis*, the desiccation-tolerant capacity of *In vitro* culture was still limited, and the comparison of the desiccation tolerance in mosses regenerated from different cultures remained unexplored.

Here, we describe the *In vitro* culture of *S. caninervis* and use the developmental characteristics of gametophytes regenerated on tissue culture (grown on agar) and sands, respectively. We compared the morphological and physiological traits related to desiccation tolerance between tissue culture and sand regeneration. This research will lay the foundation for future efforts to explore the underlying mechanism of abiotic stress resistance by moss and the development of moss-dominated crusts.

Materials and Methods

Explants: Gurbantunggut Desert in Xinjiang, China (44°36′N, 88°15′E) was the collection site for *S. caninervis* individuals. In this study, we raised a minimum of three asexual generations that were grown till maturity to develop a single clonal line. After 4 weeks of cultivation, shoots were **selected** for experimentation. Mosses were grown in a plant growth chamber (BING LIN, ZRG-L, China) and with a 12 h photoperiod (22°C light/12°C dark), with a relative humidity (RH) of 65% and a photosynthetic photon flux density (PPFD) of approximately 100 μmol·m^{-2}·s^{-1}. To multiply and establish subcultures for developing adequate quantities of moss for desiccation tolerance comparison, we used gametophytes from the original clonal line.

Tissue culture conditions: For the *In vitro* culture of the desert moss *S. caninervis* were grown on solidified Knop medium (0.25 g/L KH_2PO_4, KCl, $MgSO_47•H_2O$, 1 g/L Ca $(NO_3)_24•H_2O$ and 0.0125 g/L $FeSO_47•H_2O$) at pH 5.8 in closed Petri dishes (Nomura & Hasezawa, 2011). Young leaves of gametophytes (as explants) were rinsed with deionized water, disinfected in 75% (v/v) ethanol for 1 min, washed thrice using sterile water, and then again disinfected using 3% (v/v) sodium hypochlorite for 1 min, washed thrice using sterile water, and cultured in the sterile Petri dish containing Knop medium. The explants were grown in a controlled environment (22°C light/12°C dark, 65% RH and 100 μmol·m^{-2}·s^{-1} PPFD). All shoots in agar were completely dehardened over the duration of the experiment. After culturing for 4 weeks, the areas and numbers of the protonemata were observed and measured using digital image analysis software.

Sand cultures: The sand used for cultivating moss was collected near the metapopulation (called *in situ* soil). It was air-dried and sieved, and then autoclaved (dry mode, 1 h at 132°C). The sand surface was moistened with distilled water. The hydrated moss leaf was placed with adaxial surface facing upwards on the moistened sand surface. It was then watered every alternate week with sterile distilled water to prevent the sand surface from drying. The cultures were incubated in a growth chamber, with a 12-h photoperiod and specific growth conditions (22°C light/12°C dark, 65% RH, and 100 μmol·m^{-2}·s^{-1} PPFD); the plants growth was monitored, the numbers and areas of protonemata were recorded after 4 weeks.

Determination of water content changes during desiccation and rehydration: To evaluate the water dynamic characters of the tissue-cultured moss and sand-cultured moss, air dry, fast desiccation, and rehydration treatments were conducted.

Air dry: In the initial treatment, the explants from the regenerated gametophytes were maintained fully hydrated with distilled water. The fully hydrated moss was exposed to air on a Petri dish, and the weight was measured at intervals (0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h) until there was no further change in the mass. These materials were transferred to pre-weighed Petri dishes (3.5 cm in diameter) and quickly weighed to obtain the weight of the dehydrated moss. The Petri dishes were placed at ambient conditions (23°C, RH = 20%). The water contents of triplicate samples were calculated, and survival tests were performed. The experiments were replicated three times.

Fast desiccation: The fresh gametophytes were placed uniformly on 3-mm filter paper on activated silica gel in a covered Petri dish, which was then incubated at room temperature (RH = 2%, 23℃, -537 MPa) (Liang *et al*., 2021). The weight was recorded at regular time intervals (0, 20, 30, 60, 90, and 120 min) till no further decline in the mass was noted. The experiment was repeated thrice and these were considered as three biological replicates.

Rehydration: Fully dehydrated samples were placed in Petri dishes containing distilled water. After immediately removing the moss surface water using filter papers, the weight of the rehydrated samples was measured quickly at time intervals (0, 10, 20, 30, 40, and 60 s), and the samples were returned to the water in the Petri dish until there was no further change in the mass. Triplicate samples were performed.

We determined the shoot water content (WC) on a dry mass basis by weighing a group of 30 shoots and then reweighing it after oven-drying for 3 d at 80°C. The WC was calculated by the formula [(Equilibrated mass – Oven dry mass) / Oven dry mass] × 100, which yielded a percentage dry weight (DW) (Coe *et al*., 2020).

Chlorophyll and trehalose content assays: We transferred the mosses regenerated in tissue and sand cultures, respectively, to Petri dishes containing filter papers moistened with 8 mL filtered water and dehydrated for 1, 2, and 4 h (at 23°C, 100 μmol·m^{-2}·s^{-1}, and RH = 25%). For control, we used hydrated moss (0 h). A UV-visible spectrophotometer (Biomate 3S, Thermo Fisher Scientific, Waltham, MA, USA) was used to analyze the supernatants at 649 nm and 665 nm. Chlorophyll content was measured according to Ritchie's method. (Ritchie, 2006). The chlorophyll content, and concentrations of the chlorophylls a and b were calculated using the formulae: Chl a = 13.95 × OD_{665} − 6.88 × OD_{649}, Chl b = 24.96 × OD_{649} − 7.32 × OD_{665}. Chlorophyll content (mg·g^{-1}) = chl a + chl b.

Meanwhile, the content of trehalose was measured by the anthrone colorimetric method at multiple desiccation times (0.5, 1, 2, 4, 8, 12, 24, and 48 h) at 23°C, 100 μmol·m^{-2}·s^{-1}, and RH = 25%. Briefly, weighed about 0.1 g tissue and added 1 mL extract, homogenized in ice bath, stood at room temperature for 45 min, shook 3-5 times, centrifuged at 8000 g at 23°C for 10 min after cooling, and took the supernatant using a UV-Visible spectrophotometer measured absorbance values at 620 nm. Then, calculated trehalose content (trehalose content = [V1 × (A − 0.0729) ÷ 8.8976] ÷ (W × V1 ÷ V2). V1: sample volume; V2: extract volume; A: absorbance values; W: sample weight). The well-watered moss was used as a control (0 h). Each sample had at least three plants, and all the experiments were conducted thrice.

Determination of endogenous ABA: For ABA extraction, regenerated gametophytes were transferred to clean Petri dishes and air-dried at room temperature for 0, 0.5, 1, 2, 4, 8, 12, and 24 h. The control (hydrated) and dehydrated samples were harvested, and immediately flash-frozen in liquid nitrogen.

Endogenous ABA extraction and measurement were carried out by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) (Agilent 1290, USA). Briefly, 1 g of each sample was accurately weighed and ground to generate a fine powder, and sonicated with 10 mL of methanol containing 1% (v/v) formic acid for 2 min, followed by extraction for 12 h. The supernatants were then collected after centrifugation and purified by solid phase extraction (SPE) (C18, 300 mg, Xiboshi). The SPE cartridges were conditioned and equilibrated with 6 mL of methanol and subsequently with 6 mL of purified water. After equilibration, 500 μL of the supernatant was applied to the cartridge, which was then washed with 5 mL of 10% (v/v) methanol. ABA was eluted from the SPE cartridge with 5 mL of methanol containing 1% (v/v) formic acid. The purified extract was dried using a nitrogen evaporator and reconstituted with 1 mL of methanol containing 1% (v/v) formic acid. An aliquot of 5 μL purified samples were subjected to UPLC-MS analysis. The experiment was repeated three times and treated as three biological replicates.

Regeneration and statistical analyses: All the cultures raised in Petri dishes were placed in the growth chambers (Jiangnan, China). The growth chamber conditions were altered as per the experimental requirements. From 4 weeks after cultivation, protonemata and gametophytes at all stages were observed using a dissecting microscope (Olympus, SZXZILLT), and the surface structures of regenerated mosses were viewed using a scanning electron microscope (ZEISS SUPRA55VP). The data for secondary protonemata were initially recorded every 3 days and then on a weekly basis after 21 days. At the end of the experiment, the number of protonemata were counted by counting those directly penetrating the substrate as described by Stark *et al*., (Stark *et al*., 2004) and the areas were measured using ImageJ v1.5 software (Wayne Rasband, 2010). We cultured 30 explants in three Petri dishes for each cultural condition. Three replicates were used for the orthogonal test design and were analyzed by range analysis. Statistical analyses were performed using SPSS v18.0 (SPSS Inc, Chicago, IL, USA). Data were compared using a one-way ANOVA analysis and differences were considered statistically significant at $p<0.05$, distinct differences at $p<0.01$.

Results

The morphological comparison

The regeneration potential of the moss: During the moss culture, we observed that the explants in tissue culture first underwent protonemata stage, and then the protonemata developed into a gametophyte on Knop medium (Fig. 1a). In contrast, mosses grown on sand produce both protonemata and shoots simultaneously from the detached tissues (Fig. 1b). Therefore, the tissue-cultured explants had large numbers and areas of protonemata under the same growth times (Table 1). Emergence regeneration depended on the type of culture, with sand-cultured mosses taking a comparatively lesser time than tissue-cultured mosses, and the regeneration potential from sand-cultured mosses was greater than that from tissue-cultured mosses.

Scanning electron microscope observation: By using scanning electron microscope, we observed that the regenerated moss from sand culture displayed more verrucose and spinous leaf surface, in contrast to the regenerated moss from tissue culture, which had smooth leaf surface (Fig. 2). These phenomena might imply that the different culture conditions would produce different morphological characteristics of regenerated mosses.

Table 1. The numbers and areas of protonemata produced by the desert moss *Syntrichia caninervis* in different media ($N = 30$).

	Protonema number			Protonema area (mm^2)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Tissue	9	54	30 ± 2	126.2	341.1	172.5 ± 11.6
Sand	4	11	6 ± 1	19.4	84.5	42.8 ± 4.1

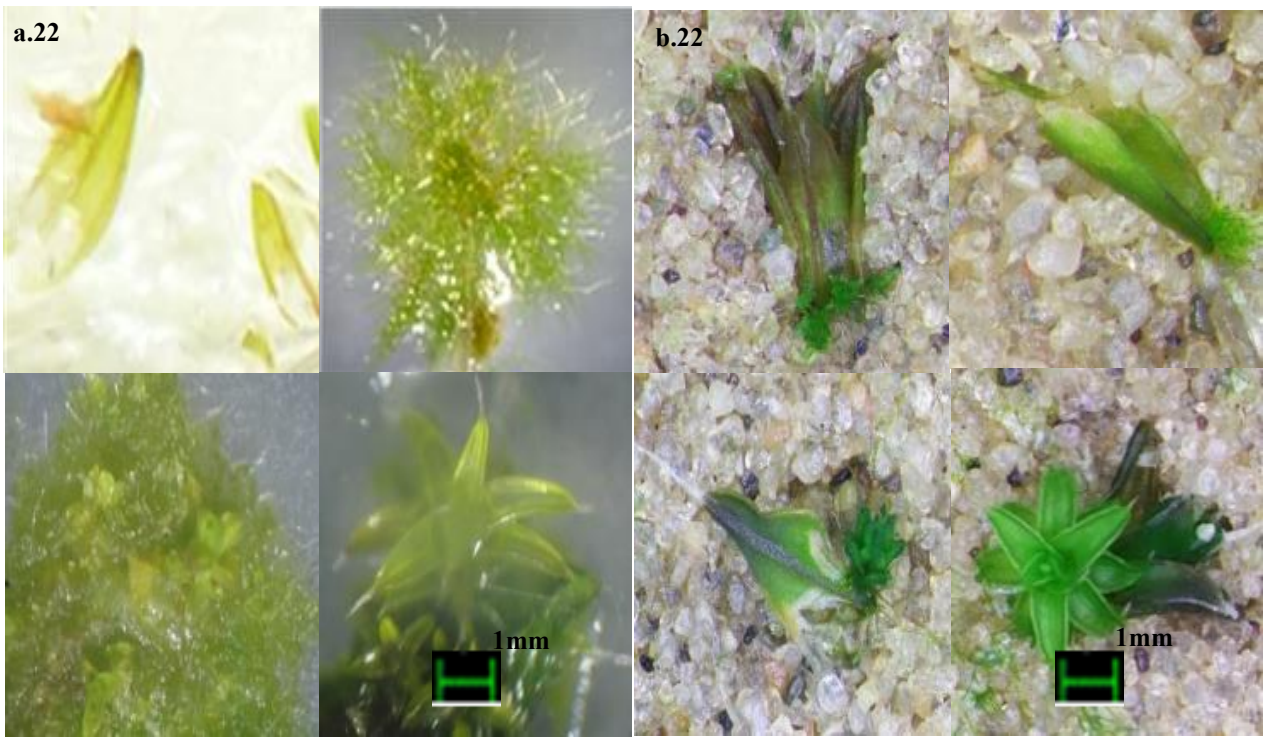


Fig. 1. Protonemata and shoots emergence from *Syntrichia caninervis* explants cultured in different media. (a) Tissue culture. (b) Sand culture.

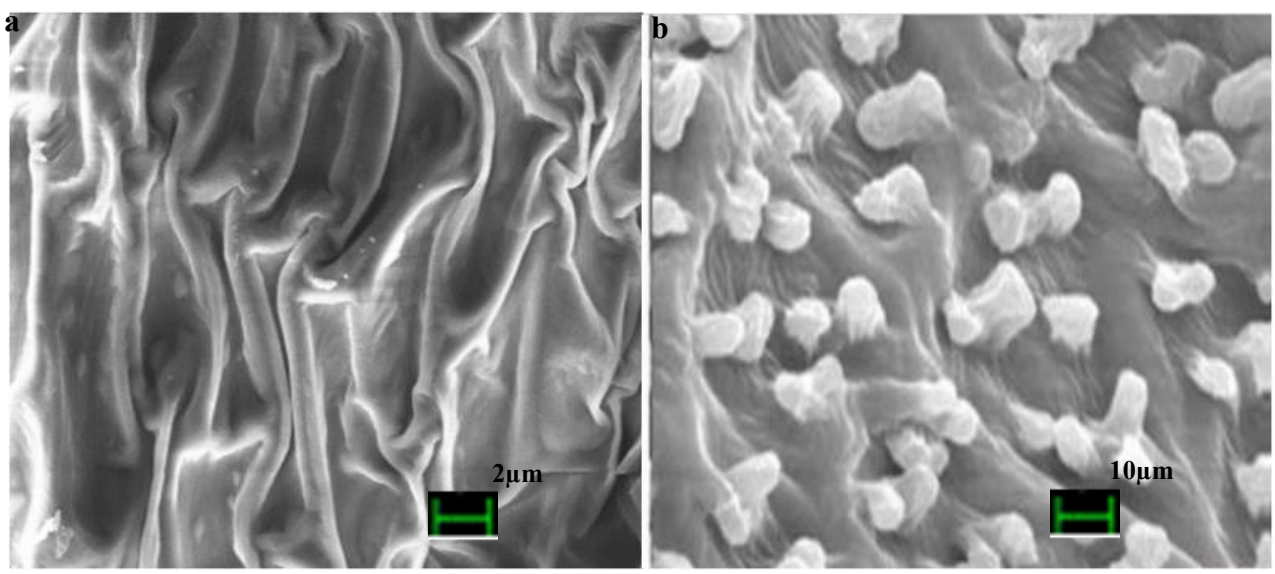


Fig. 2. Scanning electron microscopy images of moss leaves. (a) The leaf surface of the tissue-cultured moss is smooth. (b) The leaf surface of the sand-cultured moss is more verrucose and spinous.

Analysis of physiological traits related to desiccation tolerance

Changes in the water content of regenerated explants: The WC of poikilohydric mosses helps understand the water condition in biological membranes and their ability to perform metabolic activities (Coe *et al*., 2020). Changes in the water content of regenerated mosses were measured during desiccation and rehydration under ambient conditions. The water content profiles of tissue-cultured mosses in air dry and fast desiccation of the regenerated explants were the same as sand-cultured mosses, similar to water content increase pattern during rehydration (Fig. 3). However, during air dry, the water content was slightly higher in tissue-cultured mosses than in sand-cultured mosses; both decreased progressively until 4 h when the water content reached its lowest value and then remained almost constant (Fig. 3a).

Changes in chlorophyll, trehalose, and ABA contents: Total chlorophyll contents of both tissue-cultured and sand-cultured mosses were gradually decreased under desiccation treatment from 0 to 4 h. However, the chlorophyll content in tissue-cultured mosses was always higher than that of sand culture-derived gametophytes at all time points (Fig. 4).

The accumulation of trehalose during desiccation treatment was also determined in tissue- and sand-cultured mosses. In sand-cultured mosses, trehalose accumulated slowly during the early stage before 6 h, but in the following stage, from 6 h to 48 h, it accumulated linearly, which was the dominating period of trehalose accumulation. In contrast, the trehalose content in tissue-cultured mosses gradually increased from 0 to 4 h, peaked at 6 h, and then decreased slightly to a stable level (Fig. 5). At all-time points during the desiccation treatment, the trehalose level in sand-cultured mosses was much higher than that of tissue-cultured mosses, especially after 6 h (ranging from 1.6 to 2.8-fold), suggesting a protective role of trehalose synthesis during desiccation treatments.

The ABA contents were significantly increased after desiccation treatment compared with the 0 h in both *In vitro* cultures. Meanwhile, the ABA content in sand-cultured mosses was significantly higher than that of tissue-cultured mosses. In sand-cultured mosses, the ABA content was determined to be relatively constant during 0 to 1 h of desiccation but substantially increased after 1 h, reaching values as high as 17.61 $ng \cdot g^{-1}$ DW at 12 h, which was more than a 2-fold increase compared with 0 h. After reaching this level, the amount of ABA remained constant. By contrast, the ABA content in tissue-cultured mosses increased from 2.73 $ng \cdot g^{-1}$ DW in the control to 7.05 $ng \cdot g^{-1}$ DW after 12 h of water loss. Subsequently, it declined to 5.35 $ng \cdot g^{-1}$ DW at 24 h but was still higher compared to hydrated control (0 h) (Fig. 6).

Discussion

In recent years, it has been found that *S. caninervis*, a resurrection moss distributed in desert zones, can tolerate a certain amount of cellular desiccation, and can survive prolonged periods of desiccation to levels at which all the bulk water is lost from their tissues (Silva *et al*., 2021). Such characteristics make *S. caninervis* an excellent model species to study both desiccation tolerance and underlying mechanisms of cell repair. Accordingly, an appropriate culture platform is a prerequisite for further research.

In this study, we found that the moss leaves emerged protonemata after 7 days of cultivation, and they extended after another 7 days, then, the protonemata differentiated into gametophytes in tissue culture. However, the profile in sand culture was the detached leaves simultaneously produced both shoots and protonemata, this process progressed more quickly than in Knop medium (Table 1; Fig. 1). Our results showed that tissue and sand cultures produced shoots by two different methods: the former used protonemata and the latter, leaf meristems. These two regeneration methods are also dependent on the type of media. In tissue culture, Knop medium is used and ammonium tartrate is added as a supplement, which promotes the development of chloronema. Therefore, the cell type develops directly from regenerating moss protoplasts (Hohe & Reski, 2005). By contrast, *in situ* soils contain less organic matter and more inorganic salts, and resemble moss reproduction in wild environment.

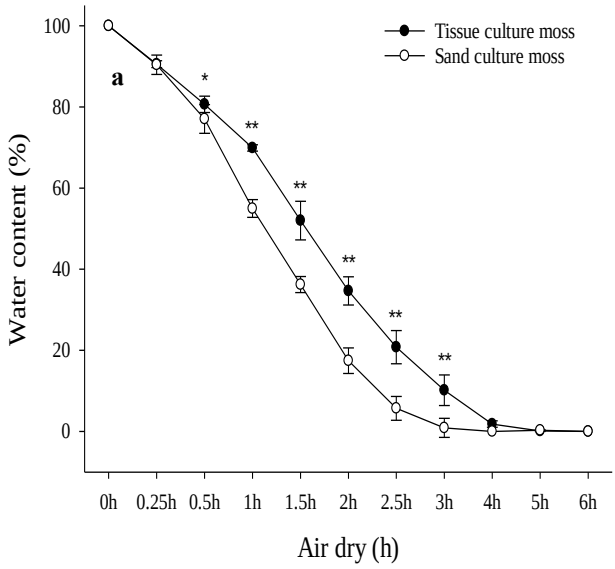
The verrucose and spinous leaf surfaces are also important characters in addition to the rhizoids, which participate in desiccation tolerance (Tao & Zhang, 2012). In this experiment, we found that the sand-cultured moss has verrucose and spinous leaf surface, which is not inherited but acquired through environmental effects. In addition, the appearance of the sand-cultured moss was very similar to the wild sample when the next generation originated from the germinated gametophyte but not from the protonemata (Fig. 1). The regenerated gametophyte observed variation indicate that *S. caninervis* occurring in different environments appear to differ morphologically (Stark *et al*., 2005) and exhibits phenotypic plasticity (Reynolds & McLetchie, 2011).

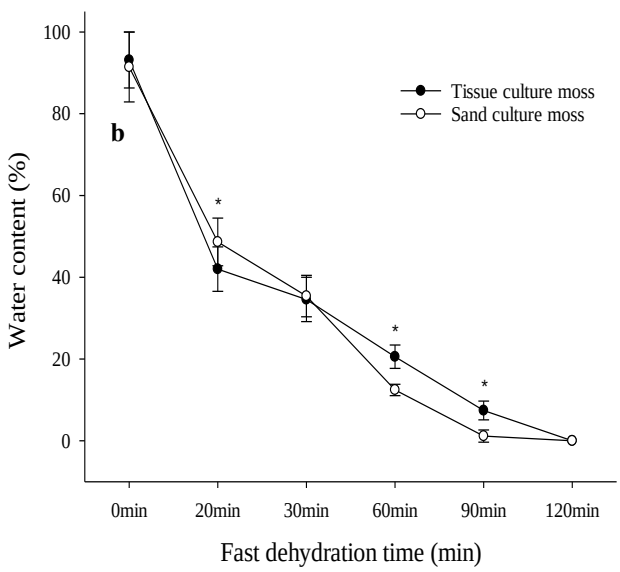
The response to water changes was the same between the tissue-cultured moss and sand-cultured moss during the processes of desiccation and rehydration (Fig. 3). This finding implied that the regenerated moss possesses the same desiccation-tolerant characteristics as the wild moss, even though there are some morphological differences between them. Therefore, the genetic research of desiccation tolerance using the regenerated gametophyte is feasible.

In plants, trehalose is extremely rare compared to sucrose. Trehalose is preferred over sucrose by most organisms because it has fewer tendencies to form crystals than does sucrose, it is considered an important factor in tolerance (França *et al*., 2007). In *Physcomitrella patens*, two active trehalose-6-phosphate synthase enzymes (*PpTPS1* and *PpTPS2*) existed (Tran *et al*., 2020), and the accumulation of trehalose was significantly changed in abiotic stresses (Arif *et al*., 2018). So far, studies in trehalose metabolism are limited in bryophyte during a desiccation event.

In this study, we have shown that desiccation leads to elevated levels of trehalose, and the sand-cultured mosses accumulated much more trehalose than tissue-cultured mosses (Fig. 5). Therefore, we speculated that trehalose prevented protein from denaturing during desiccation probably by reducing cellular osmotic potential (Kosar *et al*., 2019). The hydration potential of trehalose during desiccation is high that helps stabilize dry proteins and cellular membranes by replacing surface-bound water (Shortlidge *et al*., 2012). Hence, we speculated that sand-cultured accumulated much more trehalose is because its biological structures have high hydrophilic.

ABA is important in imparting desiccation tolerance in plants (Chen *et al*., 2019). In 2008, the whole genome of *Physcomitrella patens* was published and this promoted interest and new insights related to the function of ABA in Bryophytes (Rensing *et al*., 2008). The more recent interest in the role of ABA in regulating the acquisition of desiccation tolerance indicated that endogenous ABA levels increased during dehydration. However, ABA does not appear directly involved in acquisition of tolerance but maybe important once it is established (Rathnayake *et al*., 2019; Xiao *et al*., 2018). *S. caninervis* experiences physiological stress from constant exposure to extreme environmental conditions (Coe *et al*., 2020; Stark *et al*., 2012), it provided an ideal opportunity to determine if ABA is a part of a constitutive rather than and inductive mechanism of desiccation tolerance. Through UPLC-MS, we observed the ABA content involved in two regeneration gametophytes, that occured late in the desiccation process, which was similar to what was seen in *P. patens* (Shinde *et al*., 2012). We found that the ABA content of sand-cultured mosses was about 3-fold higher than that of tissue-cultured mosses at each time point (Fig. 6). These data are also consistent with previous observations of dehydration-induced increases in ABA in *Funaria hygrometrica* (Werner *et al*., 1991) and *Exormotheca holstii* (Hellwege *et al*., 1994).





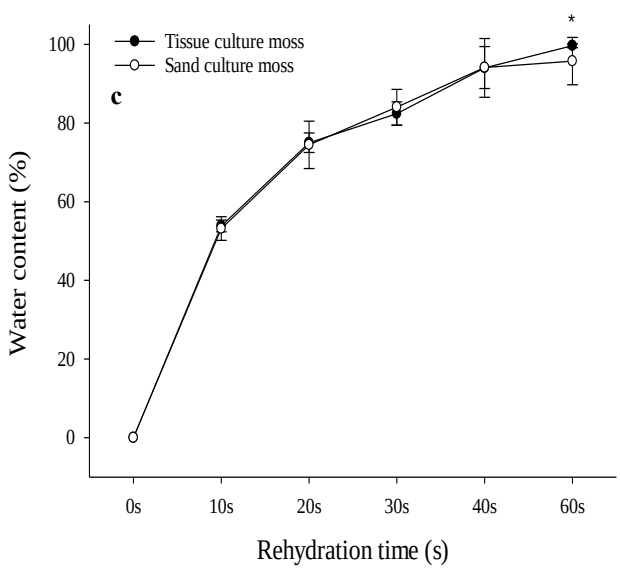


Fig. 3. The water change pattern of the regenerated explants from tissue culture and sand culture. (a) The water content (WC) of the regenerated explants during air dry. (b) The WC during fast desiccation. (c) The WC during rehydration.

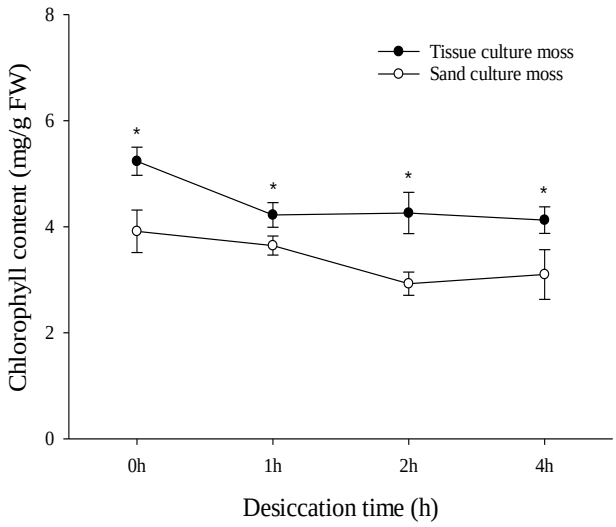


Fig. 4. Changes the chlorophyll content in tissue-cultured and sand-cultured mosses with desiccation time.

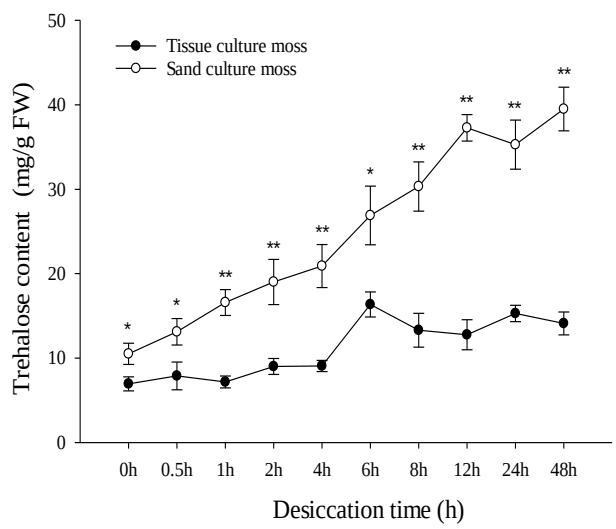


Fig. 5. Changes in the trehalose content in tissue-cultured and sand-cultured mosses with desiccation time.

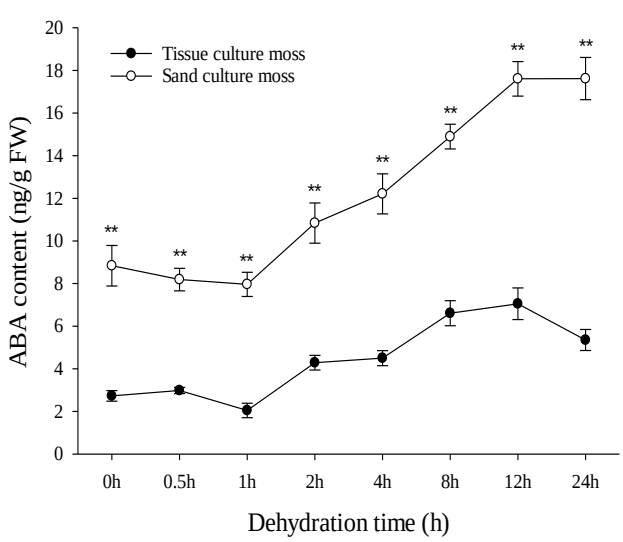


Fig. 6. Changes in the ABA content in tissue-cultured and sand-

cultured mosses with desiccation time.

This result demonstrates that ABA is important in stress response of *S. caninervis*, and sand-cultured mosses have higher endogenous ABA contents, which might be associated with stronger desiccation tolerance. These data support the hypothesis that ABA is not involved in the direct initiation of desiccation tolerance in bryophytes but maybe involved in its maintenance or perhaps in directing the production of protective metabolites and proteins that accumulate during drying (Rathnayake *et al*., 2019).

In addition, the chlorophyll content in tissue-cultured mosses was generally higher than that of sand-cultured mosses (Fig. 4), possibly because the culture medium contained Fe element, which can induce the production of chlorophylls (Molassiotis *et al*., 2006). Overall, the sand-cultured moss exhibits verrucose and spinous leaf surface and has higher ABA and trehalose contents under desiccation treatment, which indicates that sand-regenerated gametophyte is very similar to the wild sample, which has stronger desiccation tolerance.

Conclusion

In conclusion, our results demonstrate that *S. caninervis* is reproducible in agar and sands. Similar desiccation-tolerant gametophytes can be regenerated in these environments. However, the trehalose and ABA contents of sand-cultured mosses were significantly increased relative to tissue-cultured mosses, suggesting that the regenerated moss from sand culture was more desiccation-tolerant than that from tissue culture. This study not only provides sustainable and consistent materials for ongoing studies on the mechanism of desiccation tolerance and functional genomics of the desiccation-tolerant moss *S. caninervis*, but also paves the way for further studies on developing moss-dominated soil crusts in arid regions.

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Author contributions

YGZ and HLY conceived and designed the experiments, YGZ and LZ conducted them, HLY and LZ analyzed the data, and YGZ contributed to the writing of the manuscript.

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