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Evaluation of Abiotic Stress Response in In Vitro Culture of *Hylocereus undatus*

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ABSTRACT

Hylocereus undatus or white dragon fruit is a vine-like cactus that is commonly grown as both an ornamental plant and a fruit crop. However, their response to various abiotic stresses is understudied. Once transferred to the soil, the plants are exposed to different abiotic stresses caused by extreme salinity, drought, pH changes, and oxidative responses. This will either affect its growth or fruit production. The present study aims to evaluate the effects of various abiotic stress which are common in agricultural areas in vitro. The seeds of white dragon fruit were germinated in Murashige and Skoog (MS) basal medium supplemented with of 2.0 mg/L 6-benzylaminopurine (BAP) and 0.5 mg/L 1-Naphthaleneacetic acid (NAA) before the shoots formed were induced in MS with different abiotic stress which were drought, salinity, pH and oxidative stress. The shoot and the root length were recorded at the fourth week of culture. Interestingly, slight drought stress with 20 g/L polyethylene glycol (PEG) 8000 induced shoot growth but further increase of PEG 8000 reduced shoot length significantly. Shoot length was the most inhibited at 200 mM NaCl and root failed to grow at this concentration. Shoot and root growth under pH stress was the lowest at pH 8. The length of shoot and root under oxidative stress were seen to decrease gradually with increase of H_2O_2 concentration but this was only significant in roots. In conclusion, roots were more vulnerable to some abiotic stresses than shoots were. A greater magnitude of stressors can be tested for further information of *H. undatus* tolerance to abiotic stresses.

Keywords: *Hylocereus undatus*, abiotic stress, Murashige and Skoog, white dragon fruits, stress tolerance

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INTRODUCTION

Dragon fruit or pitaya is a kind of cactus vine that belongs to the Cactaceae family (Patwary et al., 2013). This plant is appealing because of its distinct look (Liaotrakoon, 2013). Dragon fruit species and varieties can be distinguished by the colour of their skin and pulp. There are three types of dragon fruit which is *Hylocereus undatus* (red peel with white flesh), *Hylocereus polyrhizus* (red peel with red flesh), and *Hylocereus megalanthus* (red peel with red flesh).

According to Rifat et al. (2019) this fruit is perceived as one of the most significant economic fruit species worldwide. Despite being one of the most extensively farmed crops in Malaysia, dragon fruit is not considered an indigenous species. Red and white flesh dragon fruits are the two main varieties that can be grown in Malaysia. On the other hand, red peel with red flesh and red peel with white flesh are typically regarded differently based on the area or county (Yusof et al., 2020). Dragon fruits demands are increasing rapidly due to their nutritive and medicinal properties (Sonawane, 2017).

For agricultural plants, abiotic factors are the primary yield-limiting elements (Waqas et al., 2019). Abiotic stress generates an imbalance of pro-oxidant and antioxidant chemicals, often known as oxidative stress, via mechanisms that have been well-reviewed (Nadarajah, 2020). Abiotic stress conditions usually favour stomatal closure, enhancing the activity of the photorespiratory pathway and activating hydrogen peroxide (H_2O_2) generation(You & Chan, 2015). Plant growth and yield distribution are impacted by temperature restrictions, drought, floods, salinity and heavy metal pressure (Waqas et al., 2017). A future decline in the efficiency of substantial yields, particularly of major food crops, has been predicted by measures based on a combination of harvest yield models and climate change, which may have serious consequences for agricultural production (Tigchelaar et al., 2018).

Drought is defined as a prolonged period of decreased water availability to plants, which has an impact on agricultural yield. Drought stress is a condition that occurs when plants experience a prolonged period of inadequate water supply. Under drought conditions, plant tries to conserve water by reducing transpiration, which results in the closure of stomata. However, this reduction in transpiration also reduces the plant's ability to absorb carbon dioxide, which is necessary for photosynthesis (Abdelraheem et al., 2019).

High salinity is the most detrimental abiotic stress to agricultural productivity in deserts and moderate climates, causing losses in the most critical phases of plant development, such as germination and seedling growth (Ibrahim, 2016). Salinity stress reduces seed metabolism and inhibits reserve accumulation because of the reduced influx of water generated by the osmotic response (Freire et al., 2018).

Gentili et al. (2018) reported that pH is an important factor related to plant development. The most important pH-dependent processes include nutrient accessibility and absorption. For instance, most micronutrients are more easily accessed by plants in acidic mediums than they are in neutral or alkaline mediums, which promotes plant growth and development. The pH of a plant can affect a variety of traits, such as height, lateral spread, biomass, flower size and quantity, pollen production, and many other traits (Jiang et al., 2017).

According to Apel and Hirt (2004), when agricultural plants are subjected to harsh abiotic circumstances, they produce an excessive amount of reactive oxygen species (ROS) and accumulate them. When ROS production exceeds a plant's capacity to scavenge excess ROS, excess ROS leaks into other areas of the plant tissue. Excessive ROS levels are damaging to plants and may disrupt the physiological, morphological, and metabolic processes of the cell. In plant cells, the primary sites of ROS generation are mitochondria, chloroplasts, and peroxisomes. ROS have distinct half-lives and oxidizing potentials, such as hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl radical. Extreme H_2O_2 causes autophagy and programmed cell death in chloroplasts and peroxisomes. The role of H_2O_2 in signalling, such as during stress adaptation and pathogen defence, has received a lot of attention, but the signalling pathways mechanisms are not entirely explicable (Hao et al., 2014; Nagano et al., 2016).

In vitro propagation of *H. undatus* is a prospective way to fulfil current market demand because they are free from diseases and uniform in terms of quality. However, their tolerance to various abiotic stress has not been investigated. Once transferred to the soil after hardening, the plants are exposed to different abiotic stresses caused by extreme salinity, drought, pH changes, and oxidative responses. This will either affect its growth or fruit production. Therefore, the present study aimed to ascertain the effects of drought, salinity, pH, and oxidative stress on *in vitro* grown *H. undatus*. The level of stresses the plant can tolerate until data on the implications of abiotic stress on *H. undatus* are obtained.

MATERIALS AND METHODS

Seed

The seeds of local dragon fruit were extracted from fresh ripe fruit that was obtained from local supermarket in Terengganu. The fruit was stored at room temperature a few days earlier to seed extraction since lower temperatures significantly decrease the viability of the seeds as explants for the *in vitro* culture.

Preparation of growth medium

MS medium (Murashige and Skoog, 1962) supplemented with of 6-benzylaminopurine (BAP) 1-Naphthaleneacetic acid (NAA) was used as growth medium in the present study. MS medium consisted of MS powder (DUCHEFA Biochemie) (4.4 g/L), 2.0 mg/L BAP and 0.5 mg/L NAA, sucrose (30 g/L) was brought to pH 5.8 before adding Phytagel™ (3g/L) and dissolved in a microwave. About 10 mL of the media were per test tube. The media were autoclaved for 15 minutes at 105 kPa and 121 °C.

Sterilization of *Hylocereus undatus* seeds

Freshly extracted seeds were surface-sterilised by dipping in 70% ethanol for two minutes, quick immersion in 1% Clorox mixed with two drops of Tween® 20. The seeds were then rinsed three times with sterile distilled water to remove remaining chemicals traces. The sterile seeds were dried in the final step by blotting on filter paper in the laminar air flow (Halliru et al., 2021).

Inoculation of *Hylocereus undatus* seeds

After sterilisation, three seeds were inoculated per test tube on MS media supplemented with BAP and NAA to establish cultures for the initial shoot initiation. The seeds were incubated for four weeks at a temperature of 25 ± 1 °C with a 16-hour photoperiod at a photosynthetic photon flux density (PPFD) of 25 mmol m^{-2} s^{-1}, which was supplied by cool white fluorescent tubes. The explants were monitored every week and the cultures' viability and media contamination were monitored and recorded.

Cultivation of *Hylocereus undatus* shoots for different abiotic stress

Shoots that had been initiated were subjected to salinity stress by culturing on the same MS medium composition as for the shoot initiation but added with different concentrations of chemicals to induce stress conditions. Cultures were also grown under the same temperature and light photoperiod as in during shoot initiation. Shoot length, root length, and the number of the root were recorded after 4 weeks. To mimic drought or osmotic stress, 10, 20, 40 g/L polyethylene glycol (PEG8000) was filter-sterilized with 0.45 mm filter and added in the slightly cooled sterilized growth medium. Treatment was labelled as Dr1, Dr2 and Dr3 respectively. For salinity stress, NaCl of 50, 100, 150 and 200 mM concentrations was added to the growth medium during media preparation to create salinity stress conditions and labelled as Sal1, Sal2, Sal3 and Sal4 respectively. For pH stress, growth medium of pH 4, 5 and 8 was prepared by adding hydrochloric acid (HCl) and/or sodium hydroxide (NaOH). Treatment was labelled as pH 4, pH 5 and pH 8 respectively. For oxidative stress, slightly cooled growth medium was added with filter-sterilized hydrogen peroxide of 20, 40, 60 uM concentrations and treatment was labelled as Oxi1, Oxi2 and Oxi3 respectively. Ten replicates were prepared for each treatment.

Data Analysis

The cultures were observed on a regular schedule and results were recorded based on visual observation and morphological changes in survival rate and shoot and root length. Every week until the fourth week of culture, the average shoot and root length were recorded. Data collected were analyzed with One Way Analysis of Variance (ANOVA) at significant level of $p < 0.05$ using SPSS software (version 22.0) (IBM Software, NY, USA). Result is presented as mean ± SEM (standard error mean).

RESULTS AND DISCUSSION

The effect of drought stress induction in *H. undatus* growth

In this study, a total of ten replicate of shoots of *Hylocereus undatus* were used as an explant. Each explant was cultured on Murashige and Skoog (1962) media with a different molecular weight of polyethylene glycol (PEG 8000). As shown in Fig. 1, the longest shoots were observed in Dr1 at 1.35 ± 0.30 cm followed by control at 0.95 ± 0.27 cm, Dr2 at 0.85 ± 0.37 cm and the shortest in Dr3 at 0.70 ± 0.16 cm. Interestingly, Dr1 which contain 10 g/L PEG8000 had significantly ($p < 0.05$) improved shoot growth compared to control treatment and other concentrations of PEG8000. Similar findings were made by Nerd and Neumann (2004), who discovered that stems continued to grow even in the face of extreme aridity and a soil water potential of about 1.5 MPa. Dragon fruit is able to maintain its water reserve within the stem because severe water deficits in the soil only slightly affect the stem's water supply (Nerd & Neumann, 2004). The mature stems' water reserves can be mobilised by the new stems. Dragon fruit is different from the majority of other plants that slow down or stop growing during drought. According to our findings, stem elongation of dragon fruits decreased under drought before stem expansion did. This suggests that water reserves may be mobilised to elongation at the expense of expansion.

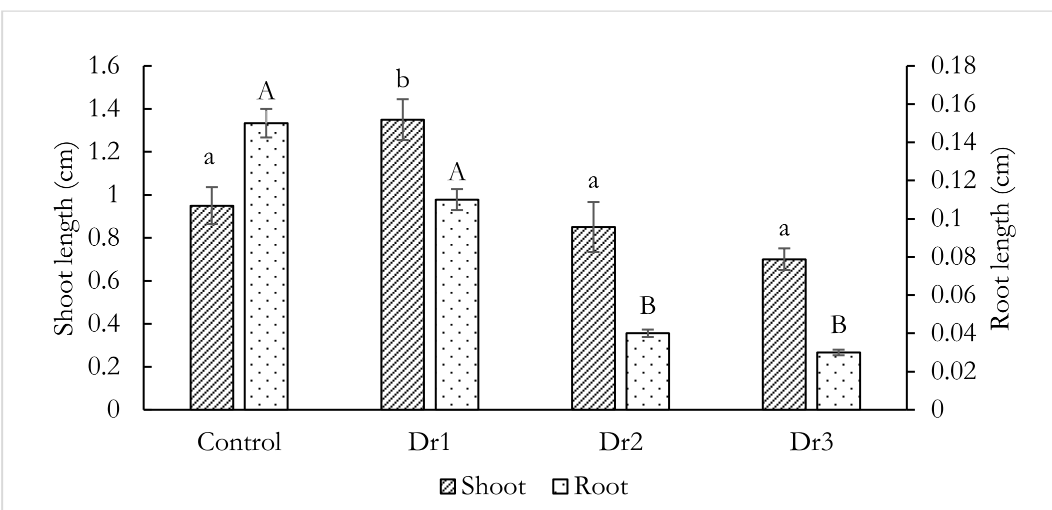

Fig.1. Mean shoot and root length of *H. undatus* treated with different molecular weight of PEG 8000 for drought stress induction. Control = 0, Dr1= 10, Dr2= 20, Dr3= 40 g/L PEG8000. Different letters indicate statistically significant differences ($p \leq 0.05$).

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Root length decreased as concentration of PEG8000 was increased and it significantly reduced root length at 20 g/L concentration (Dr2). However, doubling of the PEG8000 concentration did not show significant effect on the root growth. PEG-8000 caused a decrease in water potential, making it more challenging for plants to absorb water. Lack of water during the vegetative stage can slow the rate of leaf widening and lengthening, which inhibits root growth as evidenced by a decreased in root height. Drought stress will affect aspects of growth morphology, anatomy and physiology (Basal et al., 2020). According to Wang et. al., (2008), the level of PEG concentration was inversely related to the germination percentage value. The osmotic potential value around the seeds becomes increasingly negative as the PEG concentration is raised, making it challenging for the seeds to absorb water.

The effect of salinity stress induction in *H. undatus* growth

Salinity stress was induced by inclusion of NaCl in growth medium at different concentrations. The effect of increasing NaCl concentration was rather erratic in terms of shoot length as Sal3 with a higher NaCl concentration (150 mM) showed greater shoot length than Sal2 (100 mM). However, the difference was not significant. As expected, Sal4 with 200 mM NaCl showed the highest reduction of shoot length, approximately 40% as compared to the control (Fig. 2). The effect of salinity was more prominent in root growth as at the maximum concentration applied (200 mM) root growth was completely stunted in Sal4. The decrease in root growth was followed by Sal3, Sal2 and Sal1 with NaCl concentration of 50, 100 and 150 mM respectively. Although there was gradual decrease of shoot length as NaCl concentration increased, the differences between treatments were not statistically significant.

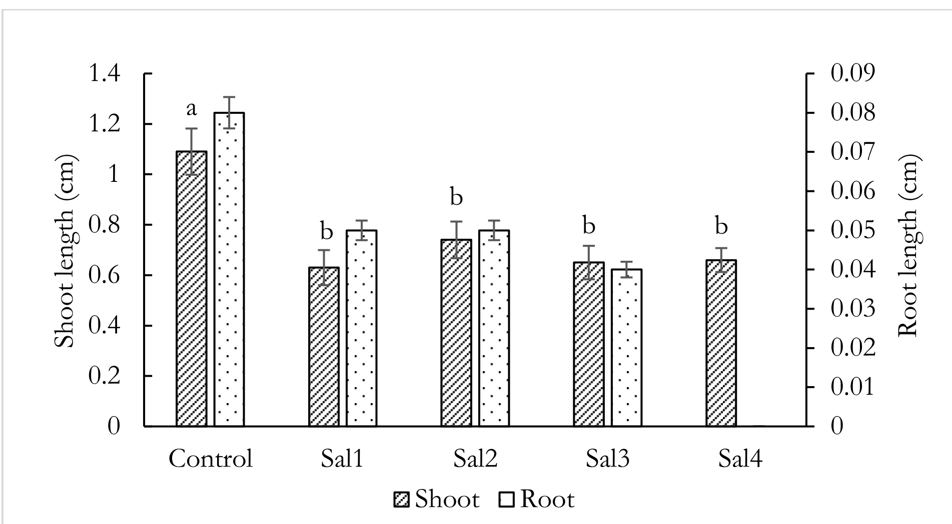

Fig. 2. Shoot and root length of *H.undatus* treated with different concentration of NaCl for induction of drought stress. Control = 0, Sal1= 50, Sal2= 100, Sal3= 150, Sal4=200 mM NaCl. Different letters indicate statistically significant differences ($p \leq 0.05$).

This phenomenon is not surprising as some plants develop alternative processes that allow them to strengthen their survival mechanisms and become tolerant of salt stress conditions (Kashyap et al., 2020). In the case of dragon fruit, its physiological makeup explains how it can endure in saline environments. Dragon fruit, like other cactus species, is a CAM (Crassulacean Acid Metabolism) type of plant which undergo different photosynthetic metabolism than the usual C4 plant that aids in the concentration of carbon dioxide. Also, in CAM plants water is used more efficiently for other metabolic activities. The ability of these plants to store water and other substances in their tissues ensures that they will continue to grow and develop even when stressed by salt. Plants that can withstand salinity can selectively accumulate ions and maintain osmotic balance, ensuring water absorption (Llanes et al., 2021). Saguaro and golden barrel cacti, which rely on osmotic adjustment based on increased osmotic potential and higher concentrations of sodium and chloride in the stem tissue, were found to be tolerant to all salinity levels (Schuch and Kelly, 2008).

Because of increased osmotic pressure in the soil, an excess of salts in these environments directly restricts plant absorption of water and nutrients (Ma et al., 2020). This explains complete restriction Sal4 root growth. According to Hu and Schmidhalter (2004) low osmotic potentials of the soil solution cause a water deficit in plant tissue under saline conditions. As a result, cell turgor pressure is reduced. Because cell growth is directly related with turgor pressure in growing tissues, decreased turgor is the primary cause of plant cell expansion inhibition under high salinity. High electrical conductivity in high salinity can inhibit nutrient absorption and cause problems for plant development, limiting plant growth due to reduced root growth. The increase in the osmotic potential of the soil reduces nutrient absorption by plants because it reduces the external water potential, which compromises the availability of water within the plant, resulting in negative effects on the capacity to translocate nutrients within the plants and causing an increase in solute levels (Ibrahimova et al., 2021).

The effect of pH difference in *H. undatus* growth

In this study *H. undatus* growth were observed when subjected to normal pH 5.8, two acidic pHs which were pH 4 and 5 and one alkaline pH which was pH 8. The reason why the pH cannot be decreased or increased further was because it interfered with solidification of Phytagel™. Too much acidity or alkalinity will not solidify it. The dragon fruit species grew best in pH between 6.6 and 7.0, which is higher than the ideal range for most fruit species (Reis et al., 2020). However, pH levels between 5.5 and 7.5 were found to be beneficial to the growth of white dragon fruit. Therefore, this explains significant reduction of shoot length when *H. undatus* was grown in pH other than the control which was 5.8 (Fig. 3).

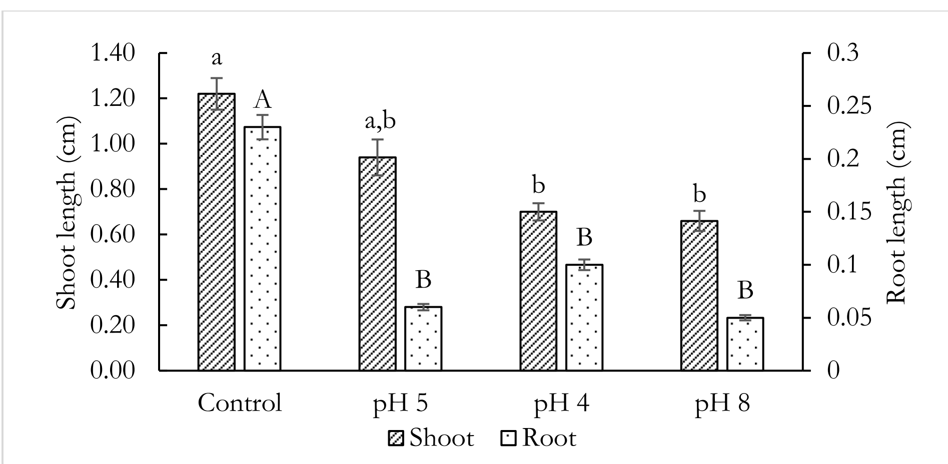

Fig. 3. Shoot and root length of *H. undatus* treated with different pH. Control pH was 5.8. Different letters indicate statistically significant differences ($p \leq 0.05$).

Similarly, there was significant reduction of root length observed compared to control but no significant difference between treatments with different pH on the root length. Effects of pH on growth and development have not yet received much attention. The few studies that have been done on the effect of pH on the development process found that the percentage of development was completely unchanged by pH levels ranging from 3.0 to 9.0 in *Hylocereus* spp. (Ortiz et al., 2015). However, Ortiz et al. (2018) found that one of the three genotypes of *Hylocereus* spp. studied had a significant pH effect on vigour, as measured by Geological Survey of India, with weak performance at pH 4.0, 5.0, and 6.0. *Hylocereus* spp. shoots are sensitive to changes in substrate pH. As a result, pH levels less than 4.5 and greater than 7.5 harmed the viability and vigour of dragon fruit genotype shoots.

The effect of oxidative stress induction in *H. undatus* growth

In this study, oxidative stress was conferred by H_2O_2, a type of reactive oxygen species (ROS). H_2O_2 is a by-product of oxidation reaction in plant cells and can accumulate if not sufficiently detoxified. In general, treatment with various concentration of H_2O_2 did not significantly affect shoot growth of *H. undatus* (Fig. 4). However, it significantly restricted root growth at 60 uM H_2O_2.

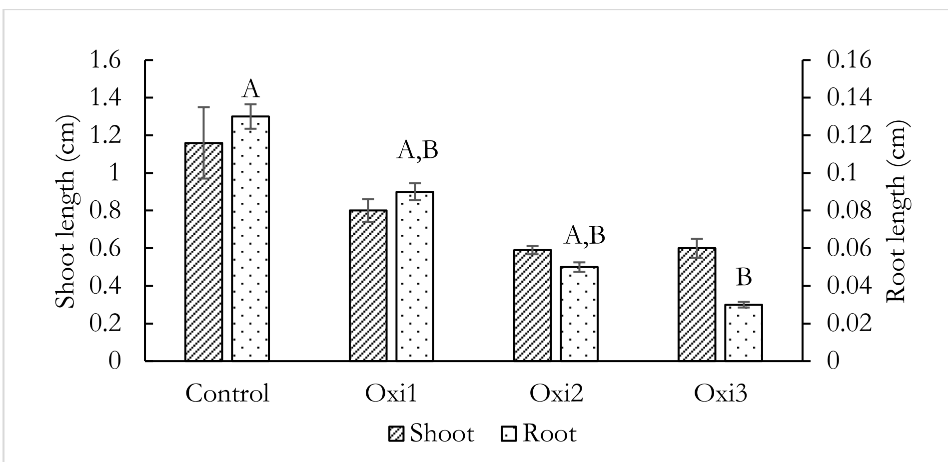

Fig. 4 Shoot and root length of *H. undatus* growth treated with different concentration of H_2O_2 for induction of oxidative stress. Control = 0, Oxi1= 20, Oxi2 = 40, Oxi3= 60 uM H_2O_2. Different letters indicate statistically significant differences ($p \leq 0.05$).

According to Desikan et al. (2003), stress regularly triggers the production of ROS like O_2^- and H_2O_2 in plant tissues. However, it also functions as a signalling molecule, triggering a defence system to get plant cells' redox homeostasis back to normal. Furthermore, H_2O_2 stimulates the biological processes including programmed cell death (Desikan et al., 1998), ABA-mediated stomatal closure (Pei et al., 2000), auxin-regulated gravitropic responses (Joo et al., 2001), mechanical wounding response (Orozco-Cardenas et al., 2001), and systematically acquired resistance (SAR). Therefore, this signalling function might be demonstrated in the shoots rather than in roots where the destructive effect of H_2O_2 was demonstrated. The other reason might be that the concentrations used in this study was not adequate to cause any significant effects in the shoots.

CONCLUSION

Based on the result that have determined, *H. undatus* growth were affected by the four abiotic stresses tested. For drought stress, both shoot and root length were significantly reduced at 60 g/L of PEG 8000. For salinity stress, the least shoot length was shown at 200 mM NaCl but no growth of root was observed at this concentration at all. pH level other than optimum pH of 5.8 significantly affected both shoot and root growth, but a greater reduction of growth was seen in root. Similarly, oxidative stress caused lesser effects in shoot growth than root as increasing H_2O_2 reduced shoot length but insignificantly but concentration of 60 uM H_2O_2 significantly reduced root length. Therefore, it can be concluded that compared to shoot growth, root growth is more sensitive to abiotic stresses tested in the present study. A greater magnitude of stressors can be tested for further information of *H. undatus* tolerance to abiotic stresses.

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