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Original Article

Morpho-physiological and glucomannan biosynthesis-related gene expression of Porang (*Amorphophallus muelleri* Blume) Under Salinity Stress

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ABSTRACT

Porang (*Amorphophallus muelleri* Blume) is a tuber crop that possesses significant potential as an alternative food source, primarily attributed to its substantial glucomannan content. To support the development of porang in Indonesia, it is crucial to focus on expanding its cultivation in saline areas to enhance productivity. This study aims to assess the morphophysiological responses of porang under various saline concentrations over a defined period. Porang was grown in soil treated with various NaCl concentrations of 0, 50, 100, 150, and 200 mM for 14 days until harvest. The study revealed that salinity stress had a detrimental impact on tuber mass and diameter, root length, plant height, leaf area, stomatal closure, and stomatal density. In terms of physiological changes, the total chlorophyll content, net assimilation rate (NAR), glucomannan content, and transpiration rate were reduced. The study of gene expression showed *SuSy2* expression increased up to 1.55-fold at 150 mM, whereas *CSLA3* increased up to 3-fold at 100 mM. To conclude, porang has the potential to be cultivated in saline soil up to 50 mM. These outcomes serve as valuable information and genetic resources for the future development of improved porang varieties.

Keywords: *CSLA3* gene, *Glucomannan,* Salt stress*, Plant growth, Saline soil, SuSy2* gene.

Introduction

Indonesia is renowned for its rich diversity of indigenous plants, including various cereals and tubers, which hold great potential as food sources. These local plants have the capacity to contribute significantly to ensuring food security within the country (Wuryanto & Arifin, 2017). Porang (*Amorphophallus muelleri* Blume) is a native Indonesian plant belonging to the Araceae family (Mutaqin *et al*., 2021). Porang plants grow well in montane areas and forests under the shade of surrounding tall plants (Santosa *et al*., 2018; Wahidah *et al*., 2021; Supriyati, 2016). In addition, porang is known to have high glucomannan levels of around 70-90% depending on the growth period (Gusmalawati *et al*., 2019). Glucomannan in porang plants is used mainly in the food

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industry as a thickener, gelling agent, emulsifier, and flour. Meanwhile, in the pharmaceutical field, glucomannan has been proven to reduce lipid and cholesterol levels, decrease blood glucose levels, as well as prevent and inhibit cancer (Supriyati, 2016). The beneficial properties of glucomannan found in porang plants have gained significant attention recently. As a result, porang has become increasingly popular, leading to its surge in global demand.

Based on data from the Agricultural Quarantine Agency in 2021, the export value of porang has increased by 160%, rising from 5.7 thousand tons in 2019 to 14.8 thousand tons in 2021 (Muhammad, 2021). In addition, the Minister of Agriculture has exported 60 tons of porang to countries including China, Japan, Vietnam, Thailand, Hong Kong, Malaysia, and Korea, among others (Utami, 2021). This promising prospect encourages people to cultivate porang plants. However, the current challenge lies in the diminishing availability of agricultural land caused by land conversion. Conversely, the challenge of increasing production, which involves encroaching upon forested areas and risking ecosystem damage (Laurance *et al*., 2013), can be addressed by utilizing sub-optimal land, such as saline soils in Indonesia, for Porang cultivation (Soedarjo *et al*., 2020). Rachman *et al.* (2007) estimated that the total area of saline land in Indonesia is approximately 440,300 hectares, with 304,000 hectares classified as slightly saline and 140,300 hectares as saline.

Stress conditions such as saline condition, drought stress, or water deficit stress potentially disturb essential physiological functions, leading to increased oxidative stress, decreased water content, and adverse effects on plant development, altering root architecture, and compromising the functionality of aerial parts (Rodrigues *et al*., 2019; Saputro *et al*., 2023). Salinity acts as a limiting factor for the growth of porang, and elevated salinity levels induce stress. Salinity stress hampers root water uptake, leading to osmotic stress and disrupting nutrient balance, resulting in the accumulation of high salt concentrations within the plant (Parvaiz & Satywati, 2008). The presence of excessive Na^+ ions due to salinity stress inhibits the uptake of essential K^+ ions, crucial for growth and development, ultimately leading to decreased productivity and potential plant mortality (Saddiq *et al*., 2021).

There are two main plant characteristics related to salt tolerance, Halophytes and Glycophytes. Halophytes are plants that complete their life cycle in salt concentrations, typically dominated by NaCl, of ≥200 mm (as defined by Flowers & Colmer, 2008). These plants exhibit a unique ability to withstand high concentrations of Na^+ and Cl^- in their shoots (Flowers *et al*., 2015). Halophytes appear to have a greater ability to maintain net photosynthesis by protecting and stabilizing both photosystems under saline stress conditions and have a high antioxidant capacity. While glycophytes are considered non-tolerant plants where many plants fall into this category, including porang. Maintaining high cytosolic K+ /Na+ ratios by compartmentalization especially in shoots and osmotic adjustment by osmoprotectant have been strongly suggested to be crucial for salt tolerance of glycophyte plants (Horie *et al*., 2012; Bose *et al*., 2014).

Therefore, plants develop mechanisms for adaptation through morphological, anatomical, physiological, and molecular alteration. Generally, plants respond with decreased morphological parameters such as root length, plant height, and number of leaves, which results in decreased chlorophyll content, photosynthetic rate, and transpiration rate (Mandal *et al*., 2019; Kumar *et al*., 2021). These conditions are caused by the inability of roots to absorb essential elements such as Ca^{2+}, Mg^{2+}, and K^+, which play a role in cell division and enlargement, as well as by the disruption of physiological processes such as chlorophyll formation (Anugrahtama *et al*., 2020). Porang plants thrive in soil conditions with electrical conductivity below 35 ds/m or 50 mmol (Siswanto & Karamina, 2016).

In addition, molecular biomarkers can be used as an early detection tool for plant responses to extreme conditions such as drought, salinity, and acidity (Nurhidayati *et al*., 2017; Chutimanukul *et al*., 2021). The molecular response shows the dynamics of gene expression during periods of stress (Xing *et al*., 2018). The porang plant, known for its abundant glucomannan production, harbors genes pivotal to the biosynthetic process. This study specifically examines gene expression, with a focus on those associated with glucomannan biosynthesis. The aim is to provide valuable insights into the correlation between salt exposure and glucomannan production. *SuSy2* and *CSLA3* genes are considered important genes in glucomannan biosynthesis. The *SuSy2* gene encodes the sucrose synthase enzyme and has the function of breaking down sucrose into glucose and fructose as the first step in glucomannan biosynthesis (Srednicki & Borompichaichartkul, 2020). The *CSLA3* gene plays an important role in the final catalytic step of glucomannan biosynthesis (Gille *et al*., 2011). Hence, understanding the morphological, anatomical, physiological, and molecular responses of porang plants when subjected to salinity stress becomes highly important.

Material And Methods

Plant materials and cultivation of porang plant

Bulbils of the porang variety Madiun_1 were used for the experiment since it was the first released variety and considered the most cultivated in Indonesia (Nugrahaeni *et al*., 2021). The bulbils were obtained from Kepel Village, Kare District, Madiun, East Java Province, Indonesia. The bulbils used in this study were relatively uniform, ranging in mass from 8–10 grams (Sumarwoto & Maryana, 2011). The composition of the growth medium comprised garden soil,

fertilizer, and husk charcoal in a ratio of 1:1:1 (Nurhidayati *et al.*, 2022). The medium was stirred evenly, and then 3 kg of homogenously mixed medium were placed in polybags (size 14 × 28 cm). After the growth medium was prepared, the bulbils were planted 5 cm deep (Hidayah, 2016). The bulbils were planted with the bud facing up (Utami, 2021). The plants were grown in a greenhouse for 120 days with a temperature of 27°–29°C. The NaCl treatment was conducted for 14 continuous days. Plants were watered in the morning, with a standard volume of 100 mL per polybag, administered daily. The experiment followed a Completely Randomized Design (CRD) with one test factor, namely salinity stress treatment at different concentrations of 0, 50, 100, 150, and 200 mM. Each treatment was repeated 5 times so that a total of 25 plant samples were obtained.

Salinity Stress treatment

The stock solution was prepared by dissolving NaCl in water with concentrations of 50, 100, 150, and 200 mM. Several concentrations were used since there is no information available on porang's sensitivity to salt. Plants were selected based on several criteria, including equal height (±65 cm), amount of leaves (±4), and main stem diameter (±26 cm) after 120 DAP (Days After Planting). Salinity stress treatment was carried out by pouring 100 mL of NaCl stock solution per polybag in the root area for 14 consecutive days, while control plants were watered using distilled water. Plants were harvested and certain parameters were measured after 14 days of treatment.

Morpho-physiological Parameters

Root length and plant height were measured using a sewing meter in centimetres (cm), fresh weight of stem tuber was measured using an analytical balance in grams (g), stem tuber diameter was measured using a digital calliper in millimetres (mm), while leaf area was measured in square centimetres (cm^2) using the gravimetric method (Irwan & Wicaksono, 2017). Two leaves from the first two branches were selected to represent the measurement or four leaves per plant. In total, with three individuals per replication, as many as 12 leaf blades were measured. Furthermore, the leaves were collected at the end of the treatment. Each leaf was drawn on pieces of paper as leaf replicas, and then leaf replicas and pieces of paper were weighed using an analytical balance. The leaf area was calculated with the following formula:

$$Leaf\ area = \frac{Weight\ of\ replicas}{Weight\ of\ pieces\ of\ paper} x\ Paper\ area$$

(Irwan & Wicaksono, 2017)

The stomatal observation was conducted at the end of the treatment using the stomatal printing method. Sampling was carried out in the morning from 8 until 10 AM local time (Indrayani & Perdani, 2018). Stomatal samples were taken from the abaxial surface of the leaf. The leaf surface was cleaned with a tissue, smeared with nail polish, and dried for 10 minutes. The abaxial surface was affixed with tape, then the mould was placed on a glass slide for observation using a light microscope at 400× magnification (Setiawati & Syamsi, 2019). The percentage of opened and closed stomata was calculated by the formula according to Perkasa *et al.* (2017):

$$Percentage\ of\ open\ and\ closed\ stomata = \frac{Number\ of\ open\ or\ closed\ stomata}{Total\ number\ of\ stomata} \times\ 100\%$$

while stomatal density was calculated using the following formula (Perkasa *et al.*, 2017):

$$Stomatal\ density = \frac{Number\ of\ stomata}{¼\pi d^2}$$

Chlorophyll extraction was performed using 1g of leaves added with 10 ml of 85% acetone and then ground. The mixture was incubated in the dark condition, temperature 4°C for overnight. The mixture was filtered with Whatman paper to obtain the extract. The extract was placed into a cuvette and the absorbance was measured at wavelengths of 645 nm and 663 nm. Total chlorophyll was calculated using the following formula (Hu *et al.*, 2013; Perez *et al.*, 2018):

Chlorophyll a (mg/g) = $(12.7 \times A_{663}) - (2.59 \times A_{645})$

Chlorophyll b (mg/g) = $(22.9 \times A_{645}) - (4.7 \times A_{663})$

Total chlorophyll = $(8.02 \times A_{663}) + (20.2 \times A_{645})$

The measurement of transpiration rate utilized the gravimetric method, as described in Nurhidayati *et al.*, (2017), involving the sealing of the pot and soil tightly to minimize water loss, except from the shoots where transpiration occurs. The potted plants were weighed at specific intervals. The transpiration rate in porang plants was measured every 3 days. The process involved initially weighing the polybag before compression on day 1. On day 2, stakes were added to each polybag, and the plastic cover was tightly sealed to prevent water loss, except from the canopy. On the 3rd day, the final weight of the polybag was measured, and the leaf area was assessed (Ayu *et al.*, 2017):

$$Transpiration\ rate = \frac{Evaporation\ rate\ (initial\ weight - final\ weight)}{Leaf\ area}$$

The dry weight of porang plants was measured by weighing the roots, stems, and leaves of the plants which had been dried in an oven at a temperature of 100 °C until they reached a constant weight (Nugroho *et al*., 2017). The net assimilation rate (NAR) can be examined through calculations using the following formula (Aziez *et al*., 2014):

$$NAR = \frac{W2 - W1}{T2 - T1} \times \frac{lnLA2 - lnLA1}{LA2 - LA1}$$

where T1 = Observation time on previous day, T2 = Observation time on day, LA1 = Leaf area at T1; LA2 = Leaf area at T2, W1 = Total dry weight of plants at T1, W2 = Total dry weight of plants at T2.

Glucomannan content

The extraction of glucomannan was performed according to the protocol by Nurlela *et al*. (2020), with modifications. The stem tubers were thinly sliced and dried under the sun to remove the moisture content. The dried samples were ground and sieved using a 40-mesh sieve, preparing the material for extraction. The extraction was done by mixing 1 g of powder with 15 mL of 60% ethanol for an hour and then filtered. The extraction was repeated three times. The extract was dried using an oven at 45 °C for 12 hours, then the sample was sieved using a 60-mesh sieve. The determination of glucomannan content was carried out using the colorimetric method with 3,5-dinitrosalicylic acid. The glucomannan extract was prepared by dissolving 0.2 g glucomannan flour in NaOH formic acid buffer solution (0.1 mol/L, 100 mL) and stirring for 4 hours. Centrifugation was conducted at 4000 rpm for 30 minutes. The supernatant is the glucomannan extract. Glucomannan hydrolysate was prepared by mixing 5 mL of glucomannan extract with 2.5 mL of 3M H_2SO_4, shaken with vortex, heated in a water bath for 90 minutes, then cooled to room temperature, and 2.5 mL of 6M NaOH were added. Finally, deionised water was added to a final volume of 25 mL and glucomannan hydrolysate was formed. Both the glucomannan extract and glucomannan hydrolysate were measured by the colorimetric method, using deionised water as a blank. The protocol was the same for both the glucomannan extract and glucomannan: 2 mL of each sample was mixed with 1.5 mL of 1% DNS, then heated for 5 minutes with boiling water, 5 mL of deionised water was added, then cooled. The three solutions were analysed using spectrophotometry at a wavelength of 500 nm. Glucomannan content was calculated by the following formula (Wardani *et al*., 2021):

$$GM\ content\ (\%) = \frac{5000f(5T - T0)}{m}$$

f = Correction factor (0.9); T = Glucose content of glucomannan hydrolysate (mg); T0 = Glucose content of glucomannan sample solution (mg); m = Mass of extracted glucomannan

Gene expression analysis

Porang stem tubers were ground in a mortar and subsequently added to the extraction buffer (RNA Mini Kit (Plant), Geneaid) to obtain total RNA. The extraction process followed the protocol from the manufacturer. The quantity and purity of RNA were measured using a NanoDrop 2000 device (Thermo Fisher Scientific, MA, USA). The purity score for extracted RNA was 1.7–2.0, predominantly at 1.9. The integrity of RNA was observed by loading 2 µL of total RNA into an electrophoresis gel; the RNA that showed 28S and 18S was considered intact RNA, while the RIN was not measured. The intact RNA was subsequently mixed with DNAse and observed again in an electrophoresis gel. To perform the real-time quantitative PCR (qrtPCR), primers for the amplification of *CSLA3* and *SuSy2* were designed according to Table 1.

Table 1. Sequences of primers used for the amplification of *SuSy2* and *CSLA3* genes in *A. muelleri*.

Name of Genes	Primers sequences	Amplicon length (bps)
Sucrose Synthase 2 (*SuSy2*)	Forward : 5'GGGAGGATTCCTATGGTGTTC3'	188 bp
	Reverse : 5'GCTGAGTGTGGAACCTAGAAT3'	
Cellulose Synthase Like A-3 (*CSLA3*)	Forward : 5'GCTGAGGAGTGCTTGATGACA3'	185 bp
	Reverse : 5'CACTGTGGAGGACATGGACTT3'	
Elongation factor (EF1- α)	Forward : 5'AAGTTCCTGAAGAATGGCGAT3'	111 bp
	Reverse : 5'GCTGAGTGTGGAACCTAGAAT3'	

The qRT-PCR was carried out with the RNA-direct™ SYBR Green RealTime PCR Master Mix Toyobo kit. Each reaction contained 2 μL of RNA as template, in a total volume of 20 μL reaction mixture. The amplification program was performed at 95 °C/30 sec followed by denaturation at 95°C/15 s, annealing at 52°C/15 s, and elongation at 74°C/15 sec (40 cycles). In order to normalize the qPCR data, an elongation factor (*EF1- α*) was selected as a housekeeping gene. The experiments were repeated three times. The calculation of relative gene expression was done based on $2^{-\blacktriangle\blacktriangle Ct}$ methods as described by Livak and Schmittgen 2001.

Data Analysis

The experiments were conducted in a randomized block design method with 4 replicates, each comprised of 5 concentration treatments. The data obtained from observations of morphological and physiological parameters were subjected to statistical analysis using the One-Way ANOVA (Analysis of Variance) test at a 95% confidence level. If the ANOVA results indicate a significant effect ($P \leq 0.05$), further testing will be conducted using the Tukey test at a 95% confidence level (α=0.05%) (Little & Rubin, 1987). Conversely, data from observations of anatomical and molecular parameters were qualitatively analyzed descriptively (Little & Rubin, 1987).

Results

Morpho-physiological responses of *A. muelleri* under salinity stress

The effects of salinity stress on the morphology of *A. muelleri* are shown in Table 2. The increment of NaCl concentration had a detrimental effect on plant performance (Fig.1A-O). According to the statistical test, all parameters were significantly different at NaCl concentrations of 150 and 200 mM when compared with the control. At a concentration of 200 mM NaCl, all parameters experienced the largest decreases: 49.6% in root length, 40.8% in plant height, 71% in leaf area, 65.6% in weight of stem tuber, and 44.1% in stem tuber diameter. The above results showed that salinity stress reduced morphological parameters such as root length, plant height, leaf area, weight, and diameter of stem tuber in *A. muelleri* with increased NaCl concentration.

The performance of stomatal conductance under salt stress was observed at the end of the treatment as shown in Fig. 2A-C. Moreover, the opened stomatal pore state in the leaf was shown in figure 2A, while the closed stomatal pore state is shown in figure 2B. The percentage of closed stomata in porang grown in saline soil increased linearly with increased salinity (Fig. 3A), but the stomatal density was not significantly affected (Fig. 3B). The percentage of closed stomata increased with increased NaCl concentration by 22% at 50 mM, 73% at 100 mM, 93% at 150 mM and 100% at 200 mM of NaCl. An increase in density was observed in all treatments compared to the control, although no significant differences between the treatments were identified (Fig. 3B).

The chlorophyll content (Fig. 3C), net assimilation rate (NAR) (Fig. 3D), glucomannan content (Fig. 3E), and transpiration rate (Fig. 3F) were all decreased with an increase in NaCl concentration. The results of the statistical analysis showed that the salinity stress treatment significantly affected the parameters of chlorophyll content, glucomannan content, and NAR compared to the control. Physiological observations show varying levels of tolerance depending on the concentration given. Total Chlorophyll at 50 mM was not significantly different compared to the control since only reduced as much by 3.11%. The highest decrease was occurred in 200 mM with 92,6%. Moreover, the NAR parameter showed no significant difference in control in 50 mM and 100 mM. The highest decrease in NAR occurred in plants treated with a 200 mM concentration of 0.001 g/cm^3/day. In the transpiration rate, the most drastic decrease was at a concentration of 200 mM by 6.3% to 76.6% every three days of measurement compared to the control. Furthermore, a notable decline in glucomannan levels becomes significant starting at 50 mM of NaCl, with higher concentrations further suppressing glucomannan production to the point of non-detection.

Table 2. Effect of different levels of salinity stress on morphological parameters of *A. muelleri*.

NaCl Treatment (mM)	Root lenght (cm)	Plant height (cm)	Leaf area (cm²)	Weight of stem tuber (g)	Stem tuber diameter (mm)
Control	18.5±0.66a	80±1.73a	436.4±36.7a	16.6±0.54a	34.9±3.3a
50	14.7±0.33ab	71±2.74ab	366.5±27.7b	12.6±0.76ab	30.1±4.8ab
100	12.6±0.38bc	66.8±1.28ab	248.8±31.9c	8.2±0.56b	25.7±1.8bc
150	11.2±0.19bc	51.4±0.63c	153.2±24.4d	7.8±0.30b	21.8±1.3cd
200	9.3±0.38c	47.4±0.61c	126.3±22.5d	5.7±0.26b	19.5±1.10d

Different letters indicated significant differences among the treatments ($P<0.05$)

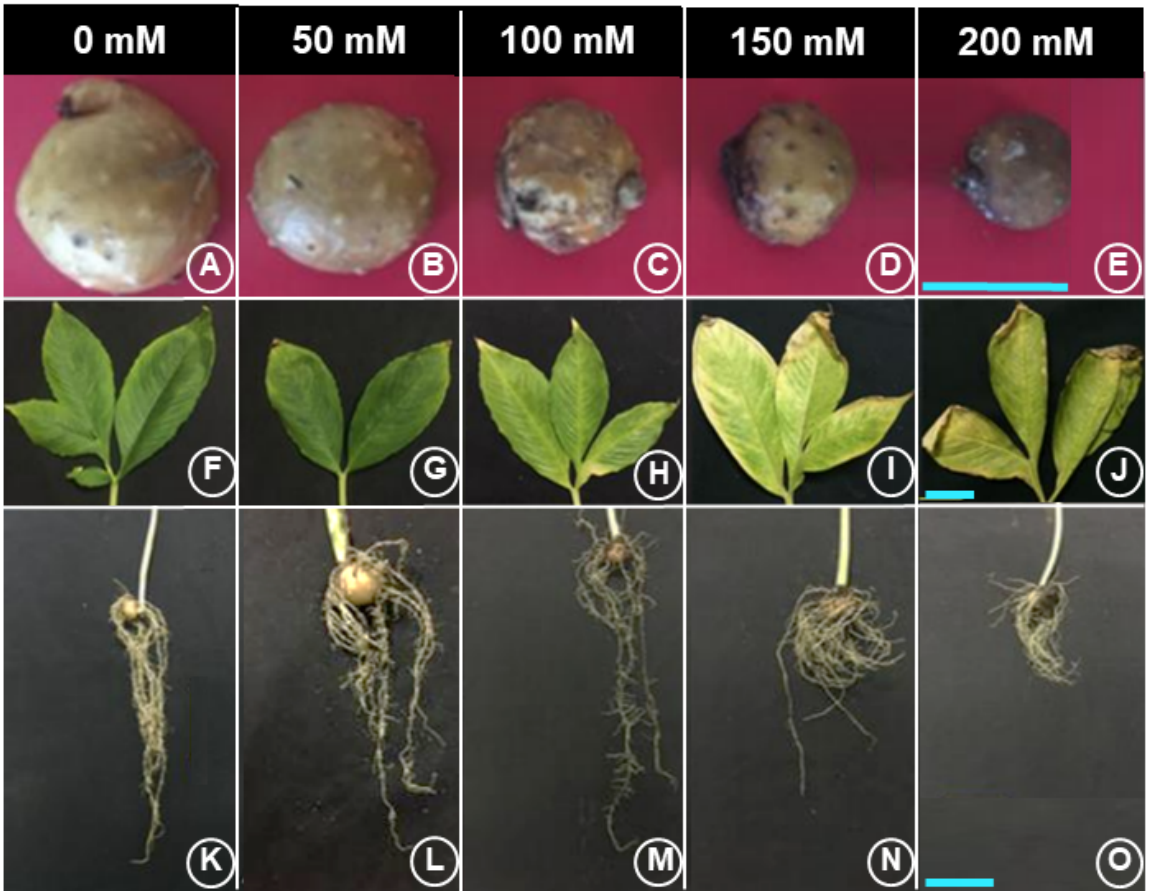


Figure 1. Response of *A. muelleri* under salt stress. A-E. Stem tuber size; F-J. Leaf performance; K-O. Root performance. (white bar = 5 cm).

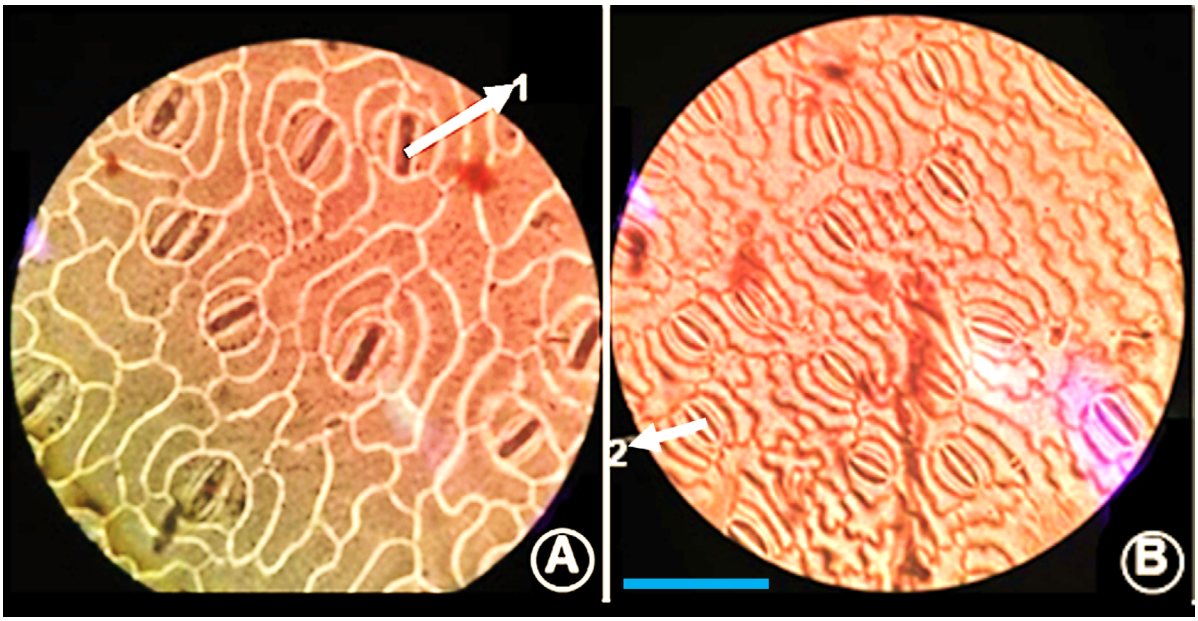


Figure 2. Stomata of *A. muelleri* under salt stress, M= 400×. A. Opened stomata in control plant; B. Closed stomata in 200 mM of NaCl. 1. Opened stomatal pore state on leaf; 2. Closed stomatal pore state on the leaf. Blue bar = 60 µm.

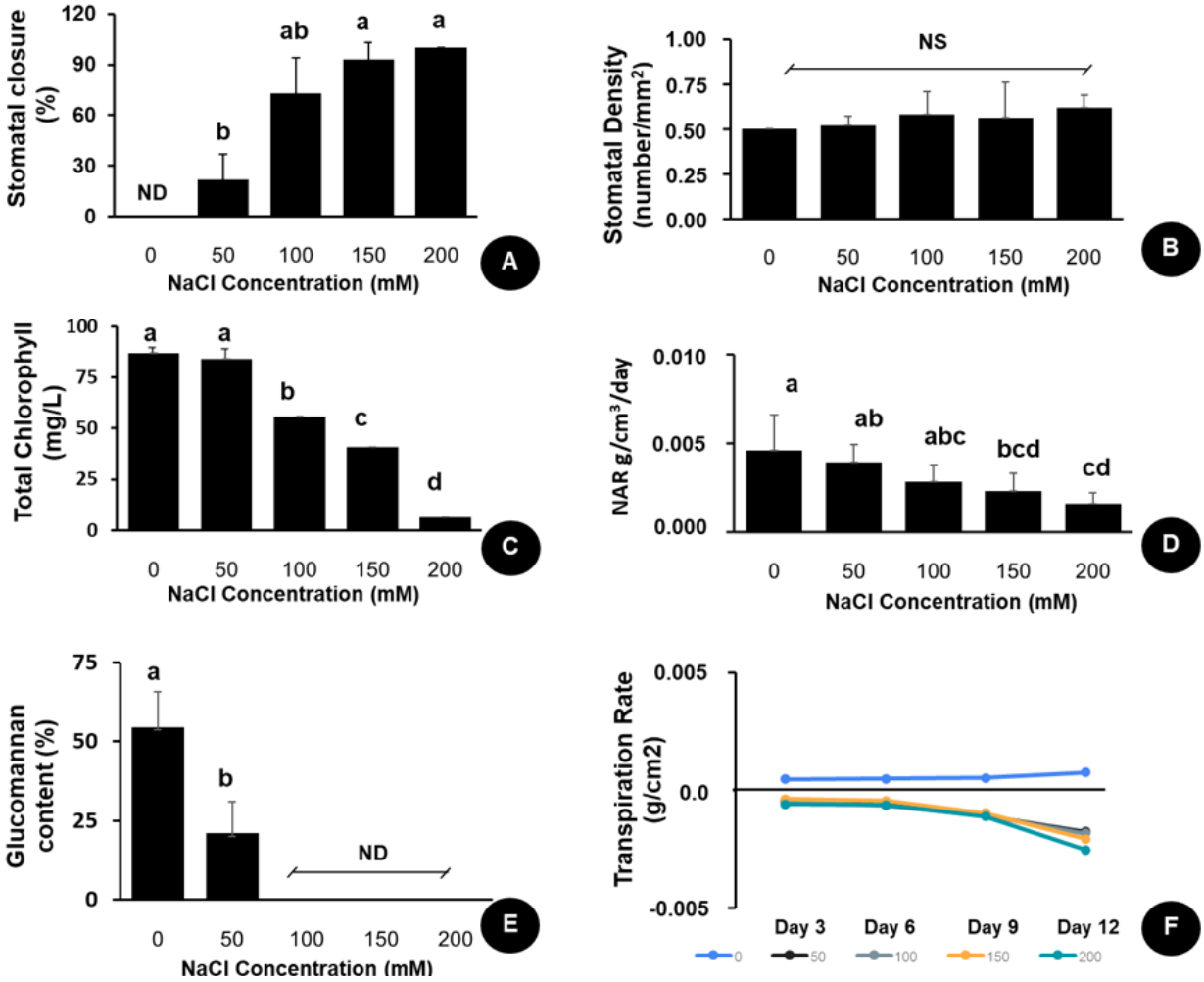


Figure 3. Effect of salinity on the anatomical and physiological parameters of *A. muelleri*. A. Stomatal closure; B. Stomatal density; C. Total chlorophyll; D. Net assimilation rate; E. Transpiration rate; F. Glucomannan content. ND = Not Detected; NS = Not Significant.

Molecular responses of *A. muelleri* under salinity stress

Porang plants are known for glucomannan content and salinity stress can affect the expression of relevant genes. The relative gene expression evaluation was carried out on genes that play a role in glucomannan biosynthesis, namely *SuSy2* (Sucrose synthase) and *CSLA3* (Cellulose synthase-like A3). The *AmSuSy2* transcript was decreased from 1-fold (control) to 0.91-fold at 50 mM NaCl concentration, considered as not significantly different (Fig. 4A). Meanwhile, the NaCl concentration of 100 and 150 mM significantly increased the *AmSuSy2* transcript when compared to control by 1.5 and 1.55-fold. However, the *AmSuSy2* transcript was decreased by 1.2-fold after treatment with 200 mM NaCl, a statistically similar gene expression when compared to control. Meanwhile, the *AmCSLA3* transcript was significantly increased from 1-fold to 1.91-fold and 3-fold in the treatments of 50 and 100 mM NaCl concentrations, respectively. However, the *AmCSLA3* transcript was decreased at a concentration of 150 mM by 1.93-fold, but higher than the control expression, while its expression was stabilised under the treatment with 200 mM NaCl (Fig.4B).

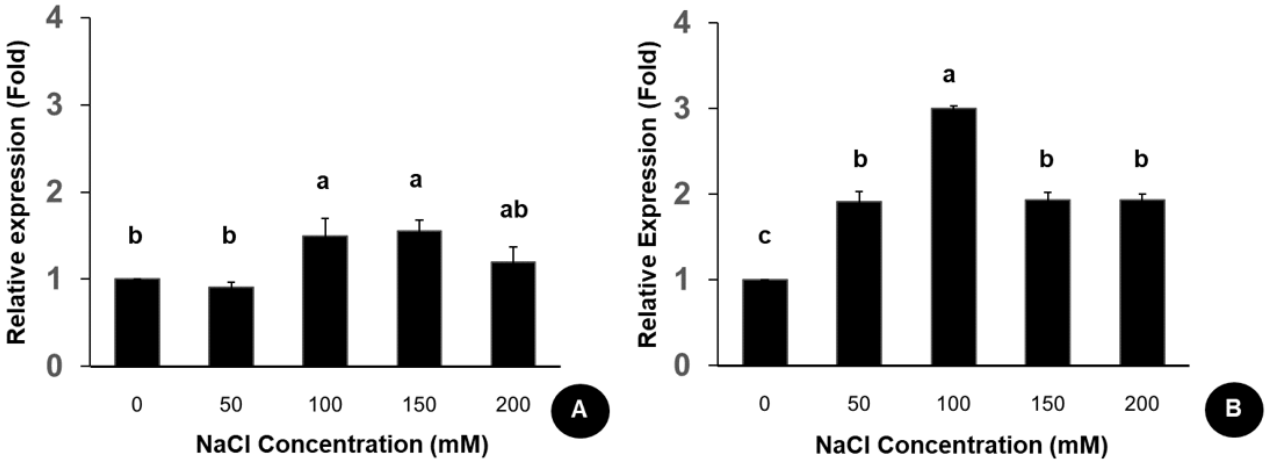


Figure 4. Effect of salinity on gene expression of *A. muelleri*. (A) *SuSy2* relative expression, (B) *CSLA3* relative expression. Relative expression was analysed in three replications.

Discussion

Porang plants demonstrate survival at a salt concentration of 50 mM. Morpho-physiological observations revealed that at this concentration, only two parameters, namely leaf area and glucomannan content, exhibited a significant decrease. However, at 100 mM, all parameters, except plant height, showed a significant decline. At concentrations of 150 mM and 200 mM, all parameters differed significantly from the control, indicating that salt induces a reduction in both growth and glucomannan levels. Salinity stress stands out as a highly detrimental abiotic stress factor impacting plant growth, productivity, and physiology caused by the high concentration of dissolved salts in the soil. This stress leads to an increase in osmotic pressure, the generation of reactive oxygen species (ROS), ion toxicity, and disruptions to biochemical processes. These factors affect the plant's ability to absorb water, resulting in water deficits that inhibit cell division and elongation activities (Dachlan *et al*., 2013; Munns & Tester. 2008; Kotagiri & Kolluru 2017). Salinity stress can damage vital plant organs, including roots, stems, and leaves, leading to impaired water absorption and suboptimal photosynthesis, ultimately resulting in plant death (Ferdosi *et al*., 2021). Our research successfully documented the relationship between salt concentrations and glucomannan production in porang plants, results that are considered novel.

In porang plants, salinity stress causes a decline in all these morphological parameters.

Salinity stress causes a reduction of IAA activity, leading to a decrement in cell elongation activity (Roro *et al*., 2017). Salinity stress is also thought to increase cytokinin oxidase, which plays a role in cytokinin degradation, so that cell division is inhibited (Zhu *et al*., 2022). Dobranszki *et al*. (2008) reported that under saline conditions the growth of roots and leaves was reduced and even damaged so that the photosynthesis process was reduced. Therefore, the accumulated of photosynthate was limited and as a result, the supply of nutrients was reduced and growth inhibited (Mzabri *et al*., 2017).

Stomatal density was not significantly affected according to statistical analysis, although the open stomata decreased in saline conditions. The increase in closed stomata during stress is believed to be a response to salinity-induced decrease in water potential, leading to cellular dehydration and subsequent stomatal closure. The stomatal closure mechanism helps to reduce water loss from leaf tissues and maintain osmotic balance in the plant, thereby assisting in coping with salinity stress and preserving water resources (Acosta *et al*., 2017).

Moreover, salinity stress is thought to increase the accumulation of ABA and H_2O_2, both of which induce the opening of the Slow anion channel 1 (SLAC1) and lead to potassium ions leaving the guard cells, resulting in stomatal closure (Imes *et al*., 2013; Hedrich & Shabala, 2018). Meanwhile, the stomatal density increased because the smaller leaf size causes a smaller leaf area so that the distance among stomata is closer, which increases stomatal density.

Chlorophyll content in porang plants decreased under salinity stress. The reduction in chlorophyll content in salinised plants can be attributed to an increase in the activity of the enzyme chlorophyllase which can degrade chlorophyll (Heidari, 2012). Li *et al*. (2019) related root length to chlorophyll content, showing that a decrease in root length causes chlorophyll content to decrease. This phenomenon occurs due to the inhibition of roots in absorbing nutrients from the soil, thus affecting the

availability of the elements N and Mg, which play a role in chlorophyll synthesis.

Maylani *et al*. (2020) reported that the high number of closed stomata caused a reduction in the transpiration rate. The net assimilation rate (NAR) is defined as the dry weight in a unit of leaf area over a period of time. NAR is used as an important indicator to evaluate plant productivity under various environmental conditions (Jinhu *et al*., 2014). The results showed that the NAR of porang plants decreased under salinity stress. The decrease in NAR yield is thought to be caused by a decrease in photosynthetic pigments, namely chlorophyll. Salinity stress reduces chlorophyll biosynthesis and increases chlorophyll degradation (Qin *et al*., 2020). Leaf area correlates with NAR yield. Leaves that have a smaller size will produce low levels of assimilation. Higher salinity levels cause a decrease in leaf area, resulting in a decrease in photoassimilate yield and causing reductions in the photosynthesis rate and the yield of NAR because CO_2 is a compound that is later converted into the final product of photosynthesis by RuBisCo (Zahra *et al*., 2022).

Glucomannan is a polysaccharide obtained from *Amorphophallus* spp. It has a straight chain structure with β-1,4 glycosidic bonds between mannose and glucose units (Zhang *et al*., 2014). Glucomannan in *A. muelleri* plants is present in large quantities and can be degraded into glucose and mannose, whose energy can be used in the germination and budding processes. Our results showed that glucomannan in porang plants decreased with increasing salinity concentration. It is suspected that glucomannan is used as a food reserve and an energy source in unfavourable conditions such as high salinity conditions.

Glucomannan as a food reserve was reported by Chua *et al*. (2013) who analysed glucomannan in the anatomy of *Amorphophallus konjac* stem tubers during the growth and development of porang. Observations on the porang tuber peel indicate that increased salinity leads to a tougher and rough-textured peel, attributed to peel thickening, which acts as a protective measure against pathogens and damage (Dangi *et al*. 2018). This thickened peel aids in minimizing the impact of salinity stress. Additionally, the reduction in glucomannan content appears to be closely linked to various parameters in this study, including leaf area, stomata, net assimilation rate, and chlorophyll content. The decline in glucomannan levels is believed to result from a decrease in photosynthate yield induced by salinity stress.

Lower glucomannan levels at higher salt concentrations raise an interesting question, as the tuber size remains almost the same. Dahro *et al*. (2016) stated that the decrease of glucomannan content occurs during stress as the glucomannan formation pathway is inhibited, leading to other pathways such as starch formation. To answer the question, we analysed the expression of genes that play a role in glucomannan biosynthesis, namely *SuSy2* (Sucrose Synthase) and *CSLA3* (Cellulose Synthase-Like A3). *SuSy2* functions to convert sucrose into UDP-glucose and fructose (Stein & Granot, 2019). *SuSy2* gene expression in porang plants was up-regulated at salinity concentrations of 100 mM and 150 mM.

Increases in *SuSy2* gene expression in *Sorghum bicolor* is thought to be caused by transcription factors that respond to stress. Specific transcription factors that respond to stress such as MYC and MYB are attached to stress regulatory elements so that there is an increase in *SuSy2* gene expression when under stressful conditions (Yang *et al*., 2012; Zhao *et al*., 2018). Meanwhile at a concentration of 200 mM *SuSy2* gene expression decreased. A decrease in *SuSy2* gene expression under salinity stress conditions was also reported by Winter & Huber (2000), who found that transcription factors have a role in inhibiting the expression of the *SuSy2* gene. The transcription factor is SNF1-related PK (SnRK1). SnRK1 is a kinase whose expression is not influenced by the signal from ABA, rather SnRK1 is expressed when there is a signal from low glucose levels, high sucrose levels, or salinity stress (Kulik *et al*., 2011). While *CSLA3* (Cellulose Synthase-Like A3) is a superfamily gene that plays a role in the biosynthesis of mannan polysaccharides (Liepman *et al*., 2005; Lerouxel *et al*., 2006; Goubet *et al*., 2009).

Specifically, *CSLA3* plays a role in the final step of the glucomannan biosynthetic pathway, which transfers mannose residues from GDP-mannose to UDP-glucose (Sawake *et al*., 2015). *CSLA3* gene expression increased at concentrations of 50 to 100 mM and then decreased (down-regulated) at a concentration of 150 mM. Previous research conducted by Guerriero *et al*. (2014) showed that alfalfa plants treated with 100 mM NaCl salinity stress showed the expression of *CESA6* (Cellulose Synthase-Like A6) increased 1.2-fold compared to the control at 24 hours of stress exposure, then increased again by 1.5-fold at 72 hours of stress exposure compared to the control but decreased expression at 96 hours of stress exposure by 1.3-fold compared to the control. *CESA6* gene expression is highly dependent on the availability of UDP-glucose produced by the *SuSy2* gene. Therefore, it can be concluded that the decrease in *CSLA3* gene expression in porang plants is thought to be a result of the decreased availability of the UDP-glucose substrate. The dynamic changes in the expression of both genes relate to glucomannan production in porang plants. Further transcriptomic analysis is necessary to understand the big picture of glucomannan production under salt stress.

The morphological response exhibited significantly lower values than the control, particularly in root length, plant height, leaf area, stem tuber weight, and stem tuber diameter. In terms of physiological changes, salinity stress resulted in significantly lower values compared to the control, particularly in chlorophyll content, glucomannan content, percentage of stomatal closure, and stomatal density, net assimilation rate (NAR). The molecular response highlighted the dynamic expression of *SuSy2* and *CSLA3* genes. The *SuSy2* gene exhibited downregulation at concentrations

of 50 mM and 200 mM, showing a reduction of 0.91 to 1.2 times compared to the control. Additionally, the *SuSy2* gene demonstrated upregulation at concentrations of 100 mM and 150 mM, with an increase of 1.5 to 1.55 times compared to the control.

In conclusion, porang demonstrates a potential capacity to grow in soil conditions where salinity concentrations can reach up to 50 mM. This implies that the cultivation of porang could be successfully expanded to regions with moderate salt levels, expanding its possible agricultural uses and emphasizing its versatility to sub-optimal soil types.

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Authors' Contribution

T.N and T.B.S Literature study, conception, design the research, data collection, gene expression analysis and wrote the manuscript; Z.F; A.A and K.I.P contributedon phenotyping of morphological and physiological responses of Porang under salt stress, data analysis, and wrote the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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