EFFECTS OF SALINITY STRESS ON CARBOHYDRATE METABOLISM IN *Cryptocoryne elliptica* CULTURES

Aziz Ahmad*, Azmi Ismun and Mariam Taib
School of Fundamental Science, Universiti Malaysia Terengganu,
21030 KUALA TERENGGANU, MALAYSIA
*Email: aaziz@umt.edu.my

ABSTRACT

Salinity limits water availability in plants, and lead to osmotic stress. Soluble carbohydrate or sugar plays crucial roles as osmolytes in salinity stress by regulating osmotic adjustment and carbon storage in plants. The involvement of salt in the biosynthesis and degradation of starch and sucrose in plants is poorly understood. In the present study, effect of salinity levels on the activity of main enzymes involved in carbohydrate metabolism was investigated. An aquatic plant, *Cryptocoryne elliptica* plantlets was challenged with salinity levels at 100 and 200 mM of NaCl for five weeks. Results showed that the salinity stress had decreased the plantlet growth and the activity of glucosidase and galactosidase enzymes. Under salinity conditions, the starch content, the activity of amylase, invertase, hexokinase and glucose-6-phosphate dehydrogenase enzymes were significantly increased during the recovery phase of salinity stress. Salinity conditions had no significant effects on the phosphoenolpyruvate carboxylase activity. These results suggest that *C. elliptica* regulates carbohydrate metabolism under salinity conditions, and has moderate response towards sodium ion, Na^+ in the culture medium.

Keywords: invertase, amylase, glucosidase, galactosidase, phosphoenolpyruvate carboxylase (PEPC), hexokinase, glucose-6-phosphate dehydrogenase (G6PDH)

INTRODUCTION

Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination, plant growth and development, and crop yield. Salinity affects more than 45 million hectares of irrigated land worldwide, and 1.5 million hectares are taken out of production yearly as results of high salinity levels in the soil (Munns and Tester 2008). Majority of crops are glycophytes, which unable to grow in high salt concentration and severely inhibited or even killed by 100 to 200 mM NaCl. On the contrary, halophytes are known to have a capability of survival and growth on salinized soils of costal or excess of 300 to 400 mM NaCl. This is determined by the effective coordination between various physiological processes, metabolic pathways and protein or gene networks to specific mechanism of salt tolerance developed during the phylogenetic adaptation (Kumari et al. 2015). Under salt stress, halophytes accumulate salts, whilst glycophytes tend to exclude the salts (Zhu 2007). The main type of soil that affects plant growth is the saline soils, where soluble salts are chiefly NaCl and Na_2SO_4, and appreciable quantities of Cl^- and SO_4^- of Ca_2^+ and Mg^{2+}, to pose negative effect on growth of most crop plants (Parihar et al. 2015). The major inhibitory effect of salt on plant growth is the ability of plant to take up water, which refer to the osmotic or water-deficit effect of salinity (Wang et al. 2015). Secondly, the salt-specific or ion-excess effects of salinity, which is due to excessive amount of salt entering the plant transpiration stream. The presence of excessive soluble salts in the soil increased the uptake of Na^+, Cl^- or SO_4^{2-}, induces specific ion toxicities and competes with the uptake and metabolism of essential mineral nutrient such as phosphorus, potassium, nitrogen and calcium by plants (Gupta and Huang 2014). Effects of salinity on nutrient availability, competitive uptake, transport or distribution, physiology and biochemical events at various development stages within the plants have been extensively reviewed (Kumari et al. 2015; Parihar et al. 2015; Slama et al. 2015).

Plants that have the ability to grow and complete their life cycle on a substrate that contains high concentration of soluble salt is considered as salt-tolerant. Salt tolerant plants may possess some unique stress-responsive genes that regulate the salt uptake and transport, osmotic or protective function and stimulate plant grow in saline soil (Munns 2005). The osmolytes, osmoprotectants or compatible solutes are molecules that have a protective function (Slama et al. 2015). They are N-containing solutes such as proline and glycine betaine (Nounjana et al. 2012); sugar such as sucrose, trehalose and raffinose (Gupta and Huang 2014); straight-chain polyhydric alcohol (polyols) such as mannitol and sorbitol (Conde et al. 2007) and cyclic polyhydric alcohol or cyclic polyols (Kumari et al. 2015). Sugars play roles in osmotic adjustment, stabilizing membranes upon stress (Wang et al. 2015), and affecting sugar-sensing system that regulates the expression of a variety of genes involved in photosynthesis, respiration, and synthesis and degradation of starch and sucrose.

Starch and sucrose catabolism are catalysed by amylase, invertase, glucosidase, galactosidase, hexokinase and glucose-6-phosphate dehydrogenase (Fig. 1). Alpha-amylase catalyses the breakdown of starch or a soluble α-glucan to maltose. Whilst soluble α-glucan is catalysed by glucosidase to glucose. The enzyme galactosidase catalyses the hydrolytic cleavage of the terminal-linked galactose moiety from galactose-containing oligosaccharides (Gao and Schaffer 1999). Glucose is a substrate of hexokinase, which produces hexose phosphate. It is converted to sucrose by sucrose-phosphate synthase (SPS) or reverted to hexose-phosphate (Stanley et al. 2005). A glucose-6-phosphate is catalysed by glucose-6-phosphate dehydrogenase (GDPD) to 6-phosphoglucono-δ-lactone with the formation of NADPH (Liu et al. 2007). In plants, sucrose can be hydrolysed by different invertase isozymes to fructose and glucose for sink metabolism (Roitsch and Gonzalez 2004). Phosphoenolpyruvate carboxylase catalyses the irreversible β-carboxylation of phosphoenolpyruvate (PEP) in the presence of HCO_3^- and Mg^{2+} to yield oxaloacetate (OAA) and inorganic phosphate (Pi) (Masumoto et al. 2010). Other proteins related to carbohydrate and energy metabolism of plants contributing to the salt tolerance are bisphosphoglycerate-independent (PGAM), fructokinase-1 (FRK), triosphosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase (PGK) and enolase (Kumari et al. 2015). To date, the specific activity of carbohydrate metabolism enzymes during salinity stress is remained limited especially on aquatic plants.

Cryptocoryne (Araceae, Crytocoryninae) is a common aquatic plant, grows in slow running rivers and streams, and seasonally inundated forest pools under extremely acid conditions with pH 4 or so (Ipor et al. 2007), which is recognised as sodic soils. These soils contain Na^+ salts are capable of alkaline hydrolysis, mainly Na_2CO_3 (Parihar et al. 2015). The survival of Crypts plant under this condition determines its sustainability. In the present study, effects of salinity on the activity of proteins that catalysed the metabolism of starch and sucrose in *Cryptocoryne elliptica* were examined.

MATERIALS AND METHODS

Plant Material and Treatment

The established culture of *Cryptocoryne elliptica* plantlets, which was previously collected from Pondok Tanjung Forest Reserve, Perak was used in the present study. Cultures were proliferated and maintained on B5 Gamborg medium (Gamborg et al. 1968) containing 20 mg/L sucrose. Medium was adjusted to pH 5.7 prior autoclaving at 121 ^{0}C for 20 min. The salinity treatment medium was added with NaCl at concentrations 0, 100 and 200 mM. The four weeks old *C. elliptica* plantlets were used as explants and aseptically transferred into treatment medium. All cultures were incubated in a growth chamber at 28 ($\pm$ 1.0) ^{0}C under 16 hrs photoperiod of white-light illumination with 55 - 70 µmol photon $m^{-2}s^{-1}$ light intensity provided by fluorescence light. The determination of fresh weight, starch, reducing sugar and total protein content, and activities of amylase, invertase, glucosidase, galactosidase, hexokinase,

phosphoenolpyruvate carboxylase and glucose-6-phospahte dehydrogenase were carried out weekly until five weeks. Cultures in NaCl-free medium were used as controls.
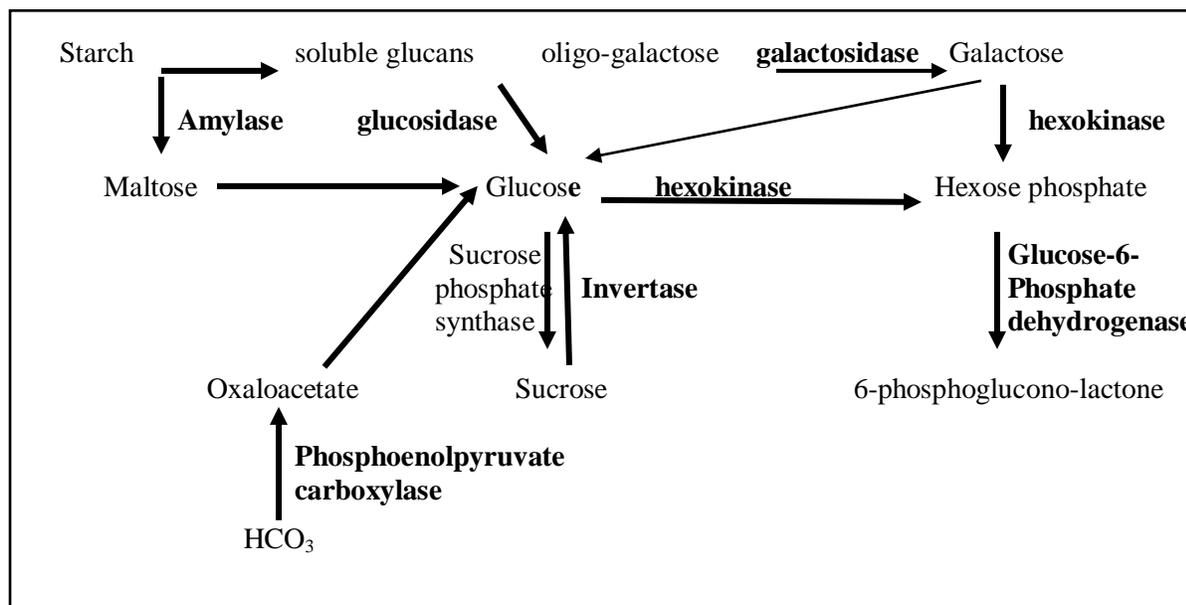
Starch   soluble glucans   oligo-galactose   **galactosidase** Galactose

Amylase   **glucosidase**   **hexokinase**

Maltose   Glucose   **hexokinase**   Hexose phosphate

Sucrose phosphate synthase   **Invertase**   **Glucose-6-Phosphate dehydrogenase**

Oxaloacetate   Sucrose   6-phosphoglucono-lactone

Phosphoenolpyruvate carboxylase

HCO_3

Figure 1. The pathway of carbohydrate metabolism in plant cells. (Modified from Stanley et al. 2005)

Starch and Reducing Sugar Content

Leaves of fresh sample were homogenised in ice-cold distilled water in ratio 1:1 (w/v) and centrifuged at 20 000 *x g* for 15 min. The starch content was quantified according to method by Farhat et al. (1999). Five hundred microliters of sample aliquot were mixed with 0.5 mL of 1M acetic acid and 1.0 mL of iodine solution [0.2 % iodine in 2 % KI] and distilled water to 50 mL in volumetric flask. The solution absorbance was measured at 620 nm and starch content were calculated based on standard curve generated using 100 mg corn starch solution dispersed in 1.0 mL EtOH, 9.0 mL of 1M NaOH and distilled water to 100 mL. Reducing sugar was determined according to Krishnaveni et al. (1984). Five hundred microliters of supernatant were mixed with 0.5 mL of arsenomolybdate reagent and boiled in a water bath for 20 min. Absorbance was measured at 520 nm. Reducing sugar was calculated based on standard curve of glucose.

Amylase, Invertase, Glucosidase and Galactosidase Assays

Leaves of fresh sample were homogenated with extraction buffers; McIlvaine buffer (pH 5) for β-glucosidase; McIlvainne buffer (pH 3.5) for β-galactosidase, acetate buffer (pH 4.7) for amylase and invertase at ratio 1:1 (w: v). Crude enzyme was obtained by centrifugation at 20 000 *x g* for 15 min and used for enzyme assay. Amylase (EC 3.2.1.1) activity was measured according to method by Doehlert and Duke (1983). One millilitre of 1% (w/v) starch dissolved in 100 mM acetate buffer (pH 4.7) was added to 250 µL of extracted crude enzyme. The mixture was incubated at 27 ^{0}C for 15 min, added with 2.0 mL of dinitrosalicyclic acid (DNS) reagent boiled and in a water bath for 5 min. The volume was adjusted to 10 mL with 1.0 mL potassium sodium tartrate and distilled water. Absorbance was read at 560 nm using spectrophotometer and amylase activity was calculated against maltose standard.

Invertase (EC 3.2.1.26) activity was measured according to Perumalla et al. (1994). One milliliter of 2 % (w/v) starch dissolved in 20 mM acetate buffer (pH 4.7) was added to 250 µL crude enzyme. The solution was incubated at 27 ^{0}C for 30 min, then 1.0 mL Nelson-Somogyi's solution was added and boiled for 5 min. The solution mixture then cooled to room temperature, added with 0.5 mL Nelson's Arsenomolybdate solution and 4.0 mL distilled water. Absorbance was measured at 650 nm using spectrophotometer and invertase activity was calculated against glucose standard.

The activity of β-glucosidase and β-galactosidase were assayed according to the method by Alcantara et al. (2006). Two hundred milliliters of p-nitrophenol glycosidase (pNPG) substrate was added with 10 µL of crude enzyme extract in 10 µL of McIlvaine buffer (pH 3.2) for β-galactosidase and (pH 5) for β-glucosidase. The mixtures were incubated in a water bath at 30 ^{0}C for 20 min. The reaction was stopped by the addition of 1.0 mL of 0.2 M Na_2CO_3. The amount of p-nitrophenol released were measured using spectrophotometer at 420 nm. The specific enzyme activity was quantified by the total activity per total protein. One unit of enzyme released was defined as the amount of enzyme released 1 µmol of p-nitrophenol per minute from pNP-Gal.

Hexokinase assay

The leaf samples were homogenised in an ice-cold of 0.5 M mannitol at the ratio of 1:4 mL (w/v). Subsequently, the supernatant containing the crude enzyme was obtained by centrifugation at 20 000 x g for 15 min. The hexokinase enzyme activity was measured according to Fox et al. (1998). The reaction mixture consisted of 1.5 mL of 10 mM ATP, 3.3 mM glucose, 10 mM $MgCl_2$, 60 mM Tris-base (pH 8), 10 mM KCl and 0.5 mL enzyme-containing supernatant. The reaction was stopped by adding 1.0 mL of 0.2 M $Ba(OH)_2$ and 1.0 mL of 0.2 M $ZnSO_4$. Residual glucose remained after reaction was measured according to Somogyi method (1952).

Glucose-6-phosphate dehydrogenase assay

A fresh leaf sample was homogenised in an ice-cold phosphate buffer (pH 7) consisting of 1.0 M NaCl, 1.0 % (v/v) polyvinyl-polypyrrolidone (PVPP) and 1.0 mM of 50 mM EDTA. Supernatant obtained by centrifugation at 20 000 x g for 15 min and used for glucose-6-phosphate dehydrogenase assay according to Liu et al., (2007). The reaction mixture consisted of 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 5 mM maleimide, 10 mM glucose-6-phopshate, 0.4 mM $NADP^+$ and 0.5 mL supernatant. A unit of enzyme activity is the quantity of enzyme to reduce 0.01 µM $NADP^+$ in 60 s.

Phosphoenolpyruvate carboxylase assay

A fresh leaf sample was homogenised in a 50 mM Tris-HCl (pH 7.8) containing 50 mM $MgCl_2$, 1.0 mM EDTA and 5.0 mM of 2-mercaptoethanol at ratio 1:1 (w/v). The supernatant was obtained by centrifugation at 20 000 x g for 15 min and used for assay of phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) according to Arnozis et al. (1988). The reaction mixture consisted of Tris-HCl (pH 7.8), 10 µM $MgCl_2$, 10 mM $Na(CO_3)_2$, 5 mM PEP, 0.4 µM NADH and 0.2 mL supernatant. Absorbance was recorded every 30 sec for 3 min at 340 nm. Total protein concentration was estimated using Bradford method (Bradford, 1976) and absorbance measured at 595 nm.

Statistical analysis

The significant difference of biomass, enzyme activities among the salinity treatment and control were statistically analysed by the One-way ANOVA using SPSS Version 16.0. (SPSS Student Version 16.0). The significant differences of the mean were identified by post hoc Tukey's test at $p = 0.05$.

RESULTS

Biomass dry weight

Our experiment showed that the *C. elliptica* culture is tolerant to moderate salinity level (100 mM NaCl). As shown in Fig. 2, the growth of *C. elliptica* cultures in both salinity levels was lower than control. However, it was severely affected in 200 mM NaCl compared to 100 mM NaCl.
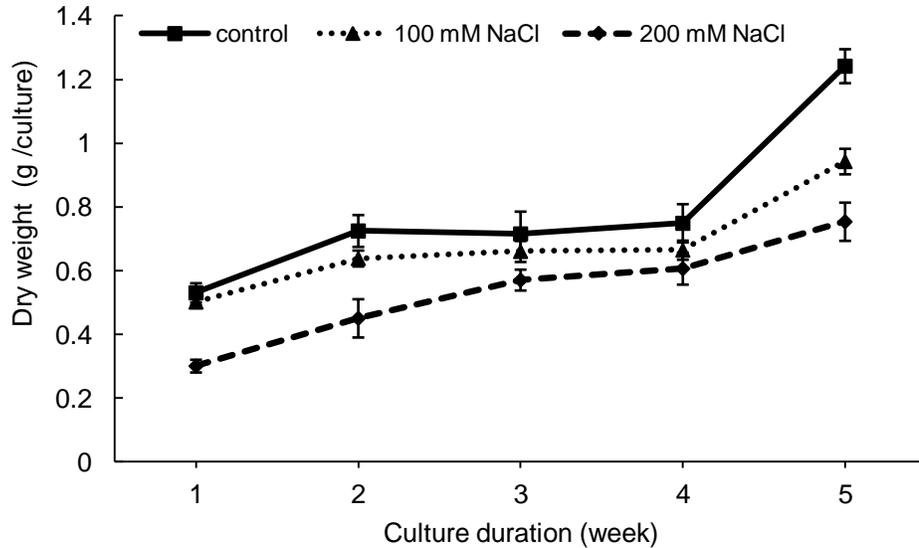

Figure 2. Effects of salinity conditions on *Cryptocoryne elliptica* biomass dry weight after five weeks of culture. Error bars represent mean ± SD (n=3).

Starch and reducing sugar content

Our results show that salinity had a significant effect on the starch content in *C. elliptica* cultures. Starch content was increased when cultured in a higher salinity level (200 mM NaCl), but was reduced in moderate salinity level (100 mM NaCl; Fig. 3a). In 200 mM NaCl, the starch was increased from 0.4-fold lower than control at the beginning of experiment to 3.1-fold higher than control at the end of experiment. Meanwhile starch content in 100 mM NaCl was lower than control, except at week five, when it was 1.2-fold higher than control.

Salinity conditions also affect reducing sugar content in *C. elliptica* culture (Fig. 3b). The content of reducing sugar in *C. elliptica* was decreased under 200 mM NaCl throughout the experiment. Whilst in 100 mM NaCl, the reducing sugar content remained unchanged until week three. It declined after this period until the end of the experiment. The content of reducing sugar in 200 mM NaCl was lesser than in 100 mM NaCl for the first two weeks. However, after week three, reducing sugar level in salinity 200 mM was higher than in 100 mM NaCl, but did not significantly differ.
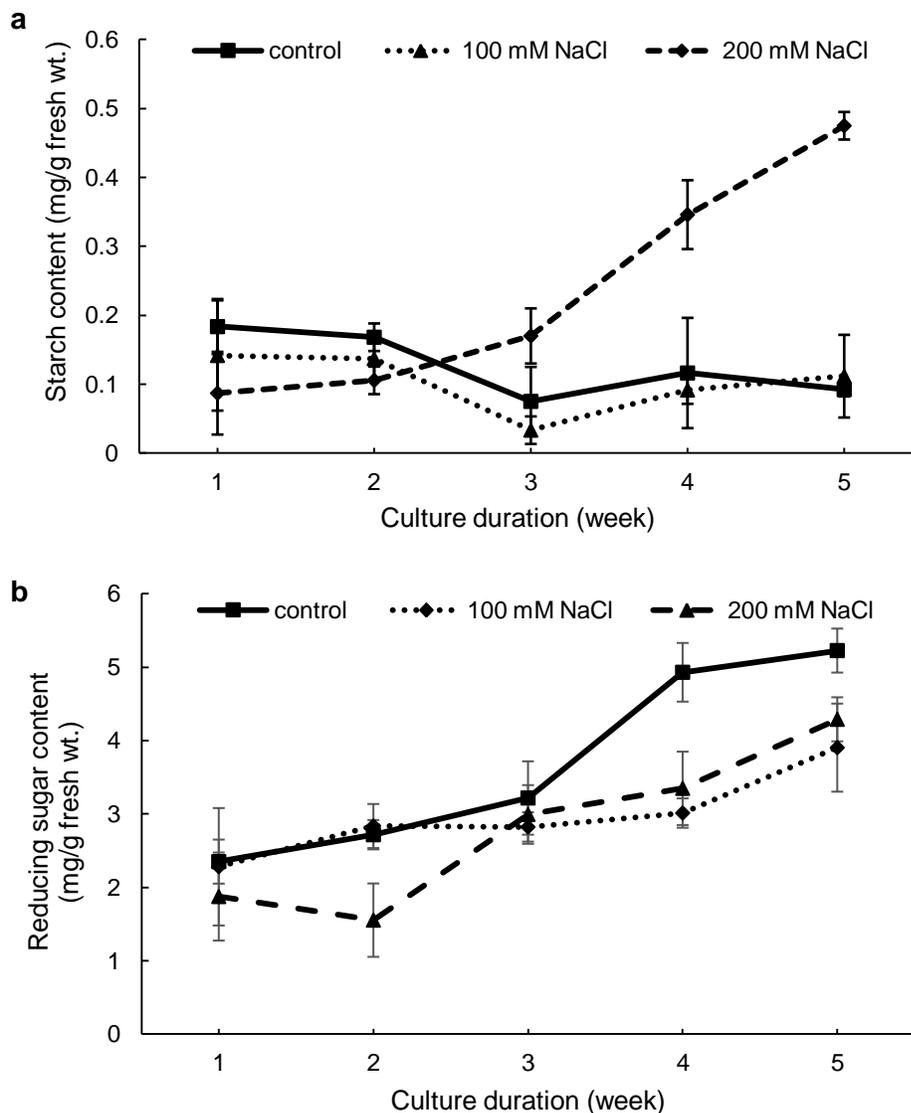
Figure 3. Effects of salinity conditions on *Cryptocoryne elliptica* after five weeks of culture; starch content (a) and reducing sugar content (b). Error bars represent mean ± SD (n=3).

Enzymes activity

As shown in Figs. 4 - 10, the activity of most enzymes was higher than the control at the first week of the treatment, except the β-glucosidase, β-galactosidase and PEPC in 100 mM NaCl. After that, their activity were drastically dropped and the lowest activity was recorded at week two of treatment. Afterwards, the activities of all enzymes were gradually increased or remained unchanged until week four. Our results show that at the beginning of the experiment, the α-amylase activity was 1.7- and 2.7- fold higher than the control for 100 and 200 mM NaCl, respectively (Fig. 4). It was decreased lower than the control at week two, but did not significantly differ between the salinity treatments. After this duration, the highest α-amylase activity was at week four, as they exhibited a similar level to the control in both NaCl concentrations.
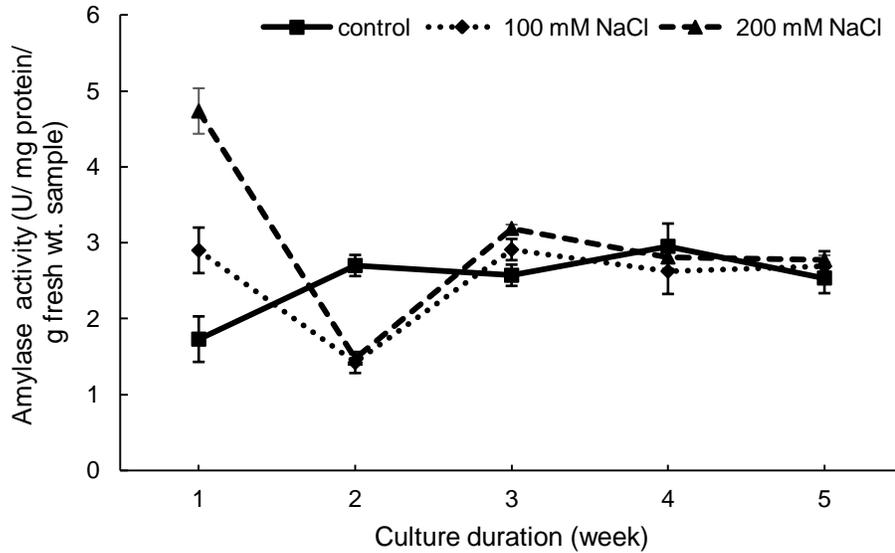
Figure 4. Effects of salinity conditions on amylase activity in *Cryptocoryne elliptica* after five weeks of culture. Error bars represent mean ± SD (n=3).

Invertase activity was the highest among the enzymes measured at the beginning of the experiment, 3.1- and 2.8-fold higher than control in 100 and 200 mM NaCl, respectively. Its activity was sharply decreased in week two, 0.5-fold lower than control in both salinity levels. However, the invertase activity was increased in after week three. Its activity was 2.0- and 1.7-fold higher than control at week four and decline until the end of experiment.
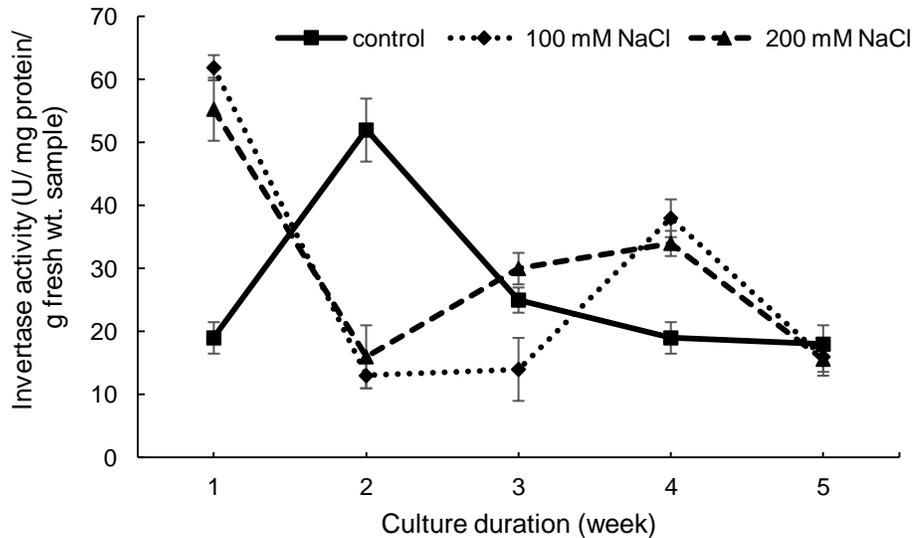

Figure 6. Effects of salinity conditions on invertase activity in *Cryptocoryne elliptica* after five weeks of culture. Error bars represent mean ± SD (n=3).

Results on Fig. 6 showed that the β-glucosidase activity in *C. elliptica* of was decreased under the salinity treatments. Interestingly, its activity in 200 mM NaCl was higher than in 100 mM NaCl. The β-glucosidase activity in 100 mM NaCl was fluctuated within 0.4- to 0.3-fold lower than control after week

two until the end of the experiment. However, its activity in 200 mM NaCl was declined from 0.9- to 0.4-fold lower than control.
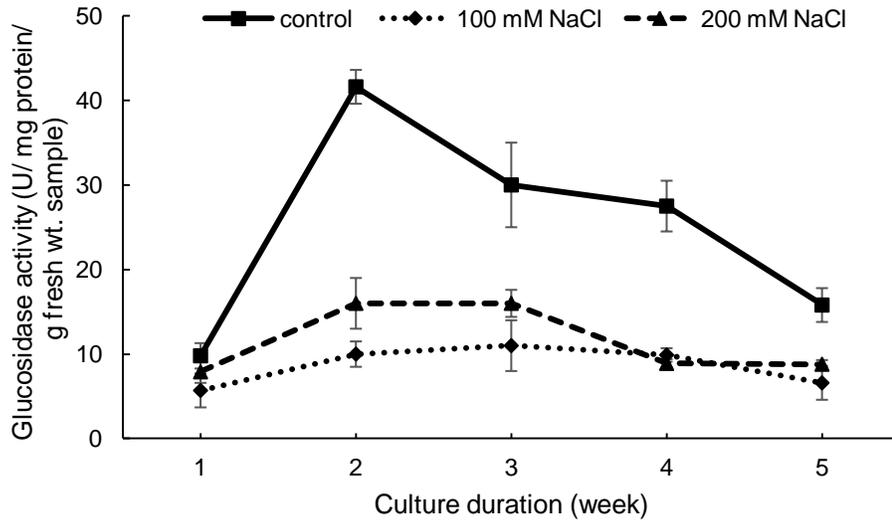

Figure 6. Effects of salinity conditions on β-glucosidase activity in *Cryptocoryne elliptica* after five weeks of culture. Error bars represent mean ± SD (n=3).

The β-galactosidase activity of *C. elliptica* was intensely suppressed in higher NaCl concentration (200 mM), but restrained in 100 mM NaCl (Fig. 7). In 200 mM NaCl, it was fluctuating within 0.2- to 0.8- fold lower than control. On the other hand, in 100 mM NaCl, its activity did not significantly differ ($p > 0.05$) from the control at week one four and five. However, it was increased from week two to week five, 0.5- to 0.8-fold lower than control, respectively.
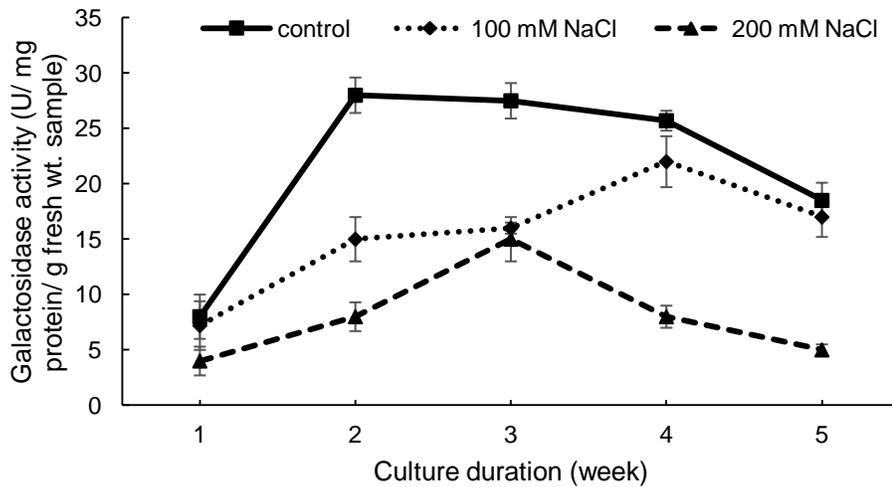

Figure 7. Effects of salinity condition on α-galactosidase activity in *Cryptocoryne elliptica* after five weeks of culture. Error bars represent mean ± SD (n=3).

As shown in Fig. 8, salinity levels (100 to 200 mM) had increased the activity of hexokinase in *C. elliptica*. The lowest hexokinase activity in both NaCl conditions was at week two, 0.83-fold lower than control. In 100 mM NaCl, it activity was 2.55- and 4.83-fold higher than control at the beginning and the end of the experiment, respectively. In 200 mM NaCl, 3.91-fold higher than control at the beginning and 1.67-fold at the end of the experiment.
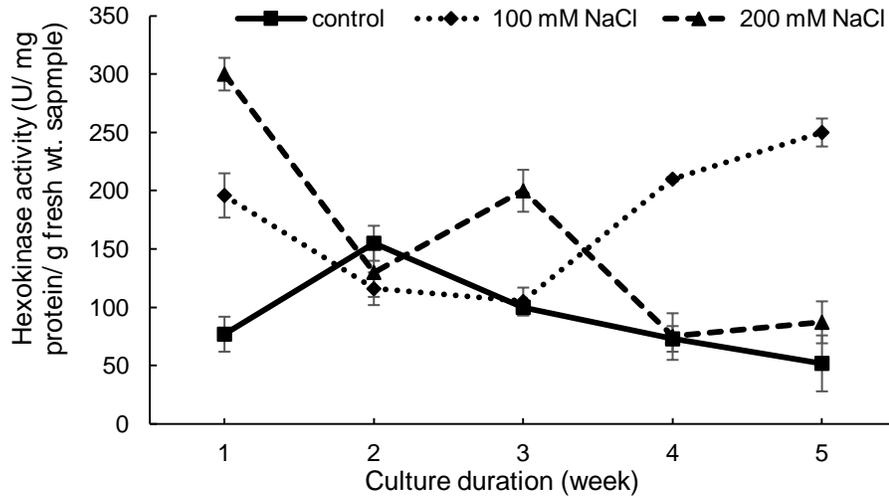

Figure 8. Effects of salinity conditions on hexokinase activity in *Cryptocoryne elliptica* after five weeks of culture. Error bars represent mean ± SD (n=3).

Results showed that the salinity conditions had a significant impact on the levels of glucose-6-phosphate dehydrogenase (G6PDH) in *C. elliptica* plantlets (Fig. 9). The G6PDH activity at the beginning of treatment was 7.84- and 4.33-fold higher than control in 100 and 200 mM NaCl, respectively. But declined to 0.23-fold lower than control at week four.
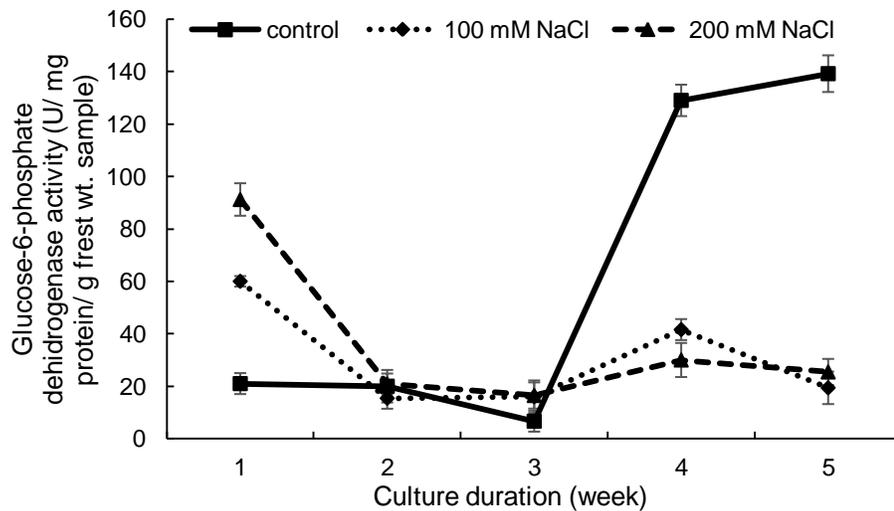

Figure 9. Effects of salinity conditions on glucose-6-phosphate dehydrogenase amylase activity in *Cryptocoryne elliptica* after five weeks of culture. Error bars represent mean ± SD (n=3).

As shown in Fig. 10, salinity condition has insignificant effect on phosphoenolpyruvate carboxylase (PEPC) activity in *C. elliptica* cultures. In higher salinity level (200 mM), the PEPC was suppressed to 0.73-fold lower than control at the beginning of the experiment, but gradually increased until week three. At this time, PEPC activity was the same as in control. In moderate salinity level (100 mM NaCl), the PEPC began at 1.11-fold higher than control but reduced to 0.86-fold lower than control at week two. However, it was regained to 1.26-fold higher than control at week three, but declined after the following week.
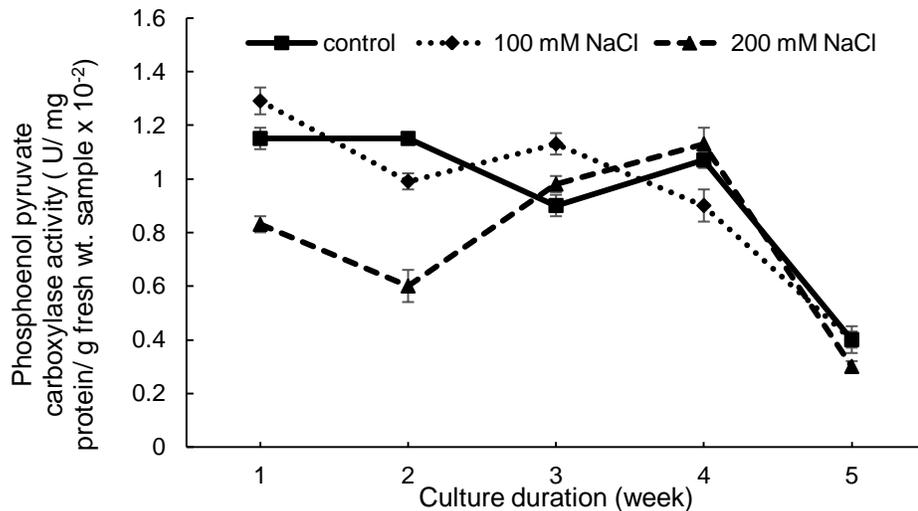

Figure 10. Effects of salinity conditions on phosphoenolpyruvate carboxylase activity in *Cryptocoryne elliptica* after five weeks of culture. Error bars represent mean ± SD (n=3).

DISCUSSION

Our results suggest that *C. elliptica* possess two stages of growth under salinity stress in tissue culture condition. The first stage is the adaptation phase, which occurs within two weeks of treatment. During this phase, most of the enzymes related to biosynthesis (NADPH), bioenergy (hexokinase) and carbon arrangement (amylase, invertase) were higher than the control. Under stress, energy requirement was reported to increase. The levels of ATP and many metabolites related to ATP biosynthesis, as well as glyceraldehyde-3-phosphate (G3P), glucose-6-phosphate (G6P), NADPH and erythrose-4-phosphate are also increased (Kumari et al. 2015). While in the second phase (the recovery phase), most of the related enzymes exhibit similar trends with the control. At this stage, this plant exhibits a capability to survive and grow in salinity conditions. Its ability to grow during salt stress was clearly contributed by carbon metabolism (Fig. 4 to 10). Carbon autotrophy and the non-reducing disaccharide sucrose play a central role in plant metabolism. Starch located in the cytosol may act as a buffer between the process of starch degradation and hexose phosphate metabolism (Stanley et al. 2005). Cytosolic amylase is responsible for degradation of cytosolic glucan (Fig. 1) and maintaining a high concentration of sugar within the cytosols. In the cytosol, maltose is converted to hexose phosphate, and either consumed by the glycolytic pathway or synthesised into sucrose for export. Sink tissues may be rapidly growing tissues, such as meristems and young leaves, which catabolise the sucrose to produce energy, or storage organs such as roots, tubers, bark and fruit, which resynthesize starch in the plastid (Slama et al. 2015).

Sugar such as sucrose and trehalose have been shown to be accumulated in plants in response to abiotic stress (Redillas et al. 2012). Sucrose with glucose was reported to act as substrates for cellular respiration,

osmolytes to maintain homeostasis and stabilize membrane (Norwood et al. 2000). Sucrose released was then hydrolysed by invertase into the hexose monomers, glucose and fructose (Roitsch and González 2004). Fructose is not involved on osmoprotection but seems to act as substrate in the biosynthesis of secondary metabolite, lignin and phenolic compounds (Rosa et al. 2009), which is also involved in regulating plant response abiotic stress**.** The disaccharide sucrose and the cleavage products such as glucose and fructose are the central molecules for the growth of sink tissues, carbohydrate translocation, metabolism and sensing in higher plants that regulate the expression variety of genes involved in photosynthesis, respiration and the synthesis and degradation of starch and sucrose (Kumari et al. 2015; Slama et al. 2015). In addition, the extracellular and vacuolar invertase isoenzymes are involved in plant life cycle and also in response to environmental stimuli either alone or/and in combination with plant hormones (Roitsch and Gonzalez 2004).

Increasing activities of invertase (Fig. 5) and sucrose phosphate synthase (Kumari et al. 2015) would lead to the accumulation of glucose. Subsequently, it triggers the activity of hexokinase. High invertase activity might induce the accumulation of glucose, a substrate of hexokinase which then contributed to increase hexokinase activity in *C. elliptica* (Fig. 8). On the other hand, β-galactosidases are involved in metabolism of various macromolecules (e.g., galactooligosaccharides, lipids and proteins in plants), raffinose oligosaccharide metabolism, stress tolerance in plants and galactolipid degradation during plant senescence (Chrost et al. 2007). Hexokinase is the main enzyme that phosphorylates glucose to glucose-6-phosphate (G6P) with the presence of ATP. The cytosolic or plastic G6DPH catalysed the oxidation of G6P to 6-phosphoglucono-δ-lacton with the reduction of $NADP^+$ to NADPH. NADPH is important in biosynthesis of cellular component and also in scavenging of reactive oxygen species (ROS). G6PDH plays a critical role in maintaining cellular reduced glutathione GSH levels and in modulating the intracellular redox homeostasis under long-term salinity stress (Liu et al. 2007). Salt stress resulted in hydrogen peroxide (H_2O_2) accumulation (Wang et al. 2008), where H_2O_2 acts as a signal in regulating G6PDH activity under salt stress. G6PDH activity is required in enhancing the activities of antioxidative enzymes, enhanced membrane leakage, lipid peroxidation, H_2O_2 and O_2 contents (Liu et al. 2012). On the other hand, our results shows that G6DPH activity was highly produced at the first two week of salinity stress, but declines then after. This might be the limited amount of substrate, the glucose-6-phospate and partitioning of substrate for ATP production. Although, the reducing sugar at this period was increased but lower than the control (Fig. 3). Under saline conditions, demand for ATP is high in responses to lower photosynthesis rate and osmotic (Kumari et al. 2015).

Our results also suggest a close communication within the cascade of reaction among amylase, invertase, hexokinase and G6DPH during salinity stress. The carbon portioning and osmolytes adjustment contributes to the survival of *C. elliptica* under salinity stress. High levels of soluble starch (Fig. 3) after week two was parallel with the lower levels of reducing sugar. It was clearly showed that sugars released from the activity of amylase and invertase contribute to the hexokinase activity, which may lead to the bioenergetics process. The available substrate may also function as a feedback inhibitor during the sugar-sensing under high salinity levels that contributed to high starch accumulation (Fig. 3a). Storage starch is very important to plants and is broken down following specific seasonal or developmental cues, such as the beginning of spring in roots and bark, and the onset of ripening in many fruits (Parihar et al. 2015). Carbohydrate are synthesized in source leaves, which involved the PEPC and translocated to sink tissues in most species in the form of sucrose to sustain heterotrophic metabolism and growth, or to be stored as sucrose or starch. In this study, PEPC activity was not affected by lower salinity levels (100 mM; Fig. 10). This is an advantage for this plant, as it is known that PEPC plays the anaplerotic role of replenishing the citric acid cycle intermediates, oxaloacetate and malate, required for nitrogen assimilation and amino acid biosynthesis (Masumoto et al. 2010). The synthesis of malate implicated in cytosolic pH regulation and stomatal opening (O'leary et al. 2011) which important for photosynthesis and cell homeostasis.

CONCLUSION

Carbohydrate metabolism plays a significant role in plants during salinity stress. *Cryptocoryne elliptica* have an ability to reactivate the activity of amylase, invertase, hexokinase and G6DPH enzymes during the recovery phase under salinity condition. Thus, this aquatic plant is moderately tolerant towards a high level of sodium ion, Na^+.

ACKNOWLEDGEMENTS

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