EFFECT OF SALINITY STRESS ON CARBOHYDRATE METABOLISM IN *CRYPTOCORYNE ELLIPTICA* CULTURES

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Salt condition could disturb water availability in plants which causes growth disturbance and also death. The plant regulates the stress by altering sugar content at metabolism level to survive the stress. This study was carried-out to determine the effects of different NaCl concentrations - 100mM and 200mM/L - on carbohydrate metabolism in aquatic plant, *Cryptocoryne elliptica* cultures, as a model plant, for 35 days. The aquatic habitats represent stressful environments which are characterized for example by low carbon availability, uncertained availability of nutrient and shaded conditions induce plants to adapt with the environment on its fully potentials. Limited studies of aquatic plants also encouraged the study to be done. The salinity treatments were also carried-out in two different medium conditions: sucrose-contained- and sucrose-free medium. In this study, the accumulation of biomass, starch content and reducing sugar content were determined. In addition, the changes in carbohydrate metabolism were determined via the activities of enzymes amylase, invertase, glucosidase, galactosidase, hexokinase, phosphoenolpyruvate carboxylase (PEPCase) and glucose-6-phosphate dehydrogenase (G6PDH). The effects of

salinity on protein content were also determined. Results showed that increased salinity decreased the biomass of *C. elliptica* in both types of media, up to 30% decrement as compared to the control. As for the starch content, salinity in sucrose-contained media increased rapidly in 200mM NaCl on week 4 and 5, but for sucrose-free media, rapid increment on week 4 and 5 occurred in explant cultured in 100mM NaCl. Reducing sugar contents in the explants cultured in the presence of sucrose are higher at the end of the experiment (5.8 mg in control, 3.9 mg in 100mM NaCl and 4.3 mg in 200mM NaCl) compared to in the absence of sucrose in media (3.0 mg in control, 3.5 mg in 100mM NaCl and 1.2 mg in 200mM NaCl). Salinity stress induced by different NaCl concentrations conveyed various activities of enzymes activities at the end of the treatment in week five. The salinity stress contributes to high activities of amylase in both sucrose-contained (1.5, 0.7, 2.8 µg /mg protein /g sample in 0mM, 100mM and 200mM respectively) and sucrose-free media (0.9, 2.6, 3.0 µg /mg protein /g sample in 0mM, 100mM and 200mM respectively). The same trend was displayed by G6PDH in both sucrose-contained (139.6, 18.7, 25.0 U /mg protein /g sample in 0mM, 100mM and 200mM, respectively) and sucrose-free media (27.1, 22.9, 43.7 U /mg protein /g sample in 0mM, 100mM and 200mM, respectively). On the other hand, in both types of media, the activities decreased as salinity increased, including invertase (49.6, 16.0, 15.6 µg /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-contained media; 13.2, 12.7, 10.1 µg /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-free media), glucosidase (15834.5, 2168.9, 6778.8 U /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-contained; 11410.2, 13894.1, 1448.9 U /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-free), galactosidase (18551.3, 37387.2, 4915.9 U /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-contained; 19948.5,

13247.2, 13248.2 U /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-free), hexokinase (51.6, 249.3, 87.2 µg /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-contained media; 204.1, 105.7, 78.6 µg /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-free media) and PEPCase (0.003, 0.012, 0.002 U /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-contained; 0.006, 0.010, 0.004 U /mg protein /g sample in 0mM, 100mM and 200mM in sucrose-free). Results also showed that the presence of sugar is favoured by the explants to regulate the salinity stress via carbohydrates accumulation and its enzyme activities including glucosidase, hexokinase and PEPCase. Protein content accumulates higher in the absence of exogenous sucrose and presence of mild salinity stress (25.9 mg /g sample). For explants in the sucrose-contained media, strong correlations occurred between amylase activity and starch accumulation (0.693), invertase activity and biomass accumulation (0.921), PEPCase and hexokinase activity (0.961), as well as between G6PDH activity with invertase (0.998). For explants in sucrose-free medium, there were strong correlations between biomass accumulation and invertase (0.819) and glucosidase (0.629). Starch also displayed strong correlations between several metabolites, invertase (0.948), glucosidase (0.999), PEPCase (0.878) and reducing sugar content (0.933). The results obtained in this study could provide useful information on how carbohydrate metabolism plays roles in the regulation of salinity stress in an aquatic plant.

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KESAN TEKANAN KEMASINAN KE ATAS METABOLISMA KARBOHIDRAT KULTUR *CRYPTOCORYNE ELLIPTICA*

AZMI BIN ISMUN

Disember 2013

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Pusat Pengajian : Sains Asas

Keadaan persekitaran bergaram mampu mengganggu kandungan air di dalam sistem tumbuhan yang boleh mengakibatkan gangguan tumbesaran dan juga menyebabkan kematian. Untuk kelangsungan hidup di dalam keadaan ini, tumbuhan mengawalatur tekanan tersebut dengan mengubah kandungan gula pada tahap metabolismanya. Kajian ini dilakukan untuk menentukan kesan kandungan natrium klorida pada kepekatan berbeza - 100 mM dan 200 mM/L - ke atas metabolisme karbohidrat di dalam kultur tumbuhan akuatik, *Cryptocoryne elliptica* sebagai tumbuhan contoh, selama 35 hari. Keadaan habitat akuatik merupakan persekitaran yang mencabar dengan keadaan karbon yang rendah, nutrient yang tidak menentu dan keadaan teduh menggalakkan penyesuaian tumbuhan menggunakan potensi sepenuhnya terhadap persekitaran. Kajian yang terhad ke atas tumbuhan akuatik juga menggalakkan kajian ini dilakukan. Rawatan kemasinan ini dilakukan di dalam dua keadaan medium: dengan kehadiran sukrosa dan tanpa kehadiran sukrosa. Dalam kajian ini, biomas, kandungan kanji dan gula penurun telah diukur. Perubahan dalam metabolisma karbohidrat juga turut ditentukan melalui aktiviti enzim amilase, invertase, glukosidase, galaktosidase, heksokinase, fosfoenolpiruvate karboksilase

(PEPCase) dan glukose-6-fosfate dehidrogenase (G6PDH). Kesan tekanan kemasinan ke atas protein juga turut diukur. Hasil kajian menunjukkan kemasinan yang meningkat merendahkan biomas *C. elliptica* di dalam kedua-dua media sehingga 30%. Di dalam media dengan kehadiran sukrosa, peningkatan kandungan kanji berlaku di minggu 4 dan 5 dalam 200 mM NaCl, tetapi di dalam media tanpa sukrosa, peningkatan yang tinggi berlaku di minggu 4 dan 5 dalam 100 mM NaCl. Kandungan gula penurun lebih tinggi di dalam eksplan yang dikultur dengan kehadiran sukrosa di hujung rawatan dalam miggu kelima (5.834 mg dalam kawalan, 3.903 mg dalam 100mM garam and 4.2931 mg dalam 200mM garam) berbanding media tanpa sukrosa (3.049 mg dalam kawalan, 3.515 mg dalam 100mM garam dan 1.243 mg dalam 200mM garam). Tekanan kemasinan yang pelbagai menyebabkan pelbagai corak aktiviti enzim pada akhir rawatan dalam minggu kelima. Ia menyebabkan peningkatan aktiviti amilase di dalam media bersukrosa (1.6, 0.7, 2.8167 µg /mg protein /g sampel dalam 0mM, 100mM dan 200mM) dan media tanpa sukrosa (0.9529, 2.5, 3.0 µg /mg protein /g sampel dalam 0mM, 100mM dan 200mM). Corak yang sama ditunjukkan oleh G6PDH di dalam media bersukrosa (139.608, 18.753, 25.0 U /mg protein /g sampel dalam 0mM, 100mM dan 200mM) dan media tanpa sukrosa (27.088, 22.921, 43.7 U /mg protein /g sampel dalam 0mM, 100mM dan 200mM). Manakala di dalam kedua-dua jenis media, kebanyakan aktiviti enzim menurun selari dengan penigkatan kemasinan, termasuk invertase (49.7, 16.0, 15.5 µg /mg protein /g sampel dalam 0mM, 100mM dan 200mM media bersukrosa; 13.168, 12.675, 10.123 µg /mg protein /g sample dalam 0mM, 100mM dan 200mM media tanpa sukrosa), glucosidase (15834.5, 2168.98, 6778.85 U /mg protein /g sampel dalam 0mM, 100 mM and 200mM media bersukrosa; 11410.21, 13894.06, 1448.92 U /mg protein /g sampel dalam 0mM, 100 mM dan 200mM

media tanpa sukrosa), galactosidase (18551.29, 37387.19, 4915.96 U /mg protein /g sampel dalam 0mM, 100 mM dan 200mM media bersukrosa; 19948.46, 13247.22, 13248.25 U /mg protein /g sample dalam 0mM, 100 mM dan 200mM media tanpa sukrosa), heksokinase (51.687, 249.382, 87.242 µg /mg protein /g sampel dalam 0mM, 100mM dan 200mM media bersukrosa; 204.115, 105.761, 78.600 µg /mg protein /g sampel dalam 0mM, 100 mM dan 200mM media tanpa sukrosa) dan PEPCase (0.00376, 0.01290, 0.00241 U /mg protein /g sampel dalam 0mM, 100 mM dan 200mM media bersukrosa; 0.006, 0.01048, 0.00430 U /mg protein /g sampel dalam 0mM, 100mM dan 200mM media tanpa sukrosa). Hasil kajian juga mendapati kehadiran sukrosa membantu eksplan dalam mengawalatur tekanan kemasinan melalui peningkatan dalam pengumpulan karbohidrat dan aktiviti-aktiviti enzimnya termasuk glukosidase, heksokinase dan PEPCase. Kandungan protein didapati tinggi dengan kehadiran sukrosa dan tekanan kemasinan yang sederhana (25.9 mg /g sampel) Untuk eksplan yang dikultur di dalam media dengan kehadiran sukrosa, kolerasi yang kuat telah diperoleh di antara aktiviti amilase dan penghasilan kanji (0.693), aktiviti invertase dan biomas (0.921), aktiviti PEPCase dan heksokinase (0.961), juga di antara G6PDH dengan beberapa metabolit termasuk invertase (0.819). Untuk eksplan yang dikultur di dalam media tanpa sukrosa, terdapat kolerasi yang kuat di antara penghasilan biomas dengan beberapa metabolit termasuk invertase (0.819) dan glukosidase (0.629). Penghasilan kanji juga menunjukkan kolerasi yang kuat dengan beberapa metabolik, invertase (0.940), glukosidase (0.999), PEPCase (0.878) dan kandungan gula penurun (0.933). Hasil kajian yang telah diperolehi mampu menyumbang maklumat yang berfaedah dalam memahami peranan metabolisma karbohidrat ketika mengawalatur tekanan kemasinan dalam tumbuhan akuatik.