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Chloroplast protein expression on two dimension electronic analysis (2DE) gels from ice plant under normal and drought stressed conditions

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[Purpose] The common or crystalline ice plant (*Mesembryanthemum crystallinum* L.) is an excellent model for investigating the functional genomics of CAM (Crassulacean acid metabolism). This species displays C3 photosynthesis when grown under non-stressed conditions and is capable of completing its entire life cycle in the C3 model of photosynthesis without ever exhibiting net nocturnal CO_2 uptake. However, under salinity or drought stress treatment conditions, plants exhibit all of the physiological features of CAM plants. This ability is referred to as "facultative CAM". Although the induction process has been well-studied, very little attention has been given to the role of the chloroplast during the drought-stress-induced transition to CAM. In this research, we isolated pure, intact chloroplast from well-watered (control) and drought-stressed (treatment) leaves of the ice plant at 6 am and 6 pm. The purified chloroplast was used for detecting the differential expression of the total chloroplast protein on two-dimension electronic analysis (2DE) gels from ice plants under normal and drought-stressed conditions.

[Materials and Methods] The washed chloroplasts were purified with Percoll gradients prepared using a Master flex R pump. The chloroplast proteins were extracted in a lysis buffer containing a protease inhibitor mix supplemented with 1 μM Leupeptin and 1 μM E64, followed by precipitation with ice-cold acetone. The chloroplast protein contents were determined by an EZQ protein quantitation kit. The proteins were extracted with Tris-saturated phenol and separated using two- dimensional gel electrophoresis (2DGE) as our previously published methods (Hong *et al.*, 2019a, b).

[Results] The results show that chloroplast an isolated from the ice plant leaves via this protocol have pure and intact. The shape of chloroplast observed by microscopy were clear and sharp. The chloroplast protein contents were highest in drought stress sample collect at 6am (4,803 mg/ml) and control sample collect at 6am (3,216 mg/ml). The typhoon scan images showed different protein expression among the different samples on 2DE gels in which proteins were well resolved for the most part from the IEF strips into the polyacrylamide gels. Image Quantity One 4.6.3 software was used to detect protein spots in gels and the result revealed diferent major protein spots from 2DE gels of the chloroplast control samples (non-drought stress treatment) and drought stress treatment collected at 6 am and 6 pm, respectively. Based on this result, it is possible to suggest that most of the chloroplast proteins extracted from this protocol were well transferred from strips to the 2DE gels, and the overall quality of proteins was superior for further proteome analysis.