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Short communication

Drought effect on electrophoretic protein pattern of *Anoectochilus formosanus*

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Abstract

Drought effect on two dimensional iso-electric focusing/sodium dodecyl sulfate-poly acrylamide gel electrophoretic (2-D IEF/SDS-PAGE) protein pattern was studied in *Anoectochilus formosanus* leaves under ex vitro condition. Spectrophotometery analysis revealed that under drought condition protein content decreased significantly. Similarly, 2-D IEF/SDS-PAGE and PDQuest software analysis indicated that out of 17 protein spots, expression of 15 protein spots were quantitatively down-regulated and 2 spots were up-regulated. Moreover, 11 protein spots were qualitatively up-regulated while 6 were down-regulated.

Keywords: *Anoectochilus formosanus*; Drought; 2-D IEF/SDS-PAGE

1. Introduction

Anoectochilus formosanus Hayata is a native perennial and terrestrial orchid plant, which is grown in the forests of Taiwan (China) and Okinawa (Japan) for their beauty because its leaves have network of colorful venation (Jewel orchid). *A. formosanus* belongs to Orchidaceae and is said to have great value in herbal medicines. In natural conditions crops are often exposed to various environmental stresses. Drought stress can be imposed by various environmental conditions such as high salinity, dehydration and freezing. To survive and develop normally, plant adapts to stress with various strategies (Sugiharto et al., 2002). From a biochemical aspect, stressed plants accumulate compatible solutes such as proline, sugar, alcohol and betaines (Rhodes and Hanson, 1993; Ingram and Bartels, 1996). These compounds help the plant to adapt biochemically to the adverse circumstances. It has been also reported that a verity of genes including LEA (late embryogenesis abundant) families, sugar metabolism and proline biosynthesis were induced by water deficit in various plant species. The proteins they encode were predicted to play an important role in the adaptive response to stresses (Ingram and Bartels, 1996; Bray, 1997; Seki et al., 2001).

Recently, proteomics has become an essential methodology for large-scale analysis of protein in the several aspects of plant biology (Pandey and Mann, 2000). Proteome analysis using differential display with two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2-D SDS-PAGE) has several advantage over other approaches commonly used for similar studies such as efficient separation of complexes protein mixture, analysis of isoforms, secondary modifications of the protein such as glycosylation and phosphorylation, alterations due to environmental perturbations, changes in molecular complexes such as protein–ligand, protein–protein interaction and proteolysis using low amount of proteins (Pandey and Mann, 2000; Shen et al., 2003). Under drought stress expression pattern of various proteins changed. Therefore, the aim of the present research was to study the changes in

Abbreviations: 2-D IEF/SDS-PAGE, two dimensional iso-electric focusing/sodium dodecyl sulfate-poly acrylamide gel electrophoresis; CHAPS, 3-[(3-cholamidopropyl)dimethylammoniol]-1-propane-sulfonate; DTT, dithiothreitol; MCE, 2-mercapto ethanol; PPF, photosynthetic photon flux; PVP, Ppolyvinylpyrrolydon; TCA, trichloroacetic acid; TEMED, *N*,*N*,*N*′,*N*′-tetramethylenediamine; TDZ, thidiazuron

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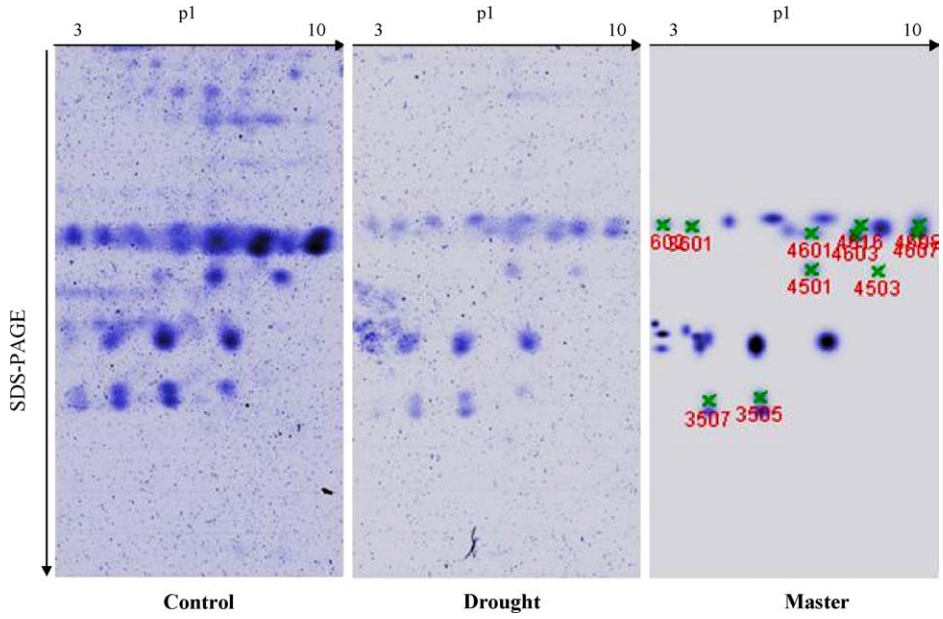

Fig. 1. Two-dimensional IEF-SDS/polyacrylamide gel electrophoretic protein pattern in *Anoectochilus formosanus* leaves that were grown ex vitro for one month under control (SMC = 76.9 ± 0.7%) and drought (SMC = 18.4 ± 1.1%) condition. Protein (500 μg) was loaded onto iso-electric focusing (IEF) dimension on immobilized pH gradient strips (ReadyStrip™ IPG Strips 7 cm, pH 3–10 NL, Catalog 163–2002, Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547), and then subjected to SDS-PAGE. The gels were stained with coomassaie brilliant blue and scanned with *UMAX powerLook 2100XL* scanner (Taiwan). Analysis of protein spot was done with the PDQuest software.

proteins under drought stress in *Anoectochilus formosanu*s leaves with the use of two-dimensional gel electrophoresis.

2. Materials and methods

2.1. Plant growth and stress treatments

Plantlets of *Anoectochilus formosanus* Hayata were propagated according to the method of Ket et al. (2004). Briefly, in vitro cultured shoots were maintained by subculturing at 3-months interval to shoot multiplication medium consisted of modified hyponex ($2\ g\ l^{-1}$ 20 N:20 P:20 K + $2\ g\ l^{-1}$ peptone) supplemented with $2\ mg\ l^{-1}$ TDZ, 3% sucrose, and 0.7% agar. After 3 months larger shoots were sub-cultured in the rooting media ($1\ g\ l^{-1}$ 20 N:20 P:20 K + $1\ g\ l^{-1}$ 6.5 N:4.5 P: 19 K + $2\ g\ l^{-1}$ peptone) supplemented with 3% sucrose, 0.5 g activated charcoal and 0.7% agar for 3 months. Six-month-old in vitro cultured plantlets (5.4 g FW and 3.9 leaves on average) were trans-planted in conical plastic pots (12 × 9 × 15 cm, Cheong Wun-5, South Korea) filled with coconut chips. These plantlets were transferred into glass house for 30 d of acclimatization under the 80 $\mu mol\ m^{-2}\ s^{-1}$ *PPF*, 60–70% relative humidity and 25 ± 2 °C temperature. For drought experiment, plants were kept at gradually imposed drought stress by withholding water supply for 30 d (soil moisture content of coconut chip, SMC = 18.4 ± 1.1%). The soil moisture content of control plants were kept at field capacity (SMC = 76.9 ± 0.7%) by irrigation at regular intervals.

2.2. Extraction of enzymatic protein and assay

Extraction of protein was done according to methods of Borland et al. (1998) and Pandey et al. (2000) with some modifications. Exactly 1.0 g control as well as droughted leaves (1st–3rd) were frozen and homogenized into the liquid nitrogen and dissolved in 4 ml extraction buffer [100 ml of 50 mM Tris–HCl (pH 7.5) containing polyvinylpyrrolydon (PVP, 16 mg), 1 mM dithiothreitol, 2 mM EDTA, 5 mM MCE, 2% PEG 20,000, 20 mM $MgCl_2.6H_2O$, 5 mM $NaHCO_3$] at 4 °C. Homogenate was centrifuged with a *Hanil Science Industrial Micro 17R* Centrifuge (Republic of Korea) at 14,000 × *g* and 4 °C for 20 min. Protein concentration was estimated by the method of Bradford (1976) using protein assay dye from BIO-RAD.

2.3. 2-D IEF/SDS-PAGE

Two-dimensional IEF/SDS-PAGE was done according to the methods of Finnie et al. (2002), Sugiharto et al. (2002) and Metodiev et al. (2002) with some modifications. Required protein extract was mixed with equal volume of 10% TCA (trichloroacetic acid) and incubated for 30 min on ice and centrifuged for 20 min at 14,000 × *g* and 4 °C. Supernatant was carefully removed and 0.3 ml of cold acetone was added and kept in ice for 30 min, centrifuged for 10 min at 14,000 × *g* and 4 °C. Supernatant was removed and pellet was used for iso-electric focusing (IEF). IEF of approximately 500 μg of protein in