Metabolome analysis

Hypocotyl and root (100 mg) were ground in liquid nitrogen and added to 50% ice-cold methanol containing internal standards. After centrifugation at 1, 5000 × g for 5 min, the supernatant was filtered through a 5-kDa cutoff filter (Millipore, Bedford, MA, USA). The metabolites were separated and detected by a CE/MS system (Agilent Technologies, Waldbronn, Germany). For the determination of anionic metabolites, a polyethylene glycol coated capillary (DB-WAX, J & W Scientific, Folsom, CA, USA) was used with 20 mM ammonium acetate (pH 6.8) as running buffer. Cationic metabolites were separated in an uncoated fused-silica capillary using 1 M formic acid (pH 1.9) as running buffer. Anonic and cationic metabolites were measured respectively in negative and positive mode. Accuracy was determined by measurement of a known concentration of selected metabolites [1].

[1] Takahashi, H., Hayashi, M., Goto, F., Sato, S. *et al.*, Evaluation of metabolic alteration in transgenic rice overexpressing dihydroflavonol-4-reductase. *Ann. Bot.* 2006, *98*, 819-825.