Supplemental Data. Borisjuk et al. (2013). Plant Cell

10.1105/tpc.113.111740

Supplemental Dataset 1

Appendix Proteome analysis

Phenolic protein extraction

For extraction of total proteins, embryos were ground in liquid nitrogen and proteins from 0.01g embryo material were extracted according to Hurkmann and Tanaka 1986.

Gel electrophoresis procedure

Total seed protein from the phenol extraction was resuspended in 350µl rehydration buffer (8M Urea, 2M Thiourea, 50mM DTT, 2% CHAPS (w/v), 5% IPG buffer 3-11n (v/v)l, 12µl/ml DeStreak reagent, bromphenol blue) and directly applied into a strip holder. Isoelectric focussing was carried out with the Ettan IPGPhor 3 (GE Healthcare, Munich, Germany) using 3 – 11nl Immobiline DryStrip Gels (18 cm). Rehydration took place at 30V for 12h and focusing during 4 steps at 500V (1h), 500-1000V (1h), 1000-8000V (3h) and 8000V (6h). Afterwards strips were equilibrated for 15 min in equilibration solution (6M Urea, 30% glycerol (87%, v/v), 2% SDS, 50mM Tric-HCl pH 8.8, bromphenol blue) with (i) 1% DTT (w/v) and (ii) 2.5% Iodacetamide (w/v), respectively. IPG strips were finally transferred horizontally onto a 16.5% tricine gel and electrophoresis was carried out for 20h at 35mA/mm gel layer (Mihr and Braun 2003). Gels were stained with coomassie colloidal (Neuhoff et al. 1985, Neuhoff et al. 1990)

Two-dimensional gel analyses

Triplicates of coomassie stained gels were scanned and analyzed using the Delta 2D Software (Decodon) for quantitation as previously described in Rode et al. 2011. Alterations in spot volume from a minimum of 1.5fold were considered to represent significant differences in protein abundance.

MS analysis

A number of 90 Spots were considered to be significantly changed between the two conditions and picked from either a gel with light grown embryos or dark grown embryos using the GelPal Protein Excision System (Genetics, Queensway, UK). Tryptic digestion of proteins and peptide extraction were carried out as outlined in Sunderhaus et al. 2010 with the following modifications: gel pieces were washed with ddH_2O to remove Coomassie and dehydrated with acetonitrile (this process was repeated after each step). Dehydrated gel pieces were incubated in 0.1M NH_4CO_3 for 15min and digestion was carried out at 37 °C overnight using trypsin (2 µg/ml Resuspension buffer [Promega, Mannheim, Germany] in 0.1 M NH_4HCO_3). Digestion was stopped the next day and peptides were extracted by addition of 5% formic acid in 50% acetonitrile and an incubation for 15 min at 37 °C. Gel pieces were further incubated two times with 1% formic acid in 50% acetonitrile for 15 min at 37 °C. Finally, gel pieces were washed with 100% acetonitrile. All supernantants were combined and dried via vacuum centrifugation. Dried peptides were resolved in 2% acetonitrile and 0.1% formic acid. MS analyses were performed using an EASY-nLC-system (Proxeon) coupled to a MicrOTOF-Q-II mass spectrometer (Bruker Daltonics). Identification of proteins was carried out using the MASCOT search algorithm against (i) UniProtKB (www.expasy.org) (ii) NCBI nonredundant protein database (www.ncbi.nlm.nih.gov/protein) and (iii) The Arabidopsis Information Resource (Tair release 9, www.arabidopsis.org).

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