Cytokinins and the CRE1 receptor influence endogenous gibberellin levels in Medicago truncatula

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Plant growth and treatments

To generate samples for real-time RT-PCR (RT-qPCR) expression analyses, 50 germinated seedlings were placed on a grid in a Magenta box in a low-nitrogen liquid medium and grown 4 days in a shaking incubator (125 rpm) at 24°C under long-day conditions (16 h light, 150 µE / 8 h dark). For CK treatment, seedlings were treated for 3h with 10^{-7}M BAP (Sigma). Roots were cut at the base of the hypocotyl and immediately frozen in liquid nitrogen.

Gene expression analysis

RT-qPCR experiments were performed as described in Fonouni-Farde et al. (2016a).

GA extraction and quantification

GA was extracted from shoots (comprising stems, leaves and apical buds) and roots (cut at the base of the hypocotyl) as detailed by Boden et al. (2014). Briefly, tissue was homogenised, covered in 80% MeOH overnight, internal standards ($[^2H_2]GA_1$ and $[^2H_2]GA_{20}$) were added and solids were removed by centrifugation. Samples were extracted by passing though C18 Sep-Pak (Waters Pty Ltd, http://www.waters.com) followed by SAX (Maxi-Clean™; Grace Davison Discovery Sciences, USA) columns. Samples were then derivatised one of two ways. In the first method, samples were derivatised as described by Boden et al. (2014) by adding di-isopropyl ethylamine and 2-bromoethyl triethylammonium bromide, heating at 80ºC for 2 h,. In the second method, samples were derivatised as described by Li et al. (2016) except that samples were incubated in 15µl overnight at 40ºC and then diluted with 15ul MQW. Following derivatisation, samples were dried and re-suspended in 1% acetic acid and analyzed using a Waters Acquity H-Class UPLC coupled to a Waters Xevo triple quadrupole mass spectrometer (MS) equipped with a Waters Acquity UPLC BEH C_{18} column. For the first derivatisation method, the UPLC program was initially 100% A (1.0% (v/v) acetic acid in water), held for 0.5 min followed by a linear gradient to 85% A: 15% B (acetonitrile) at 2.0 min and 53% A at 6 min. The flow rate was 0.35 mL min^{-1}, the column was held at 35°C, and the sample compartment was at 6°C. The MS was operated in positive ion electrospray mode with a needle voltage of 2.6 kV, and Multiple Reaction Monitoring (MRM) was used to detect all analytes with a dwell time of 66 msec. Three MRM transitions were monitored for each analyte. The ion source temperature was 130°C, the desolvation gas was N_2 at 950 L h^{-1}, the cone gas flow was 100 L h^{-1}, and the desolvation temperature was 450°C. For the second derivatisation method, the instrument was operated

and ions monitored as described by Li et al. (2016). The data were processed using MassLynx software (Waters) and peak areas for endogenous and labelled GAs were compared and combined with fresh weight (FW) of sample to calculate ng/g FW.