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Highlighting type A RRs as potential regulators of the dkHK1 multi-step phosphorelay pathway in *Populus*

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Highlights

- Isolation of ten type A RRs from poplar clone 'Dorskamp'
- Nucleocytoplasmic and nuclear localization for eight and two of them respectively
- Eight type A dkRRs could interfere the dkHK1a-b/dkHPts/dkRRB MSP

Abstract

In previous studies, we highlighted a multistep phosphorelay (MSP) system in poplars composed of two hybrid-type Histidine aspartate Kinases, dkHK1a and dkHK1b, which interact with three Histidine Phosphotransfer proteins, dkHPt2, 7, and 9, which in turn interact with six type B Response Regulators. These interactions correspond to the dkHK1a-b/dkHPts/dkRRBs MSP. This MSP is putatively involved in an osmosensing pathway, as dkHK1a-b are orthologous to the Arabidopsis osmosensor AHK1, and able to complement a mutant yeast deleted for its osmosensors. Since type A RRs have been characterized as negative regulators in cytokinin MSP signaling due to their interaction with HPt proteins, we decided in this study to characterize poplar type A RRs and their implication in the MSP. For a global view of this MSP, we isolated 10 poplar type A RR cDNAs, and determined their subcellular localization to check the *in silico* prediction experimentally. For most of them, the *in planta* subcellular localization was as predicted, except for three RRAs, for which this experimental approach gave a more precise localization. Interaction studies using yeast two-hybrid and *in planta* BiFC assays, together with transcript expression analysis in poplar organs led to eight dkRRAs being singled out as partners which could interfere the dkHK1a-b/dkHPts/dkRRBs MSP identified in previous studies. Consequently, the results obtained in this study now provide an exhaustive view of dkHK1a-b partners belonging to a poplar MSP.