



Mining expressed sequence tags of rapeseed (*Brassica napus* L.) to predict the drought responsive regulatory network

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Abstract It is of great significance to understand the regulatory mechanisms by which plants deal with drought stress. Two EST libraries derived from rapeseed (*Brassica napus*) leaves in non-stressed and drought stress conditions were analyzed in order to obtain the transcriptomic landscape of drought-exposed *B. napus* plants, and also to identify and characterize significant drought responsive regulatory genes and microRNAs. The functional ontology analysis revealed a substantial shift in the *B. napus* transcriptome to govern cellular drought responsiveness via different stress-activated mechanisms. The activity of transcription factor and protein kinase modules generally increased in response to drought stress. The 26 regulatory genes consisting of 17 transcription factor genes, eight protein kinase genes and one protein phosphatase gene were identified showing significant alterations in their expressions in response to drought stress. We also found the six microRNAs which were differentially expressed during drought stress supporting the involvement of a post-transcriptional level of regulation for *B. napus* drought response. The drought responsive regulatory network shed light on the significance of some regulatory components involved in biosynthesis and signaling of various plant hormones (abscisic acid, auxin and brassinosteroids), ubiquitin proteasome system, and signaling through Reactive Oxygen Species (ROS). Our findings suggested a complex and multi-level

regulatory system modulating response to drought stress in *B. napus*.

Keywords *Brassica napus* · EST analysis · Drought stress · Transcription factors · Protein kinases · MicroRNA

Introduction

Growth and development of crop plants are severely affected by several abiotic stresses (Seki et al. 2003; Shinozaki and Yamaguchi-Shinozaki 1996). Among the abiotic stresses, drought has a crucial devastating impact on agricultural production (Farooq et al. 2009; Farooq et al. 2012). Plants recruit complex overlapping mechanisms to reprogram their gene expression to cope with drought stress (Ahuja et al. 2010; Golldack et al. 2011). The orchestrated regulatory networks at transcriptional, post transcriptional and post translational levels are involved in forming the appropriate transcriptome and proteome of stress-exposed plants to show an adaptive response (Duque et al. 2013; Mazzucotelli et al. 2008). Understanding the role of regulatory components will be of great benefit for breeding drought tolerant crops.

Various regulatory proteins including transcription factors, protein kinases and protein phosphatases have been identified to function as the core regulatory elements of stress responses (Ashraf 2010; Hadiarto and Tran 2011; Seki et al. 2007; Shinozaki and Yamaguchi-Shinozaki 1996; Umezawa et al. 2006; Xoconostle-Cazares et al. 2010; Yamaguchi-Shinozaki and Shinozaki 2006). Also, it has been revealed that the expression of some plant microRNAs (miRNAs), as negative regulator of gene expression, alters during stress conditions such as drought, salinity and cold (Khraiwesh et al. 2012; Lu and Huang 2008; Sunkar et al. 2007; Sunkar et al. 2012). Notably, most of these miRNAs target genes encoding

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