

Combined Transcriptome and Proteome Analysis Identifies Pathways and Markers Associated with the Establishment of *Brassica napus* Microspore-derived Embryo development

Ronny Joosen , Jan Cordewener , Ence Darmo Jaya Supena , Oscar Vorst , Michiel Lammers , Chris Maliepaard , Tieme Zeilmaker , Brian Miki , Twan America , Jan Custers , and Kim Boutilier *

Business Units Bioscience; Biometry; Plant Research International, P.O. Box 16, 6700 AA Wageningen, the Netherlands; Research Center for Biotechnology, **Bogor Agricultural University** (IPB), P.O. Box 1, **Bogor** 16610, Indonesia; Eastern Cereal **and** Oilseeds Research Centre, Agriculture **and** Agri-Food Canada, 960 Carling Avenue, Ottawa, Canada K1A 0C6

* Corresponding author; email: kim.boutilier@wur.nl

Microspore-derived embryo (MDE) cultures are used as a model system to study plant cell totipotency **and** as an *in vitro* system to study embryo development. We characterized **and** compared the transcriptome **and** proteome of *Brassica napus* MDEs from the few-celled stage to the globular-heart stage using two MDE culture systems: conventional cultures in which MDEs initially develop as unorganized clusters that usually lack a suspensor, **and** a novel suspensor-bearing embryo culture system in which the embryo proper originates from the distal cell of a suspensor-like structure **and** undergoes the same ordered cell divisions as the zygotic embryo. The improved histodifferentiation of the suspensor-bearing MDEs suggests a new role for the suspensor in driving embryo cell identity **and** patterning. An MDE culture cDNA array **and** 2-D gel electrophoresis **and** protein sequencing were used to compile global **and** specific expression profiles for the two types of MDE cultures. Analysis of the identities of 220 candidate embryo markers, as well as the identities of 32 sequenced embryo-upregulated protein spots, indicate general roles for protein synthesis, glycolysis **and** ascorbate metabolism in the establishment of MDE development. A collection of 135 robust markers for the transition to MDE development was identified, a number of which may be coregulated at the gene **and** protein expression level. Comparison of the expression profiles of preglobular stage conventional **and** suspensor-bearing MDEs identified genes whose differential expression may reflect the improved histodifferentiation of suspensor-bearing embryos. This collection of early embryo-expressed genes **and** proteins serves as a starting point for future marker development **and** gene function studies aimed at understanding the molecular regulation of cell totipotency **and** early embryo development in plants.