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Physiological and metabolome changes during anther development in wheat (*Triticum aestivum* L.)

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Running head: Dynamic metabolomics of wheat anther development.

Abstract: This study used cytology, cytochemistry, and non-targeted metabolomics to investigate the distribution characteristic of polysaccharides, lipids, and all the metabolites present during five wheat (*Triticum aestivum* L.) anther developmental stages to provide insights into wheat anther development. Anthers were collected from the tetrad through trinucleate stages, and 1.5% (w/v) acetocarmine and 4′,6-diamidino-2-phenylindole staining were used to confirm the developmental stage and visualize the nuclei, respectively. Polysaccharides and lipids were detected by staining with periodic acid-Schiff and Sudan Black B, respectively. The integrated optical density of the tapetum and microspores were calculated using IPP6.0 software. Furthermore, the metabolites were identified by gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer (GC-TOF-MS). The results indicated that the interior and exterior surface cells of anthers are orderly. Pollen was rich in numerous nutrient substances (e.g., lipids, insoluble carbohydrates, and others), and formed a normal sperm cell that contained three nuclei, i.e., one