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Comparative quantitative proteomic analysis of embryogenic and non-embryogenic calli in maize suggests the role of oxylipins in plant totipotency☆

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ABSTRACT

Totipotency, the ability of somatic plant cell to generate whole plant through somatic embryogenesis, is still not well understood. In this study, maize immature zygotic embryos were used to generate embryogenic (EC) and non-embryogenic (NEC) calli. In order to compare proteomes of EC and NEC, two-dimensional electrophoresis (2-DE) in combination with mass spectrometry was used. This approach resulted into 361 quantified 2-DE spots out of which 44 were found statistically significantly differentially abundant between EC and NEC. Mass spectrometry provided the identity for 23 proteins that were classified into 8 metabolic categories. The most abundant were proteins associated with energy followed by proteins associated with disease and defense. Based on the abundances of identified proteins in this and other studies, working model for plant totipotency was proposed. One aspect of this working model suggests that increased abundances of proteins associated with pyruvate biosynthesis and suppression of embryogenic genes might be responsible for differences between EC and NEC cells. Furthermore we speculate that the increased abundance of lipoxygenase in the NEC cells results in changes in the equilibrium levels of one or more signaling molecules and is at least partly responsible for somatic cell reprogramming during totipotency.

Biological significance
Totipotency, the ability of somatic plant cell to generate whole plant through somatic embryogenesis, is still not well understood. In order to further advance understanding of this biological phenomenon, proteomes of embryogenic and non-embryogenic callus, derived from immature zygotic embryos of inbred maize line A19, were compared using 2-DE based proteomic technology. Based on the abundances of identified proteins in this and other studies, working model for plant totipotency was proposed. One aspect of this working model suggests that increased abundances of proteins associated with pyruvate biosynthesis and suppression of embryogenic genes might be responsible for differences between EC and NEC

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1. Introduction

The development of higher plants normally starts with fusion of pollen cell with egg cell to form a zygote. Totipotency is a biological phenomenon where somatic cell generates complete plant without fusion of gametes, through the process called somatic embryogenesis (SE) [1,2]. During SE, new plant develops *de novo* directly from the tissue cells that was originally demonstrated on *Daucus carota* [3,4]. Embryo development through SE follows similar morphological patterns (globular, heart, torpedo and mature) as through zygotic embryogenesis [5,6]. The development of somatic embryo occurs i) directly from the tissue cells of explants and ii) indirectly through the development of unorganized callus [7,8].

In recent years, proteomics was successfully used to understand process of SE in complex manner. In model legume, *Medicago truncatula*, differentially abundant proteins were detected between embryogenic/nonembryogenic lines [9] and lines that differ in SE potential [10]. Embryogenic line showed a higher adaptability toward the stress imposed by the culture conditions [9] and more than 60% of differentially abundant proteins showed different patterns of gene expression between lines that differ in SE potential [10]. Additionally, the reference maps with 169 proteins were established for embryogenic cell lines delivered from protoplasts [11].

Proteomic technology was used to investigate SE in economically important ornamental crop *Cyclamen persicum*. Four glycolytic enzymes, namely UDP-glucose pyrophosphorylase, fructose bisphosphate aldolase, triosephosphate isomerase, and glyceraldehyde-3-phosphate dehydrogenase were found more abundant in somatic embryos of *C. persicum* [12]. It was also suggested that auxin release might be important for late developmental stages of somatic embryos [13]. Interestingly, based on identity of 247 differentially abundant proteins between somatic and zygotic embryos of *C. persicum*, it was proposed that "small enolases" might serve as storage compounds during the embryogenesis [14].

Additionally, the morphological disorders in somatic embryos of *Acca sellowiana* [15], differences between somatic embryo and proembryonic masses-type cultures in avocado [16], differences between proteomes of embryogenic and non-embryogenic calli *Vitis vinifera* [17], early somatic embryogenesis in *Picea glauca* [18], and early somatic and zygotic embryogenesis in *Theobroma cacao* L. [19] were investigated. In Valencia sweet orange, five developmental time points of somatic embryogenesis were characterized [20].

All of these studies indicate that there is increasing interest to understand plant totipotency. The aim of the present work was to further advance these studies and propose working proteomics model that will advance our understanding of this biological phenomenon.

2. Material and methods

2.1. Plant material

Plants of inbred maize (*Zea mays* L.) line A19 were grown in the field located in Nitra, Slovakia. Maize ears were harvested at 16 and 20 days after pollination (DAF) and surface sterilized for 5 min in 70% ethanol and 20 min in 40% sodium hypochlorite solution (with a few drops of Tween-20). After the rinse in sterile distilled water (three times), immature embryos were aseptically isolated by cutting the tips of the kernels with a scalpel without touching the embryo.

2.2. Induction of callus from immature embryos and cytological examinations

Callus initiation medium N6 [21] supplemented with N6 salts, 2% sucrose, 25 mmol/L proline, 1 mg/L 2,4-D, 100 mg/L casein hydrolysate, and 1 mg/L N6 vitamins was used to induce embryogenic (EC) or non-embryogenic (NEC) calli. The pH of N6 medium was adjusted to 5.8, and 3 g/L Gelrite was added. Silver nitrate (10 mg/L) was added after N6 medium was autoclaved. The N6 medium was solidified and kept in the dark at 28 °C. Twenty immature embryos were transferred on one Petri dish of N6 media. The percentage of immature embryos forming primary callus was recorded two weeks after the culture. The developing callus was sub-cultured after 14 days into the callus maintenance N6 medium without silver nitrate. After four weeks, embryogenic calli were transferred to the maturation N6 medium (1 mg/L N6 vitamins, 6% saccharose, 1 mg/L 2,4 D, 1.4 g proline, 100 mg/L casein hydrolysate and 3 mg/L Gelrite) and kept at 28 °C in the dark.

Type of induced callus (embryogenic or non-embryogenic) was determined by microscopic observations. Calli were stained with safranin and fast green according to Jensen [22]. Additionally, embryogenic calli were stained with 2% acetocarmine and examined under a light microscope Axioplan 2 (Zeiss) with CCD camera SONY DXC-S500.

2.3. Protein extraction

Proteins were extracted in three independent experiments from embryogenic and non-embryogenic calli as described in Hajduch et al. [23]. Briefly, embryogenic and non-embryogenic calli were grounded in liquid nitrogen using mortar and pestle. Then 5 mL extraction buffer (0.1 M Tris–HCL pH 8.8; 10 mM EDTA; 0.9 M sucrose), 20 μL 2-mercaptoethanol, and 5 mL phenol were added. The mixture was centrifuged in 4000 ×*g* at 4 °C for 10 min. Proteins from the phenol phase were precipitated using five volumes of ice-cold 0.1 M ammonium acetate in 100% methanol and incubated overnight at −20 °C. The precipitate was collected by centrifugation for 20 min., 4000 ×*g* at 4 °C. Finally, the pellet was washed 2 times with 0.1 M