IDENTIFICATION OF LIPID RAFTS IN WHEAT CHLOROPLAST MEMBRANES

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Abstract. *Recently, there is increasing evidence that the biological membrane is heterogeneous and has a domain organization. Lipid rafts are a type of domain present in cell membranes. Raft structures enable many processes to occur in the cell, including signaling. Rafts are found in almost all plant cell membranes, but not all have shown their functions. In the present work, raft structures have been identified in wheat chloroplasts. This is proved by the presence of raft-forming lipids in the opalescent zone.*

Key words: *wheat, crop, chloroplast membrane, raft, photosynthesis.*

Wheat as a food crop is one of the main sources of energy for humans and animals. In world agriculture, cereal crops occupy the largest area in comparison with other crops. It is known that the importance of cereal crops is huge and diverse in food, fodder and agronomic terms [4]-[13].

Wheat productivity depends not only on agronomic conditions but also on the photosynthetic apparatus. Photosynthesis is the main process occurring in plants. Photosynthesis is the main physiological process that determines the level of crop yields, as it produces 90-95% of plant dry matter. Photosynthetic activity of wheat plants serves as a biological basis for yield formation [4], [13]. The efficiency of the photosynthesis process depends on the assimilation apparatus, the basis of which are chloroplasts, namely their membranes [4]. Membranology is one of the important areas of modern biology. Changes in the structure of membranes and their functional activity determine the direction of intracellular processes, including the operation of the genetic apparatus [1]. To date, there is increasing evidence in the literature that cell membranes are heterogeneous and have lipid-protein microdomains.

This area is actively developing and is of great interest because these structures are involved in important cellular processes (signaling, transport, apoptosis) [11]. To date, lipid rafts have been identified in many membranes of eukaryotes and prokaryotes. Currently, lipid rafts have been identified in chloroplast membranes of halophytes. However, their functions have not yet been shown. The aim of this work was to detect lipid rafts in the composition

of wheat chloroplast membranes. In the future it is planned to study the functions of the obtained microdomains.

Spring wheat variety Istiqlol-6 was used in this work. Plants were grown in a climatic chamber. Growing conditions: air temperature - 22°C, photoperiod -16/8 day/night. Illumination was 300 μmol/m-2s-1. Plants were grown until the first true leaf. Chloroplasts were then isolated using the differential centrifugation method. The purity of chloroplast fractions was controlled using an inverted biological microscope ("Axio observer Z1", Carl Zeiss, Germany). To obtain lipid rafts, chloroplast fractions were solubilized with 1% Triton X-100 for 30 min at 4 °C, plated on a sucrose gradient of 35-25-15-5% and centrifuged at 200,000 g for 2 h. Lipid extraction, identification, and analysis were performed according to the method [2].

After centrifugation, an opalescent zone was identified in the region of the 15% sucrose gradient, which contained the largest amount of rafts [10, 4]. Next, lipid analysis of the resulting zone was performed to identify raft-forming lipids such as sterols and cerebrosides (sphingolipids). It is known that high content of sterols is a distinctive feature of raft structures since sterols are necessary for "dense" packing of microdomains.

The analysis revealed that the opalescent zone contains more sterols than the membrane itself (Figure 1), which is a biochemical marker of the presence of lipid rafts in the opalescent zone. In the rafts of chloroplasts of halophytes, sterols were also predominant compared to chloroplast membranes [4].

Figure 1. Sterol content in chloroplast membranes and the resulting lipid rafts.

It was previously shown that rafts contain 25-96% of the total content of sphingolipids (cerebrosides) in the membrane. This is probably due to the

fact that sphingolipids have a high affinity for sterols, resulting in densely packed membrane regions (lipid rafts) [7]. In the rafts of chloroplasts of halophyte chloroplasts, cerebrosides accounted for about 20-30% of the sum of lipids [4].

In the lipid composition of the obtained opalescent zone cerebrosides were twice as much as in chloroplast membranes (Figure 2). These data indicate that the obtained opalescent band contains raft structures.

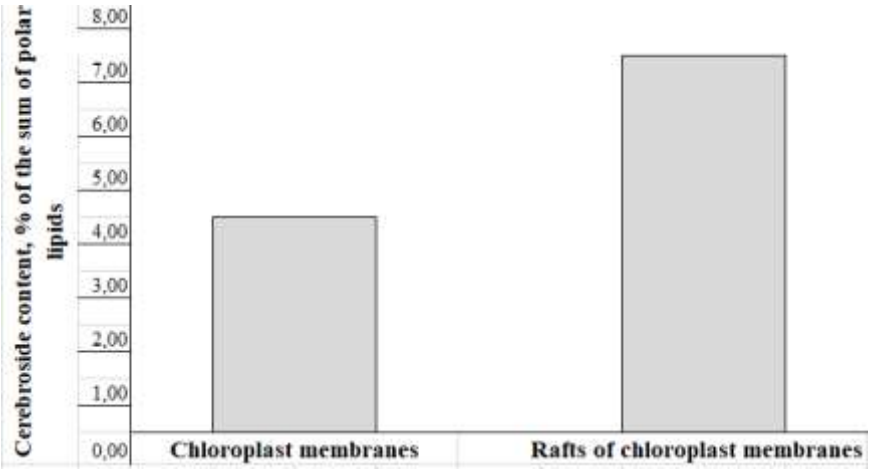

Figure 2. Cerebroside content in chloroplast membranes and in the resulting lipid rafts

According to the presented results, it can be concluded that wheat chloroplast membranes contain raft structures. This is proved by the presence of an opalescent band in a 15% sucrose gradient and the content of a large amount of raft-forming lipids (sterols, cerebrosides). In the future it is planned to determine the functions of the obtained microdomains.

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