Remodeling of the composition of the membrane's lipids of buckwheat plants (*Fagopyrum esculentum* Moench.) under conditions of phosphorous deficiency and seed bacterization with phosphate solubilizing microorganisms

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Abstract

This paper presents research results on the sensitivity of buckwheat (*Fagopyrum esculentum* Moench.) inoculated with phosphate solubilizing microorganisms (PSM) to phosphorus deficiency using the transformation of major photosynthetic tissues membrane lipids as the indicator. The analysis of glyco- and phospholipids performed has revealed the plants' ability to react to a deficit in phosphorus with the selective accumulation of sulfoquinovosyldiacylglycerol (SQDG) and digalactosyldiacylglycerol (DGDG) along with a decrease in phosphatidylglycerol (PG). Pre-sowing seed bacterization with PSM has balanced out the negative impact of a phosphorus deficiency on plants by stabilizing the PG content and reducing the difference in the PG/SQDG ratio.

Keywords: *Fagopyrum esculentum*, phosphatidylglycerol, phosphorus deficiency, phosphate solubilizing microorganisms, sulfoquinovosyldiacylglycerol

Introduction

Various regulatory mechanisms ensure the maintenance of optimal concentrations of phosphorus ions in plant cells. One of them is related to the modification of the membrane structures of all subcellular compartments and changes in the speed and direction of the metabolism of lipid components which make up their composition. In particular, the transformation of phospho- and glycolipids provides the functional activity of photosynthetic thylakoid membranes under various stresses, including phosphorus deficiency. The study of the transformations of the lipid complex of the

chloroplast thylakoid membranes is an integral indicator of the physiological state of the plants and the feasibility of their adaptive capacity.

Given the location of the main lipid classes among the membrane subcellular compartments, it is known that galactolipids – monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are predominant lipids in higher plant chloroplast thylakoid membranes and are essential as major structural components of the thylakoid membrane of lipids in chloroplasts. These lipids form a bilayer matrix for photosynthetic complexes and are associated with photosynthetic properties. Besides this, chloroplasts are the only organelles that have anionic sulfur containing lipid sulfoquinovosyldiacylglycerol (SQDG) in their structure. The phospholipid – phosphatidylglycerol (PG) is mainly present in thylakoid membranes in chloroplasts and is also found as a minor component of other membranes (Hagio et al., 2002; Hölzl and Dörmann, 2007; Kobayashi and Wada, 2016). The prime location of PG in thylakoid membranes has suggested that PG plays a critical role in photosynthesis. So, the thylakoids, which constitute the bulk surface in a chloroplast, are, in principle, composed of four lipid classes – the uncharged galactolipids – MGDG and DGDG; glycolipids – SQDG and phospholipids – PG, anionic lipids with a negative charge on their head groups. A prerequisite to maintaining the stability and membrane functioning of key proteins related to photosynthesis is to maintain a balance between DGDG galactolipid, which is capable of formating bilayer structures and non-bilayer forming with the participation of MGDG, with a sufficient content of acidic SQDG and PG in the membranes of the chloroplasts (Hölzl and Dörmann, 2007; Kobayashi and Wada, 2016).

One of the mechanisms of the beneficial action of phosphate solubilizing microorganisms (PSM) is the impact of bacteria on the availability of soil soluble mineral phosphates and phosphorus stored in the organic form, inaccessible to plants, and their subsequent removal from the reserve pool into the plants' metabolism (Walpola and Yoon, 2012; Sharma et al., 2013). Rhizosphere microorganisms serve as intermediaries between the soil and plants by being an integral part of the soil-plant-microorganism system. Therefore, it is important to use an integrated approach to studying different plant-microbial associations, where plant organisms play an important role.

In view of the above, the purpose of work was to study the characteristics of the direction of metabolic changes in the phospho- and glycolipids in the membranes of buckwheat plants under a P_i deficit and pre-sowing seed bacterization with microbial preparations based on the phosphate solubilizing microorganisms: Polymyxobacteryn and Albobacterin.

Materials and methods

Plant growth condition

The study was performed using buckwheat plants (*Fagopyrum esculentum* Moench.) of the Antaria cultivar. The plants were cultivated under greenhouse growing conditions in pots containing sand, with 2.3 mg P_2O_5 per 1 kg of sand (determined by using a modified Kirsanov method (State standard of Ukraine, 2005) and a micronutrient medium of Hoagland and Arnon (1950). The plants were planted at a

density of 15 plants per pot (0.29 m^2). Treatments with a normal phosphorous supply (31 mg of KH_2PO_4) per 1 kg of substrate were used as a control. A phosphorus deficiency was simulated by substituting plant available phosphates (KH_2PO_4) with almost insoluble and inaccessible $Ca_3(PO_4)_2$ salts using the same dosage of phosphorus. The plants were grown at 25/22 °C (16 h day/8 h night) under irradiated conditions (185 µmol*m^{-2}*s^{-1}), 30% relative air humidity and completed watering (the level of the soil water content was 70% of the field water capacity). Plant sampling was performed at the flowering stage on 21-day-old plants. Only the middle part of buckwheat leaves from the control and the experimental treatments was collected for the lipidomic study of each variant analyzed. The tissue material was kept frozen in liquid nitrogen.

Seed bacterization

Pre-sowing seed bacterization was conducted by treating the seeds with microbial preparations Polymyxobacteryn (agent – *Paenibacillus polymyxa* KB), and Albobacteryn (agent – *Achromobacter album* 1122), the phosphorous mobilizing activity of which is 52 mg P_2O_5*100^{-1} ml and 83.5 mg P_2O_5*100^{-1} ml of solution, respectively. Seed bacterization was performed in laboratory conditions in accordance with the State Standards for microbial preparations treatments ensuring the presence of 5 billion bacteria per seed. For this the microbial treatment solution was prepared by suspending 1 ml of preparation in 2 l of distilled water and then applied to 1 kg of buckwheat plant seeds.

Lipid extraction

The lipids were extracted using the Liu and Wang (2016) protocol for extraction and profiling of plant polar glycerol lipids according to the procedure of total lipid extraction. The glycolipids were separated by thin layer chromatography (TLC) and the content of MGDG, DGDG and PG was determined according to standard (Sigma-Aldrich) with TotalLab TL120.The content of SQDG was determined according to Kean (1968).

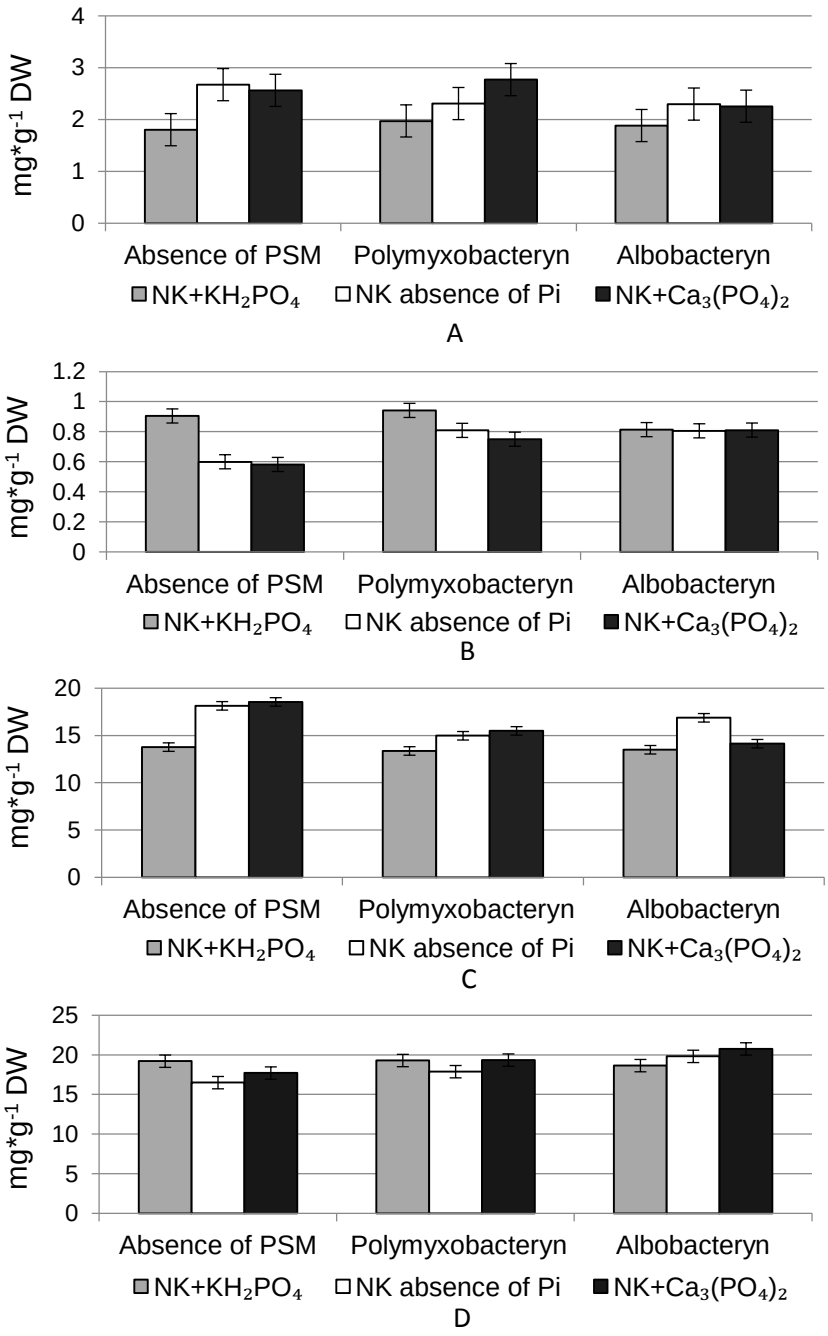
Statistical analysis

The statistical data analysis was conducted using Microsoft Office Excel software. Statistical significances between the groups were determined by two-way analysis of the variance (ANOVA). The treatments where the P values were ≤ 0.05 were considered to be significantly different. The number of biological and analytical replications in the experiment were at least three fold.

Results and discussion

The study of the transformations of lipid complex components of photosynthetic plant tissues of buckwheat under phosphate (P_i) deficiency has revealed that the lack of phosphates has triggered both lipid biosynthesis and degradation in the membranes of the buckwheat plants. The analysis of the membrane's lipids transformations has

revealed the ability of buckwheat plants to react to the P_i deficit with a selective accumulation of SQDG, DGDG, the degradation of PG and no significant changes in MGDG content (Figures 1).



DW – dry weight

Figure 1. Transformations of sulfoquinovosyldiacylglycerol (A), phosphatidylglycerol (B), digalactosyldiacylglycerol (C) and monolactosyldiacylglycerol (D) in the membranes of buckwheat plants (*Fagopyrum esculentum* Moench.) under P_i deficiency and seed bacterization by microbial preparations based on phosphate solubilizing microorganisms: Polymyxobacteryn and Albobacterin

Under the condition of a phosphorus deficiency the amount of SQDG has increased to 2.67 $mg^{*}g^{-1}$ in the absence of P_i and to 2.56 $mg^{*}g^{-1}$ in the treatments where the insoluble salt $Ca_3(PO_4)_2$ was used compared to the control plants (1.8 $mg^{*}g^{-1}$) grown under sufficient levels of mineral nutrition. Pre-sowing seed bacterization with PSM has ensured the reduction of SQDG accumulation in plants grown under P_i stress conditions. Only, in the variant with seed treatment using Polymyxobacteryn, did the SQDG content grow to 2.77 $mg^{*}g^{-1}$ in the presence $Ca_3(PO_4)_2$ in comparison to 1.97 $mg^{*}g^{-1}$ in the control (see Figure 1A).

The PG content in plants grown under conditions of a phosphorus deficiency was 66.3% and 63.2% of the control level, respectively (see Figure 1B).

Analysis of the inoculated plants has shown that, in the absence of P_i and in the presence of insoluble salt, the decrease of the PG content was not as significant upon seed bacterization by Polymyxobacteryn. Thus, PG was equal to 0.81 $mg^{*}g^{-1}$ and 0.75 $mg^{*}g^{-1}$, respectively, as compared to PG in plants grown under sufficient mineral nutritional conditions (0.94 $mg^{*}g^{-1}$).The determination of the PG content in plants inoculated with Albobacteryn has revealed no significant difference between the experimental and control treatments (see Figure 1B).

As shown by Kobayashi et al. (2015) P_i the limitation has activated glycolipid biosynthesis and has increased the ratio of glycolipids (MGDG, DGDG and SQDG) with a further decrease in PG content in pgp1-2 mutants. Analysis of one of the null pgp1 mutants (pgp1-2) has revealed that the PG deficiency in chloroplasts has strongly inhibited the formation of photosystem light-harvesting chlorophyll-protein complexes and photosynthetic electron transport (Kobayashi et al., 2015). Although the data suggest that glycolipids cannot fully compensate for the function of PG in photosynthesis, they can form thylakoid membrane networks without PG (Kobayashi et al., 2015; Kobayashi and Wada, 2016).

Taking that into the account, the decrease of the PG content can be explained by its partial compensation with another anionic lipid – SQDG in order to preserve the anionic balance in membranes, in order to form thylakoid membrane networks and in order to maintain the optimal level of photosynthetic processes in the chloroplasts. The presence of partial compensatory mechanisms of substituting anionic PG with anionic SQDG (which does not have phosphorous in its composition) in plastid membranes (Svietlova, 2012), allow buckwheat plants to adapt to stress conditions of P_i deficit. Pre-sowing seed bacterization with PSM, has contributed to the transformation of mineral phosphorous $Ca_3(PO_4)_2$ from a reserve to the metabolic pool, resulting in stress reduction for plants. Improvement of the soil phosphorus status has influenced the anionic lipid metabolism in buckwheat plants. Thus, the maintenance of the anionic balance in the membranes was ensured by PG while its partial compensatory replacement mechanism with SQDG was less obvious. This affected the PG/SQDG balance in plants. The buckwheat plants grown under conditions of a P_i deficit had half (or less) of the ratio index compared to the plants grown under optimal levels of mineral nutrition (Figure 2A). At the point of seed bacterization by Polymyxobacteryn, the PG/SQDG index decreased to 0.35 and to 0.27 in the absence of P_i and in the presence of $Ca_3(PO_4)_2$, respectively, when compared to the control value of 0.48 (Figure 2B). Seeds inoculated with Albobacteryn have reduced the difference in the PG/SQDG ratio between the control

and the experimental plants even more: in the absence of P_i the index declined to 0.35; in the presence of the insoluble salt – 0.36 in comparison to the control value of 0.43 (Figure 2C).

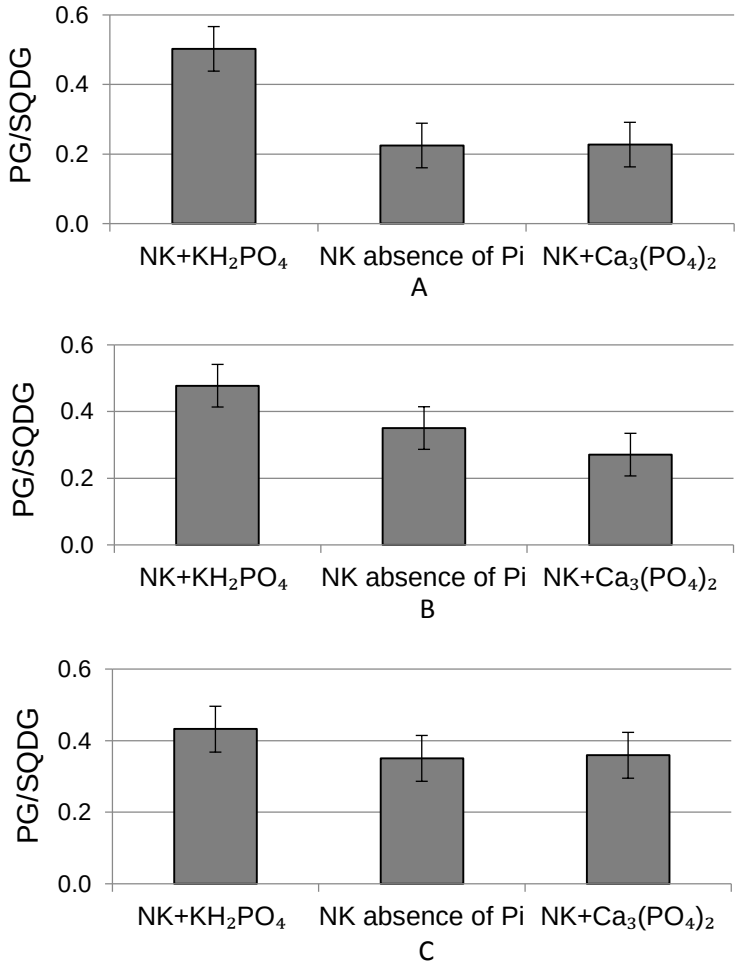


Figure 2.The phosphatidylglycerol / sulfoquinovosyldiacylglycerol ratio of buckwheat plants membranes (*Fagopirum esculentum* Moench.) under a P_i deficit (A) and seed bacterization by microbial preparations based on the phosphate solubilizing microorganisms: Polymyxobacteryn (B) and Albobacterin (C)

The P_i deficit has caused changes in the metabolism in the direction of one of the two galactolipids in the photosynthetic membranes DGDG. Its content has increased compared to the control (13.78 $mg^{*}g^{-1}$) by 18.14 $mg^{*}g^{-1}$and 18.55 $mg^{*}g^{-1}$ in the absence of phosphorus salts and $Ca_3(PO_4)_2$, respectively (Figure 1C). Under the influence of Polymyxobacteryn a smaller difference in DGDG content between the control and the P_i deficit plants was noticed; the level of DGDG under the influence of Albobacteryn used for seed inoculation in the treatment with insoluble $Ca_3(PO_4)_2$ was equal to the control which can also indicate the assumption that the plants adapt and their stress is reduced under the condition of P_i deficiency.

Taking into the account that the biosynthesis of DGDG is regulated by the transcription of DGDG synthase genes: *DGD1* and *DGD2*, its accumulation in the absence of phosphorus salts and $Ca_3(PO_4)_2$ is probably associated with a *DGD2* gene which is activated under the conditions of a P_i deficit and is not associated with the regulation of the DGDG synthesis under optimal conditions (Kelly et al., 2003).

No significant changes in MGDG content was observed in the experimental (the plants grown under a P_i deficit or in the treatments with seeds inoculated with PSM or in the control variants (Figure 1D).

The data obtained support the fact that out of two types of MGDG synthase, identified in the plants, MGDG synthase type B (*MGD2*, *MGD3*), which is expressed predominantly in non-photosynthetic tissues under conditions of a P_i deficit, is not significantly involved in the synthesis of MGDG in photosynthetic membranes, unlike MGDG synthase type A (*MGD1*) (Dormann et al., 1999; Awai et al., 2001; Kelly and Dormann, 2004), which is regulating the biosynthesis of most of the galactolipids in the presence of P_i during the growth and development of the plants.

Analysis of the transformation of galactolipids – MGDG and DGDG in photosynthetic membranes under a P_i deficiency has indicated that the accumulation of DGDG is probably associated with the activation of an alternative pathway making its synthesis possible due to the expression of a *DGD2* gene (Kelly et al., 2003). The relative stability of MGDG content in the plants under the condition of P_i deficiency indicates the prevailing role of a constitutional biosynthetic pathway but not an alternative one through the MGDG synthase type B biosynthesis (Dormann et al., 1999; Awai et al., 2001; Kelly and Dormann, 2004).

Glycolipid synthase regulate the biosynthesis of glycolipids (MGDG (EC 2.4.1.46), DGDG (EC 2.4.1.241) and SQDG synthase (EC 2.4.1.-), which use diacylglycerol (DAG) as a substrate synthesized both in plastids and imported from the endoplasmic reticulum (Douce and Joyard, 1996) PG phosphate synthase (EC 3.6.1.40), which regulates the synthesis of PG, uses DAG formed in plastids as a substrate. This ability of glycolipid synthases, combined with expression of the corresponding genes, essential for conversion of DAG as the result of PG degradation into the SQDG and MGDG and forms the molecular basis for significant transformations of membrane lipid composition under the P_i deficit (Essigmann et al., 1998; Sato et al., 2000; Awai et al., 2001; Dormann and Benning, 2002; Yu et al., 2002; Kelly et al., 2003; Jouhet et al., 2003).

Conclusions

Summarizing, the decrease of the concentration of phosphorus ions in plant cells to a critical minimum level is used as the signal for the start of stress reactions in plant organism causing changes in the direction of metabolism of phosphorus and glycolipids. The established partial compensatory mechanisms of phospholipids replacement with phosphorless glycolipids in the membranes of buckwheat plants maintain an equilibrium within the conformational changes of the macromolecules in plants' membranes and determine plants ability to adapt to the P_i deficiency. At the same time the phospholipids have served as a reserve pool of cellular phosphorus for housing salvage ions in a donor-acceptor system.

Pre-sowing seed bacterization with PSM, has possibly contributed to the transformation of mineral phosphorous $Ca_3(PO_4)_2$ from the reserve pool into the metabolic system, causing a reduction of the stress effect on the plants. An improvement of soil phosphorus conditions has influenced the anionic lipids metabolism – the maintenance of the anionic balance of the membranes was maintained by means of PG, while the partial compensatory mechanism of SQDG phospholipid replacement was less obvious, especially in those plants inoculated with Albobacteryn.

Authors' contributions

The following declarations about the authors' contributions have been made: NS: the study conception, supervision of the design of the research, preparation of the manuscript, MV: assisted in data interpretation, preparation and proof reading of the manuscript, VS and OK: biological experiments and data analysis, VG: draft of the biological methods, OS and NT: critical revision of the manuscript. The authors of the present paper contributed equally and they all declare that there are no conflicts of interest.

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