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MODERN PLANT BIOLOGY : THEORETICAL AND APPLIED ASPECTS

dedicated to the 130th anniversary of the Department of Plant and Microorganisms' Physiology and Biochemistry of V. N. Karazin Kharkiv National University

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ABSTRACT BOOK

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Address : maidan Svobodi, 4, Kharkiv, Ukraine, 61022,
V. N. Karazin Kharkov National University
E-mail: zhmurko@karazin.ua

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was studied. It was found that two lines with different photoperiodic reaction reacted in the evening irradiation in different ways: in the SD line the night nitrate accumulation increased, and during the day their decrease was accelerated, whereas in the ND line the changes of nitrate content were not observed. On interruption of the night of the emergency two lines reacted with the nitrate content unidirectionally. Nitrates accumulated in the leaves at night and during the day the rate of their recovery decreased.

RELATIONSHIP BETWEEN NITRATE AND PHOSPHATE ASSIMILATION IN SOME FRESHWATER AND SALTWATER ALGAE

Komaristaya V. P.

V.N. Karazin Kharkiv National University,
Department of Botany and Plant Ecology,
Svobody sq, 4, Kharkiv – 61022, Ukraine
e-mail: v.p.komarysta@karazin.ua

Freshwater algae *Haematococcus pluvialis* Flotow and *Chlorococcum dissectum* Korshikov accumulate natural xanthophylls valuable for food and feed. They can serve as their industrial sources: the first species is already implemented into biotechnology, the second is potential. The same applies to the saltwater alga *Dunaliella salina* Teodoresco that is one of the established sources of natural beta-carotene in the biotechnological industry.

Nitrate and phosphate in the nutrient medium for microalgae are sources of nitrogen and phosphorus, which are necessary for the yield of the biomass. For carotenogenic algae, depletion of nitrate or phosphate in the medium is an inducer of accumulation of carotenoids in the cells. Therefore, adjusting the concentration of nitrate and phosphate in the nutrient medium is an important measure to control the productivity of the culture of these microalgae.

The objective of this research is to study how the deficiency of nitrate affects the dynamics of phosphate acquisition and *vice versa*, in cultures of *H. pluvialis*, *Ch. dissectum* and *D. salina* at two levels of light intensity (2 and 8 klx) and two levels of salinity (1 and 4 M NaCl) for saltwater *D. salina*.

Cultures of two freshwater species were grown in the BBM medium (Nichols, Bold, Journal of Phycology, 1965, 1, 1, 34-38). *D. salina* was cultivated in the Artari medium modification (Масюк, Морфология, систематика, экология..., Киев: Наукова думка, 1973, 244). In the media, the concentrations of nitrate and phosphate were decreased (80 mg/L $NaNO_3$ was added to the BBM or the same amount of KNO_3 to the Artari medium, and 10 mg/L K_2HPO_4 was added to both media), and in the Artari medium, which ordinarily contains 2M NaCl, salinity was set at two levels (1 and 4M NaCl). For preventing depletion of nitrate and phosphate in the medium, the cultures were grown in the fed-batch mode, adding half of the initial dose of both nutrients every 3-4 days. In the nutrient-deficient experimental variants, the nutrients were not added to the medium. The experiments were carried out according to the full factorial design, which envisaged the addition or non-addition of nitrate and phosphate, two levels of illumination, and two levels of salinity for saltwater *D. salina*. The cultures were grown in 15 ml of the medium in Erlenmeyer flasks per 25 ml, at a temperature of 25-27 °C and a photoperiod of 16/8 hours light to the darkness. The growth dynamics of the cultures were controlled by counting the number of cells in the Goryaev hemocytometer. The dynamics of the assimilation of nutrients were judged by their residual concentrations in the medium before each addition: nitrate was measured as described (Cataldo et al., Comm. Soil Sci. Plant Anal., 1975, 6, 1, 71-80), phosphate was measured by the method (Fogg, Wilkinson, Analyst,

1958, 83, 988, 406-414). The experiments were repeated in triplicate. The normality of data distribution was checked using the Shapiro – Wilk test. Statistical analysis of the effects of cultivation conditions on the culture growth dynamics and assimilation of the nutrients was carried out using the ANOVA method. For comparison of the means, Fisher's LSD was calculated.

In all three species, deficiency of nitrogen or phosphorus in the medium expectedly inhibited the growth of the cultures. Increased salinity (4 M NaCl) partially inhibited the growth of *D. salina* culture but to a lesser extent than nutrient deficiencies.

In both freshwater species *H. pluvialis* and *Ch. dissectum*, deficiency of one of the nutrients reduced the assimilation rate of the other nutrient. A possible cause may be low cell concentrations due to inhibition of culture growth. Nitrate deficiency affected phosphate assimilation stronger than *vice versa*. Perhaps since cells' need for nitrogen is higher than their need for phosphorus, the exclusion of nitrogen stronger affects culture parameters (following the law of minimum).

As for the saltwater *D. salina*, a similar effect of suppressing the assimilation of one nutrient by lack of the other was observed only at the low salinity of 1M NaCl. An increase of salinity to 4M NaCl *per se* led to inhibition of the absorption of both nutrients, but a lesser degree. Noteworthy, at a salinity of 4M NaCl, a deficiency of one of the nutrients no longer affected the assimilation of the other. We previously observed this phenomenon even at a salinity of 2M NaCl, which is standard for the cultivation of *D. salina* (Komaristaya et al., Algologiya, 2010, 20, 1, 42-55). The independence of nitrate and phosphate uptake at a salinity of 2M NaCl led to the fact that *D. salina* cells intensively accumulated another available nutrient when one of the nutrients was deficient (Komaristaya et al., Вісник ХНАУ: Серія Біологія, 2016, 3, 39, 18 -26). That hindered the subsequent induction of beta-carotene accumulation by the deficiency of this nutrient in the medium (Komaristaya et al., The VI Open Congress of Phytobiologists of Black Sea Region, Kherson, Ukraine, 2015, pp. 46-49).

No statistically significant effect of increasing light intensity from 2 to 8 klx on the dynamics of nutrient absorption was observed in any of the studied species.

There is a discussion in the literature about the biochemical independence of nitrate and phosphate metabolism and absorption. The metabolic pathways of nitrate and phosphate are not directly related, but many experimental data indicate such a relationship. Only assumptions are expressed about its mechanism: the availability of nitrogen might limit the transport of phosphate since phosphate membrane transporters are protein molecules, therefore nitrogen is necessary for their synthesis. ATP-dependent transport of nitrate across the plasmalemma could be limited by the availability of phosphorus (Bougaran et al., Journ. of Theor. Biol., 2010, 265, 3, 443-454). Besides, the ability of the cells to absorb a particular nutrient when limited by the other one is determined by physiological limits called quotas (Elrifi, Turpin, Journal of Phycology, 1985, 21, 4, 592-602).

Our data suggest that when salinity increases in *D. salina* culture, either change in nutrient transport through the plasma membrane occurs, for example, more efficient carriers are induced, or high salinity increases cell quota for nitrogen and phosphorus.

The study of the mechanisms of the assimilation of nitrate and phosphate by carotenogenic algae, as well as the influence of salinity on these processes, will shed light on the mechanisms of induction of carotenoid accumulation in the cells to control industrial culture more effectively.

ЗВ'ЯЗОК МІЖ НІТРАТНИМ ТА ФОСФАТНИМ ЖИВЛЕННЯМ У ДЕЯКИХ ПРІСНОВОДНИХ І СОЛОНОВОДНИХ ВОДОРОСТЕЙ.

Комариста В. П.

У прісноводних *Haematococcus pluvialis* і *Chlorococcum dissectum*, а також у солоноводної *Dunaliella salina* за зниженої солоності (1 М NaCl) нестача одного з джерел біогенів

(нітрату або фосфату) призводила до часткового пригнічення асиміляції іншого, при чому у прісноводних видів нестача нітрату сильніше відбивалася на асиміляції фосфату, ніж навпаки. У *D. salina* підвищена солоність (4 M NaCl) викликала часткове інгібування поглинання обох біогенів, але менш виражене, ніж нестача одного з них. За вискої солоності асиміляція нітрату та фосфату в *D. salina* відбувалася незалежним чином: за нестачи одного з них клітини продовжували поглинати інший.

FATTY ACIDS IN GRAIN OF SOME TETRAPLOID WHEAT SPECIES

Relina L. I., Suprun O. H., Vecherska L. A., Boguslavskyi R. L.

Plant Production Institute named after VYa Yuriev of NAAS
Moskovskyi ave., 142, Kharkiv-61060, Ukraine
e-mail: lyudmila_vecherska@ukr.net

Wheat has never been considered an oil crop, however, oil from wheat germs and bran is rich in some bioactive compounds. Most of studies in this area are conducted on traditional commercial wheat varieties. At the same time, the interest of breeders, producers and consumers is returning to ancient and underutilized wheats species. There is very little information on the oil quality from such species. In the light of this, we stated the objective to assess tetraploid wheat species (*Triticum. dicoccoides var. pseudojordanicum, Triticum dicoccum, Triticum timofeevii, Triticum persicum var rubiginosum, Triticum durum var. falcatamelanopus, Triticum polonicum var. pseudocompactum* and *Triticum aethiopicum var. densimenelikii)* for fatty acid composition. Fatty acid composition was analyzed by gas chromatography. Six major fatty acids were found in the species under investigation, with linoleic acid being the most abundant. They are listed in order of decreasing amounts as follows: linoleic > oleic >palmitic > linolenic > stearic > palmitoleic. This distribution did not vary from year to year. We also detected trace amounts of 3 minor fatty acids: eicosanoic, eicosenoic and behenic acids.

There were no significant differences for 4 (palmitic, linoleic, oleic and palmitoleic) of 6 major fatty acids between *T. dicoccoides var. pseudojordanicum* and *T. dicoccum var. serbicum,* which is considered to have not been crossed with other tetraploid species and have undergone the least changes in the breeding process. *T. dicoccum var. atratum* accessions from different locations, which are morphologically very close, in many cases differ one from another in contents of 5 of 6 major fatty acids (except palmitoleic acid).

The oil value is primarily determined by unsaturated fatty acids. In this respect, *T. timofeevii* seems the most promising species for crossing with other tetraploid species to improve wheat oil quality via breeding (unsaturated/saturated ratio = 5). Nevertheless, emmer varieties Holikovska and Romanivska and durum wheat variety Spadschina, boast rather high unsaturated/saturated ratios (4.5, 4.7, and 5.1, respectively). These values are higher than those registered for durum wheat in the National Nutrient Database for Standard Reference Nutrient data for product 20076 ‘Wheat, durum’ of the United States Department of Agriculture and the database of the Italian National Institute for Research on Food and Nutrition (3.0 and 3.5, respectively) (Narducci V. et al., 2019). The ratios for *T. persicum var. rubiginosum, T. durum var. falcatamelanopus, T. polonicum var. pseudocmpactum* and *T. aethiopicum var. densimenelikii* were 3.9, 45.3, 4.0 and 3.9, respectively. Thus, these species are unadvisable to use in crossings for improvement oil quality.

No deterioration in the grain quality in terms of unsaturated fatty acid levels was detected in the course of emmer domestication, since the ratio of unsaturated acids to saturated ones in