



Seasonal Dynamic of Morpho-Physiological Properties and the Lipid Composition of *Plantago Media* (*Plantaginaceae*) in the Middle Volga Region

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Abstract

The changes in morpho-physiological properties and lipid composition have been studied in the leaves of the plant *Plantago media* collected from two different places in the Middle Volga region during the summer 2011. The plants gathered from the first plot (P1 plants) grew on plane ground in the midst of typical meadow-steppe perennial plants. The plants of the second group (P2 plants) grew on a flat slope of the South-West exposition, in the grass community. The leaves of the plants P 1 had lower specific area densities but larger areas and masses; they accumulated more levels lipid peroxide products. The changes in lipid compositions depended on the growth phase and habitats. Correlations between morpho-physiological parameters and certain lipids have been established. The amounts of galactolipids have been shown to correlate with the leaf areas. When the leaf areas were reduced, a ratio between phosphatidylcholine and phosphatidylethanolamine decreased. The result of our study showed that gradual changes of morphometrical parameters were accompanied by the alterations in biomass structure and modifications in lipids and FA.

Keywords

Fatty acids; Lipids; Morpho-physiological changes; *Plantago media*

Introduction

Species of Plantaginaceae (about 200 plants) are found all over the world representing an important part of many ecosystems. Some *Plantago* species have been found to be genetically differentiated and phenotypically plastic towards illumination [1], mineral nutrition [2] and soil humidity [3]. *Plantago media* is a perennial polycarpic plant, which is characterised by moderate demands for soil-climatic conditions. It is a Eurasian boreal species, its areal covers Europe, Siberia, Western and Central Asia [4].

It is noteworthy that species of Plantaginaceae have been used for a long time in both, traditional and alternative medicine as the basis of many remedies and drugs [5]. *Plantago major* is the most studied species of this family [6-9]. *P. media* L. is a closely related

species with multiple medicinal uses. This plant has been found to contain bitter and tannic compounds, vitamins C, K and U, flavonoids, polysaccharides and essential fatty acids (FA) [10-12]. In the course of plant ontogenesis, cellular metabolic activities change several times depending on the genetic program realization during development and on adaptive reactions during acclimation to the changing environmental conditions. Plants have evolved mechanisms to monitor their environment and to respond to the changing conditions to optimize growth and reproductive success. Such responsive reactions have been shown to occur on cellular, whole-organism and ecosystem levels [13-17]. Many cellular compounds may be involved in these regulatory and metabolic reactions, and lipids and FA are undoubtedly the best known molecules in such adaptive responses. In general, lipids play a number of important roles in all living organisms [18]. Structurally they can be divided into two major groups: the non-polar lipids (NL) and polar lipids. Of the NL, the triacylglycerols (TAG) are common storage products, which can easily catabolised to provide metabolic energy. Polar lipids and sterols (ST) are important structural components of cell membranes and they maintain the membrane specific functions. In addition to a structural function, some polar lipids may act as key intermediates in cell signalling pathways and play a role in responding to changes in the environment [19].

In earlier work, we obtained the data that showed a morphophysiological and biochemical plasticity of *P. media* leaves in respect to illumination conditions [20,21]. We also studied the daily dynamics of membrane lipids in this species collected from midland regions of Russia [22].

The aim of the present work was to study the lipid and FA composition of *P. media* leaves from two different habitats in relation to the dynamics of some morphometric and physiological characteristics during vegetation period [23]. This information can be useful for understanding the role of lipids in adaptation to different environment as well as for revealing a possible relationship between lipids and ontogenetic alterations in the morphology of leaves.

Materials and Methods

Plant material

The plants were gathered in the National Park "Samarskaya Luka" from two plots (800 × 800 m). The plants gathered from the first plot (P1 plants) (latitude 53°23'59"/ S longitude 49°35'21"/ E, altitude 142 m) grew on plane ground in the midst of typical meadow-steppe perennial plants *Festuca valesiaca* Gaudin, *Poa angustifolia* L., *Phleum phleoides* (L.) Karst., *Filipendula vulgaris* Moench etc. The plants of the second group (P2 plants) (latitude 53°23'35"/ S longitude 49°35'17"/ E, altitude 158 m) grew on a flat slope of the South-West exposition, in the grass community of *Bromopsis inermis* (Leys.) Holub, *Dactylis glomerata* L., *Elytrigia repens* (L.) Nevski and *Fragaria viridis* Duch with some *Agropyrum repens* (L.), *Amoria repens* (L.) and *Megicago foleata* L. The plants of *P. media* grew on a sparsely grassed terrace with high light (P1 plants) and a thickly grassed flat slope with low light (P2 plants). The plant material was collected three times during the summer 2011 with monthly intervals in the daytime (from 11.00 a.m. to 12.30 p.m.).

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Environmental condition

Evaluation of abiotic factors of P1 at the time of sampling showed that air temperature varied from 33° to 36° C, the illumination level – 902–1408 $\mu\text{mol m}^{-2}\text{s}^{-1}$, soil pH – 6,3–6,8, soil moisture – 8–12% depending on the month of the studies. For P 2 indices differed: the air temperature was 24–30°C, the level of illumination was 110–990 $\mu\text{mol m}^{-2}\text{s}^{-1}$, soil pH – 6,4–6,9, soil moisture – 11–19% depending on the month of the studies.

Morphometrical measures of leaves and plants

The mature leaves were collected from the mid rosette part of the similar plants (10–12 plants of the same age), and their lengths and widths were measured. A leaf area was calculated as $LA=0.66lw$, where l is a leaf length and w is a leaf width [24]. The specific leaf area density was calculated as the ratio of dry leaf mass (mg) to its area (cm^2). To determine the mass of under (M_U)- and overground (M_O) organs, the entire plant was dug out and sorted into leaves, generative shoots and roots; the masses of these organs were determined gravimetrically. The root length was the length of their longest part. The dry plant mass was determined after drying at 50°C until the constant weight.

Determination of lipid peroxidation

Lipid peroxidation intensity in the plant leaves was determined by measurement of malondialdehyde (MDA) concentrations after reaction with thiobarbituric acid [25]. Fluorescence intensity was measured using a spectrophotometer (Specol, Germany) at 532 nm.

Lipid extraction and analysis

For lipid analysis, the mid leaf part was cut in small parts and three samples of 1–2 g were chosen from the total mass. The samples were treated with hot isopropanol and kept in a cold, dark place prior to analysis.

Lipids were extracted three times using three times chloroform/methanol (1:2, v/v) by the method [26]. The combined extracts were purified from non-lipid compounds and concentrated using a rotary vacuum evaporator.

Phospholipids (PL) were separated by two-dimensional thin-layer chromatography (TLC) on 6 x 6 cm silica gel G plates using chloroform: methanol: benzene: ammonium hydroxide (130:60:20:12, v/v/v/v) in the first dimension and then chloroform: methanol: benzene: acetone: acetic acid (140:60:20:10:8, v/v/v/v/v) in the second. PL were visualised by charring the TLC plates after spraying with 10% H_2SO_4 in methanol. The quantification of phospholipids was performed by the content of inorganic phosphorus [27] with the following calculation of their molar masses.

Galactolipids (GL) were separated by one-dimensional TLC on 10 x 10 cm silica gel G plates using acetone:benzene:water (91:30:8, v/v/v) and visualised after spraying with 5% $12\text{MoO}_3 \cdot x\text{H}_3\text{PO}_4$ in ethanol. GL were quantified using a densitometer Sorbfil (Russia) with the occasional comparison of the data with those obtained from the galactose measurement. The latter was done using an anthrone reagent [28], using a spectrophotometer (Specol, Germany) at 620 nm. Monogalactosyldiacylglycerol (MGDG) (Laroden, Sweden) and galactose (Sigma, USA) were used to create calibration curves. NL were measured spectrophotometrically, and tripalmitate (Sigma, USA) was used as a standard for calibration curve. Total lipids (TL) were calculated as a sum of GL, PL and NL were quantified using a spectrophotometer (Specol 11, Carl Zeiss, Jena, Germany)

[29]. Tripalmitate (Sigma-Aldrich, USA) was used as a calibration standard.

Fatty acid profile determination

Fatty acid methyl esters (FAME) were prepared by transmethylation with 5% HCl in methanol. FAMES were purified by preparative TLC using hexane:diethyl ether:acidic acid (80:20:1, v/v/v). They were analysed using a Cristal 5000.1 gas chromatograph (Perkin-Elmer, Norwalk, Connecticut), fitted with a 105 m x 0.25 mm i.d. capillary column (Restek, USA) under isothermal conditions (column at 180°C; injector and detector at 260°C). The oven temperature was programmed: 170°C for 3 min, heated to 220°C at 4°C min^{-1} , held at 220°C for 15 min. FAMES were identified by comparing retention times with fatty acid standards (Supelco 37, Supelco, USA). The unsaturation index (UI) was calculated according to the formula, where P_j is a content of the unsaturated FA (% of total FA) and n is a number of double bonds in this FA.

$$UI = \sum P_j \cdot n / 100$$

Statistical Analysis

The data obtained are represented as means of three independent experiments ($n=3$) with standard errors (SE). The confidence of the difference between two values was estimated by a student t -test ($p<0.05$). Relationship between morphometric parameters and lipid characteristics was measured using a Spearman's correlation coefficient. The results were statistically processed using Statistica 10 (Statsoft Inc., USA).

Results

Morphometric parameters

The habitats of two plots where the plants were collected differ significantly by the phytocenological. P1 plants grew on plane ground in the midst of typical meadow-steppe perennial plants, and – P2 plants grew on a flat slope in the grass community. During the summer period, a successive shift of phenological phases occurred from the beginning of flowering in June to full-scale flowering in July and, finally, the fading in August [4].

Linear sizes of under- and overground organs of *P. media* were variable depending on the observation period (Table 1). Thus, linear leaf and root sizes of *P. media* were increased during the summer. Leaf area of P1 plants were smaller than those of P2 plants. Leaves of P2 have lowered the specific leaf area density and dry weights (DW), but they have longer roots. As seen from the mass analysis (M_U/M_O), an increase in the masses of over ground parts with a concomitant decrease in the root mass has been observed during the whole summer period.

Lipid peroxidation

The intensity of lipid peroxide production is one of the main criterions for estimation of physiological status of the plant on the cellular level. The content of MDA, the final product of lipid peroxidation, did not show any statistically significant changes in the plants from two different habitats in June (Figure 1). However, a 1.6–2.9-fold increase in lipid peroxidation in the P1 plants has been demonstrated in the middle and at the end of summer. In P2 plants, the accumulation of lipid peroxidation products has been noted only in August (Figure 1).

Table 1: The seasonal changes in morphometric characteristics of under- and overground parts of *P. media*.

Parameter	Vegetation time, month					
	VI		VII		VIII	
	P1	P2	P1	P2	P1	P2
Leaf length, mm	10.1 ± 0.5	9.6 ± 0.5	10.0 ± 0.5	11.7 ± 1.0	18.2 ± 1.6*	13.8 ± 0.9*
Leaf width, mm	2.5 ± 0.1	3.4 ± 0.1*	2.7 ± 0.1	2.6 ± 0.2	2.8 ± 0.2	3.3 ± 0.2
Root length, mm	11.8 ± 1.7	15.9 ± 1.7*	12.0 ± 2.2	13.6 ± 1.9	9.4 ± 1.0	12.1 ± 0.7*
Leaf area, cm ²	16.6 ± 1.7	21.5 ± 1.6*	17.8 ± 1.5	20.0 ± 2.2	33.6 ± 3.7*	30.1 ± 2.9*
Specific leaf area density, mg cm ⁻²	47.0 ± 4.2*	41.4 ± 0.4*	27.5 ± 2.3	21.6 ± 2.1	28.6 ± 2.1	24.1 ± 2.4*
Leaf DW, %	23.7 ± 0.1	17.4 ± 0.1*	24.0 ± 0.0	22.7 ± 0.1*	29.4 ± 0.2	22.9 ± 0.2*
M _u /M _o	1.2 ± 0.1*	2.0 ± 0.2	2.0 ± 0.2*	2.3 ± 0.5	4.6 ± 0.9*	4.5 ± 1.2*

Note: P1–Plot 1; P2–Plot 2; VI–June; VII–July; VIII–August; DW–dry weight.

* indicates statistically significant differences between the plots and/or vegetation time ($p \leq 0.05$); means ± SE (n = 3).

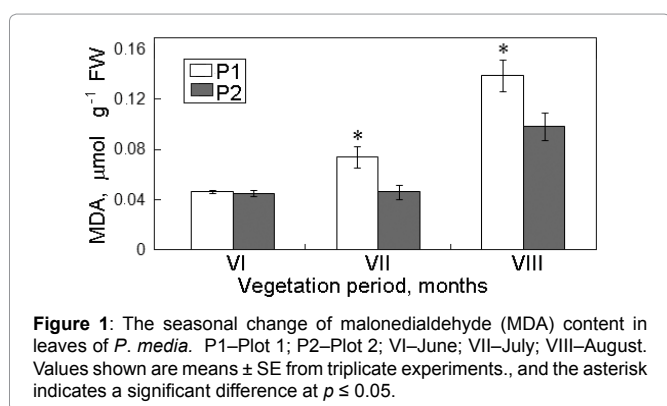


Figure 1: The seasonal change of malonaldehyde (MDA) content in leaves of *P. media*. P1–Plot 1; P2–Plot 2; VI–June; VII–July; VIII–August. Values shown are means ± SE from triplicate experiments., and the asterisk indicates a significant difference at $p \leq 0.05$.

Total lipid content and composition

The most TL were found in leaves P1 and P2 plants collected in June (32 mg g⁻¹ and 41 mg g⁻¹ DW) (Figure 2). The dynamics of lipid classes showed that leaves of plants accumulated mainly galacto- and phospholipids (GL and PL) (Figure 2). The levels of these lipids decreased in August with a concomitant increase in the level of non-polar lipids (NL). This fraction GL consists of three major chloroplast lipids, MGDG (35–46% of sum GL), digalactosyldiacylglycerol (DGDG) (~ 50%) and sulfoquinovosyldiacylglycerol (SQDG) (9–15%). Our data show that the highest amounts of MGDG and DGDG were accumulated in leaves in June, and GL content was higher by 1.5 times in P2 plants (Table 2). During summer, the levels of DGDG and SQDG varied from 7.1 to 4.3 mg g⁻¹ DW (44–40% of sum GL) and from 1.9 to 1.6 mg g⁻¹ (12%–15%) in P1 plants. In P2 plants the levels of DGDG also decreased from 10.5 to 8.5 mg g⁻¹ DW (43%–45% of sum GL), but the levels of SQDG remained constant, but changed his contribution to the pool of the GL (10%–13%).

PL are the structural compounds of non-photosynthetic membranes except phosphatidylglycerol (PG) which located in chloroplasts. In this fraction, we identified phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidic acid (PA) and diphosphatidylglycerol (DPG), or cardiolipin (Table 2). The contribution of PC to the total PL pool in leaves was maximal at the beginning of summer and was 5.5 and 5.4 mg g⁻¹ of DW (57 and 55% of the total PL). Then, its relative level decreased up to 45% in leaves of P1 plants and up to 46% in P2 plants with the parallel increase in the level of PA and DPG. The contribution of PG to the total PL fraction also decreased in the plants of both populations. However, in June its level was by 1.2 times higher in P2 plants in comparison to

P1 plants. The relative content of PE and PI was relatively constant in leaves in the plants of both populations.

Among individual NL identified in *P. media*, diacylglycerols (DAG) and sterols (ST) were the dominant classes in leaves and their contents were 1.2–2.3 mg g⁻¹ DW (18–31%) and 0.9–1.9 mg g⁻¹ DW (17%–30% of sum NL), respectively. Then followed the TAG (8%–20%) and sterol esters (ES) (11%–19%). In addition to these major lipid classes, fatty alcohols, free FA and waxes have been determined in the amounts varied from 4% to 12%. In July, the DAG content increased by 1.3 times (by the expense of ST) in leaves of plants from both populations. In leaves of P2 plants, a threefold increase in the level of TAG has been noted in July in comparison to June. The increased TAG accumulation has been noted in August, and it was more pronounced in P2 plants. The contents of SE and ST have been also noted to increase during this time period.

Fatty acids

We identified more than 10 individual FA in leaves of *P. media* is present in Table 3. Palmitic (C16:0), linoleic (C18:2) and linolenic (C18:3) acids were the dominant FA; they amounted for about 90% of the total FA. Contents of short-chain FA in plants ranged from 0.9 to 1.9%. Among saturated FA was predominant C16:0, which increased the percentage in July from 16% to 25% in leaves of the P2 plants and from 14 to 19% in P1 plants.

C18:3 was the major FA in leaves during the summer with the highest relative amount in P2 leaves in June. The level of this acid in leaves decreased at the end of summer by 1.3 times in P1 plants

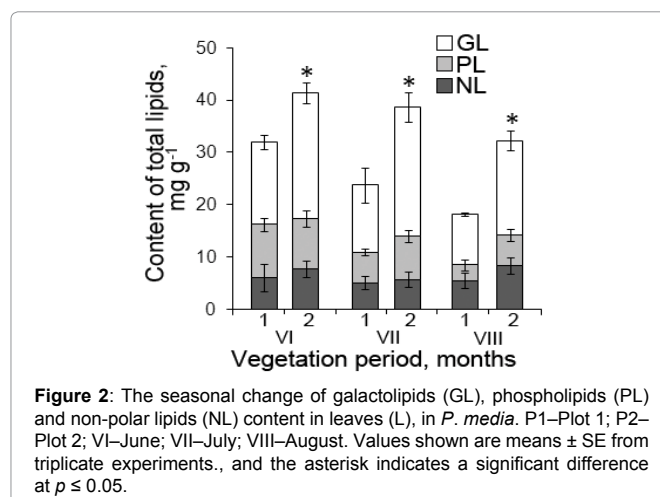


Figure 2: The seasonal change of galactolipids (GL), phospholipids (PL) and non-polar lipids (NL) content in leaves (L), in *P. media*. P1–Plot 1; P2–Plot 2; VI–June; VII–July; VIII–August. Values shown are means ± SE from triplicate experiments., and the asterisk indicates a significant difference at $p \leq 0.05$.

Table 2: The seasonal change in the content of individual components membrane and lipids in leaves in *P. media*.

Components (DW mg g ⁻¹)	Vegetation period, months					
	VI		VII		VIII	
	P1	P2	P1	P2	P1	P2
MGDG	6.8 ± 0.4*	11.1 ± 1.6	5.7 ± 1.5	11.5 ± 1.2	3.8 ± 0.5	7.0 ± 1.5*
DGDG	7.1 ± 1.1	10.5 ± 0.6	5.7 ± 1.3	10.4 ± 0.5	4.3 ± 1.2*	8.5 ± 1.6
SQDG	1.9 ± 0.1	2.5 ± 0.3	1.4 ± 1.0	2.4 ± 1.0	1.6 ± 0.6	2.5 ± 0.6
PC	5.3 ± 1.3*	5.4 ± 1.1*	2.7 ± 1.3	3.3 ± 1.0	1.5 ± 1.0	3.1 ± 1.0
PG	2.1 ± 0.7*	2.6 ± 0.2*	1.2 ± 0.2	1.0 ± 0.0	0.5 ± 0.1	1.0 ± 0.2
PE	0.8 ± 0.2	0.9 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.3 ± 0.1*	0.6 ± 0.1*
PI	0.9 ± 0.3	0.6 ± 0.1	0.7 ± 0.3	0.8 ± 0.1	0.4 ± 0.2	0.7 ± 0.0
PA	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.7 ± 0.2*	0.1 ± 0.0	0.2 ± 0.1
DPG	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1*	0.4 ± 0.0*	0.1 ± 0.0	0.2 ± 0.0
ST	1.7 ± 0.2	1.9 ± 0.4	1.2 ± 0.7	0.9 ± 0.5	1.5 ± 0.5	1.8 ± 0.8
TAG	1.0 ± 0.2	1.0 ± 0.1	0.8 ± 0.4	0.6 ± 0.1*	0.9 ± 0.3	1.6 ± 0.3*
DAG	1.8 ± 0.4	1.8 ± 0.2	2.2 ± 0.8*	2.3 ± 1.1*	1.2 ± 0.2	2.1 ± 0.1
ES	0.9 ± 0.6	1.4 ± 0.7	0.6 ± 0.1	0.8 ± 0.2	1.0 ± 0.5	1.3 ± 0.3
Other	0.3 ± 0.1	0.8 ± 0.2	0.4 ± 0.1	0.6 ± 0.3	0.8 ± 0.1	1.4 ± 0.4*

Note: P1–Plot 1; P2–Plot 2; VI–June; VII–July; VIII–August; DW–dry weight; MGDG–monogalactosyldiacylglycerol; DGDG–digalactosyldiacylglycerol; SQDG–sulfoquinovosyldiacylglycerol; PC–phosphatidylcholine; PG–phosphatidylglycerol; PE–phosphatidylethanolamine; PI–phosphatidylinositol; PA–phosphatidic acid; DPG–diphosphatidylglycerol; ST–sterol; TAG–Triacylglycerol; DAG–diacylglycerol; ES–sterol esters.

* indicates statistically significant differences between the plots and/or vegetation time ($p \leq 0.05$); means \pm SE (n = 3).

Table 3: The seasonal changes of fatty acids, (FA) composition in leaves of *P. media*.

FA (% of sum total FA)	Vegetation period, months					
	VI		VII		VIII	
	P1	P2	P1	P2	P1	P2
C<16	0.1	0.9 ± 0.1	1.3 ± 0.1	1.7 ± 0.8	1.9 ± 0.0*	1.8 ± 0.1*
C 16:0	16.0 ± 0.5	14.4 ± 1.2	24.6 ± 0.2*	18.9 ± 3.7*	20.9 ± 0.1	17.1 ± 0.0
C 16:1	0.1	0.1	1.5 ± 0.1	4.7 ± 2.1	1.9 ± 0.0	0.0
C 18:0	2.2 ± 0.0	1.7 ± 0.2	3.7 ± 0.7*	3.5 ± 0.4*	0.1	2.8 ± 0.3
C 18:1	2.6 ± 0.1	0.1	4.2 ± 0.9	7.4 ± 4.5	7.7 ± 0.2*	7.5 ± 0.1*
C 18:2	16.0 ± 0.0	15.4 ± 0.5	15.3 ± 0.6	18.8 ± 3.5	17.1 ± 0.1	17.3 ± 1.8
C 18:3	51.2 ± 7.2*	59.9 ± 1.9*	39.6 ± 3.3	35.7 ± 1.5	41.5 ± 2.1*	45.0 ± 0.1*
Other	8.4 ± 0.1	3.3 ± 0.1	9.8 ± 0.7	9.3 ± 0.0	6.7 ± 0.1	7.4 ± 1.3
LCFA	1.3	1.6	7.7	7.4	4.1	2.0
UI	2.2	1.9	1.6	1.6	1.8	1.7

Note: P1–Plot 1; P2–Plot 2; VI–June; VII–July; VIII–August; LCFA–long-chain fatty acids; UI–unsaturated index.

* indicates statistically significant differences between the plots and/or vegetation time ($p \leq 0.05$); means \pm SE (n = 3).

and by 1.3–1.7 times in P2 plants. The relative amounts of C18:2 did not change while amounts increased oleic acid (C18:1). In general the variation of the FA during the summer lead to decreased unsaturation index (UI) in plants of both populations.

Discussion

In this work, we have revealed the specific changes in morphometric and physiological-biochemical characteristics of *P. media* plants of two population groups (P1 and P2). The leaves of P2 plants smaller specific leaf area density, but larger leaf area and biomass. Additionally, P2 plants had a longer root system. Hence, at the level of the entire plant, acclimation to the environmental (phytocenological) conditions is seen as changes of the morphometrical parameters and biomass structure.

All the examined plants were in the phase of flowering. During the summer period, a successive shift of phenological phases occurred from the beginning of flowering in June to full-scale flowering in July and, finally, the fading in August [4].

The concentration of lipid peroxide may indicate a successive change of phenological phases of ontogenesis from breaking out into blossom to full flowering and blading (Figure 1).

The changes of morphometric parameters of leaves were accompanied by alterations in the lipid content and composition. It is known that leaves are usually rich in GL (Figure 2). Their content in the leaves of *P. media* plants correlated with leaf area ($r=0.77$ at $p<0.05$) and did not depend on the environmental conditions. With the general reducing the amount of TL during summer, the contribution of the GL varied from 53 to 58% and from 46 to 56% of TL in P1 and P2 plants, respectively. However, the GL composition and the content of individual GL underwent more substantial changes. The highest content of MGDG and DGDG was determined in June. In the course of growth and development, the contribution of DGDG and SQDG in the pool of GL increased.

This resulted to the fact that in the last month of summer the ratio of MGDG/DGDG reduced. It is known that DGDG and PG participate in stabilizing the structure of the light-harvesting complex of the photosynthetic apparatus. These components affect the formation and stabilization of the grana [30]. One of the functions SQDG is their participation in the stabilization of the complex ATPase [31]. The changing of the ratio of individual GL, namely the increase of DGDG and SQDG, is apparently aimed at preserving the functional structure of the photosynthetic apparatus by changing architectonics thylakoids [32].

During the summer vegetative season, a decline in the leaf content of PL in P1 and P2 plants has been also demonstrated. A strong correlation was found between changes in the morphometric parameters and contents of individual PL: when the leaf area decreased, the ratio of PC/PE, the main structural lipids of non-plastidial membranes, also decreased ($r=0.83$ at $p<0.01$). The physiological significance of the increasing proportion of PC, apparently, is the local change in membrane fluidity by changing the composition of the FA.

A common alteration in PL dynamics observed in both leaves was an increase in the content of PA. Since PA and PI are signaling molecules, their simultaneous increase in the leaves may be related to the activation of signaling systems in order to transit to the next level of regulation of physiological processes. Evidences to switching to the different metabolic status in August are the accumulation of lipid peroxidation products indicating the beginning of leaf ageing. The elevated PA content in leaves occurred with a concomitant decrease in PC and PE levels, which may indicate an activation of phospholipase D, whose preferential substrates are most often PC and PE [33,34]. The accumulation of PI can be considered as its storage; the products of PI hydrolysis are DAG, an activator of protein kinase C and inositol-1,4,5-triphosphate, a regulator of Ca^{2+} exchange in the cell [35].

In contrast to PL, the content of NL in the leaves of *P. media* increased in the course of growth. A high correlation was found between the content of NL and leaf area ($r=0.94$ at $p<0.05$), which can be explained by the formation of lipid plastoglobules whose size increases as the leaves grow [36]. It should be noted that, in whole,

the character of changes in the leaf content of GL, PL and NL in P1 and P2 plants was identical but depended on the phase of vegetation.

As for NL, an increase of DAG percentages in leaves has been demonstrated, and it may be related to the formation of structural and energetic reserves in *P. media* cells. In July, the content of sterols in the leaves declined, with the effect being reversed in August. The sterols being one of the structural components of cellular membranes. The presence of ST compounds in membranes their more rigid structure and less flexibility [37].

The functional properties of lipids also greatly depend on their FA composition. A study of the FA composition of TL depends on the environmental conditions and plant vegetation phase. It should be mentioned that changes in the leaf FA content depended not only on the phase of vegetation and environmental conditions but were specific for every FA. In the leaves, for example, the content of C16:0 varied, in different periods, from 15.9 to 24.9% in P1 plants and from 16.2 to 18.9% in P2 plants. In general, however, these results confirm the known fact of the individual role of FA in membranes [38]. In general, the variation of the FA during the summer lead to decreased UI in plants of both populations. Therefore, the composition of the FA also depends on the environmental conditions and the period of growth.

Due to the method of canonical correspondence analysis, the connection between averaged characteristics of each type of lipids and the gradients of environmental factors and their combinations was established (Figure 3). The vectors indicate the directions and the force and effect of corresponding parameters. One-directionality of the vectors, characterizing morphometric and biochemical parameters, shows a positive correlation: the closer both types of the vectors to each other are, the stronger it is. The opposite directions of the vectors indicate a negative relationship between the respective characteristics. The angle of 90° shows a very low correlation parameters.

The main factors having the greatest effect on the studied parameters on P1 were light and temperature. So, in June specific leaf area density, the relative content of C18:3 in the leaves, increased, and in August – the level of lipid peroxidation and dry mass. P2 was greatly influenced by soil moisture, the presence of which helped to

increase the width of leaves, the root length, the content of GL and PL in the leaves as compared with P1 for all three months of the research.

Conclusion

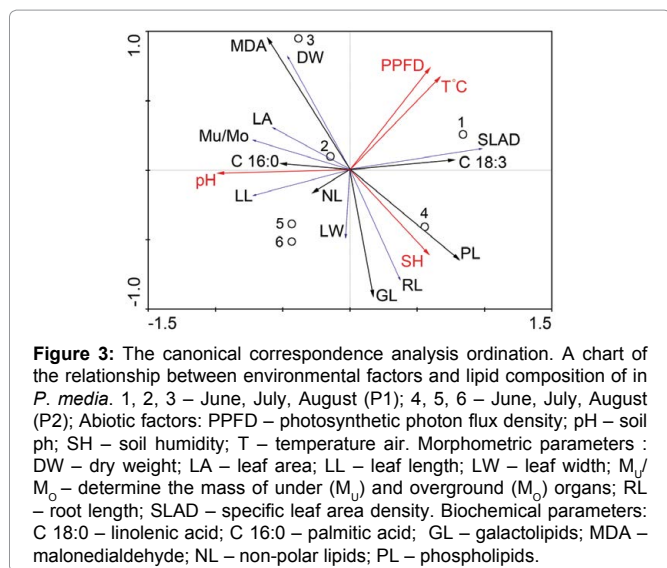
The result of our complex study showed that gradual changes of morphometrical parameters were accompanied by the alterations in biomass structure and modifications in lipids and FA. The latter depended on both, ontogenetic stage and environmental conditions. Our data supported the fact that lipid composition and content vary during the vegetative period, and these compounds are involved in acclimation to the changing environmental conditions. Our findings should be taken into account when considering *P. media* as a source of biologically active lipids and FA.

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