

## Research Article

Lipid Content Variation in *Plantago media* Leaves in Response to Light ConditionsOlga Rozentsvet<sup>1</sup>, Tamara Golovko<sup>2</sup>, Viktor Nesterov<sup>1</sup>, Elena Bogdanova<sup>1</sup>, Galina Tabalenkova<sup>2</sup>, Iljia Zakhochiy<sup>2</sup>, Igor Dalke<sup>2</sup>

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**Abstract:** The aim of the present work was to study the variation of lipid and fatty acids composition as well as morphometric characteristics of *Plantago media* leaves from the different light conditions in northeastern Russia. The leaves of the plants grown under lower light had lower leaf mass/area ratio but larger areas of lamina. They accumulated lower levels of lipid peroxide products. It was found that as the duration of sunshine increased, the content of total lipids increased ( $r = 0.78$ ), but an increase in temperature resulted in a decrease of their content ( $r = -0.70$ ), especially for plants in high-sunshine habitats. Concentration of GL in leaves of shaded plants increased with increasing precipitation but decreased with increasing temperature and the duration of sunshine. Amount of saturation FA increased with increasing temperature and the duration of sunshine. Precipitation contributed to the accumulation of unsaturation FA. Thus, the content of lipids in the leaves depended on weather and microclimate conditions.

**Key words:** *Plantago media*, fatty acids, lipids, plasticity.

## Introduction

In nature, plants are subjected to simultaneous effects of various ecological factors. 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 [1]-[5].

Estimations of the influence of climatic changes on plants are based mainly on morphogenetic criteria. Eco-physiological and biochemical characteristics are used more infrequently, though they determine the genotype plasticity and ability to survive in extreme conditions. Many cellular compounds may be involved in these regulatory and metabolic reactions, and lipids and fatty acids are undoubtedly the best known molecules in such adaptive responses. In general, lipids play a number of important roles in all living organisms [6]. Structurally they can be divided into two major groups: the non-polar lipids (NL)

and polar lipids. Of the NL, the triacylglycerols are common storage products, which can be easily catabolised to

provide metabolic energy. Polar lipids and sterols 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 signaling pathways and play a role in responding to changes in the environment [7]-[8].

Species of Plantaginaceae (about 200 plants) are found all over the world representing an important part of many ecosystems. The large natural diversity of ecotypes can be evidently explained by varying strategies of plant adaptation to climatic conditions. The information on adaptive strategies of these plants is scarce; only few publications are available on the role of lipids in the adaptation of *Plantago* species to their specific environment. For example, the lipid composition of the roots of *Plantago* species has been studied as a response to

alteration in the mineral nutrition and some ecologic factors [9].

*P. media* is a perennial polycarpic plant, which is characterized by moderate demands for soil-climatic conditions. It is an Eurasian boreal species, its areal covers Europe, Siberia, Western and Central Asia [10]. In our earlier work, we studied the daily dynamics of membrane lipids in *P. media* collected from midland regions of Russia [11].

The aim of the present work was to study the variation of morphometric characteristics, lipid and fatty acids composition of *P. media* leaves from the different light conditions in northeastern Russia.

**MATERIALS AND METHODS**

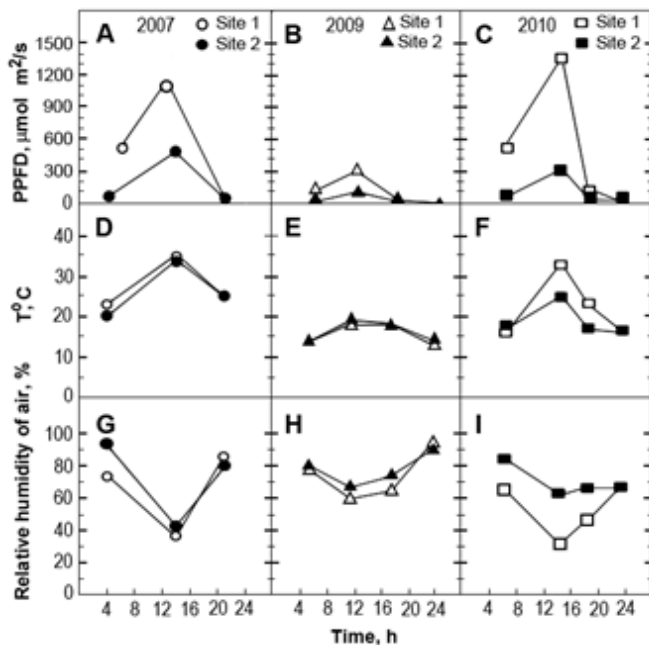
**Sampling site and plant material** The region of study was in South Timan (latitude 62°45' N, longitude 55°49' E) in the southern part of the Timan Ridge, which is an important orographical structure of the northeastern section of the European part of Russia [12]. The mean annual air temperature in the region of study is -1.5°C; the mean temperature of the warmest month (July) is +15°C. The frost-free season lasts 76 days, on average.

The characteristics of weather conditions during the study were provided by the Meteorology Centre of Komi Republic (Table 1).

Parameter	2007			2009			2010		
	June	July		June	July		June	July	
	III	I	II	III	I	II	III	I	II
Average air temperature, °C	17.6	24.6	21.3	10.6	11.0	16.7	16.4	16.3	16.2
Deviation from the norm	2.0	9.0	-5.0	-4.0	-5.0	0	2.0	1.0	0
Precipitation, mm	84.0	1.0	18.0	18.0	50.0	17.0	4.0	7.0	2.0
Precipitation, % of the norm	443.0	5.0	78.0	82.0	217.0	74.0	18.0	30.0	9.0
Duration of sun shining, h	90.0	141.0	97.0	100	42	110	105	102	115

The centre was about 20 km away from the sites of study. Microclimatic parameters (air temperature and humidity) at the sites were measured with a portable meteorological station (Data Logger LI-1400, USA) (Fig. 1).

*P. media* plants grew on a sparsely grassed flank of the southeastern exposition (open site 1, S1 – light type) and a thick-grassed terrace at the mountain foot (shaded site 2, S2 – shadow type). The site area was approximately 700 m<sup>2</sup>. Leaves from 12–15 typical plants were collected. To measure the linear sizes (length, width) and leaf area, images of 10 leaves were collected together with a scale rule using a digital camera (DMC-TZ3 Panasonic, Japan). The data were processed in Image Tool for Windows, v. 3.00 software. The leaves were further weighed and dried at 80°C. The leaves of S1 and S2 plants were compared by linear size, area, and leaf mass/area ratio (LMA, g m<sup>-2</sup>).



**Fig. 1:** The habitats of growing plants of *Plantago media* plants in open and shaded sites. PPFD – photosynthetic photon flux density.

**Biochemical 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 biomass. The samples were treated with hot isopropanol and kept in a cold, dark place prior to analysis.

Lipid peroxidation intensity in the plant leaves was determined by measurement of malondialdehyde (MDA) concentrations after reaction with thiobarbituric acid [13]. Fluorescence intensity was measured using a spectrophotometer Specol (Germany) at 532 nm.

Lipids were extracted three times using three times chloroform/methanol (1:2, v/v) by the method of [14]. The combined extracts were purified from non-lipid compounds and concentrated using a rotary vacuum evaporator.

The quantification of phospholipids (PL) was performed by the content of inorganic phosphorus [15] with the following calculation of their molar masses. 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 [16] using a spectrophotometer Specol (Germany) at 620 nm. Monogalactosyldiacylglycerol (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 NL, GL- and PL.

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 105m x 0.25mm 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<sup>-1</sup>, held at 220°C for 15 min. FAMES were identified by comparing retention times with fatty acid standards (Supelco 37, Supelco, USA).

#### Statistical analysis

Statistical significance between groups was assessed by Student t-test ( $p < 0.05$ ). The data are present as means  $\pm$  standard errors ( $n=10-12$ ). Relationship between morphometric parameters and lipid characteristics was measured using a Spearman's correlation ratio.

## Results

### Habitats of growing plants

The experimental period of 2007 was the warmest and sunniest; in 2009, it was cold, with short periods of sunshine. In 2010, there was a relatively small amount of precipitation interspersed in sunny and warm weather (Table 1). The weather conditions exerted a substantial effect on the microclimate in the plantain habitats. As seen in Figure 1, S1 plants (light type) received more light and heat on clear, sunny days during 2007 and 2010, especially in the first half of the day. The intensity of photosynthetic active radiation (PAR) at the plant level was 400–500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  already by the early morning hours; by midday, it reached 1000–1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The maximal light of S2 plants (shadow type) was three times as low as that of S1 plants. The differences in the light regime of the two plantain habitats were also maintained on overcast days with dense cloud cover (2009) (Fig. 1B). The relative humidity at the plant level varied widely, dropping considerably at the midday hours, especially on sunny days (2007, 2010) (Figure 1G and I). Thus, the habitats of *P. media* plants differed substantially by microclimate conditions, predominantly by light regime. The environmental conditions constantly changed throughout the day and varied depending on the weather.

### Morphometric data

*P. media* S1 plants formed smaller leaves compared to S2 plants (Table 2). In all years of study, the lamina of S2

plants was approximately twice as long as that of S1 plants. The differences in the lamina width were evident in 2009 and 2010.

The specific leaf area (SLA) of S1 plants was 2–4 times lower than that of S2 plants. However, it is worth noting that the year-wise differences in the SLA were more pronounced in S2 plants than in the S1 ones.

**TABLE II**

The Morphometric Characteristics of Leaves of *P. media* from the Open (S1) and Shaded (S2) Sites

Parameters	2007	2009	2010
Site 1			
Leaf length, mm	55 $\pm$ 3	53 $\pm$ 2	46 $\pm$ 2
Leaf width, mm	23 $\pm$ 2	24 $\pm$ 2	19 $\pm$ 1
SLA, mm <sup>2</sup>	830 $\pm$ 80	880 $\pm$ 80	650 $\pm$ 40
LMA, g/m <sup>2</sup>	87	120	93
Site 2			
Leaf length, mm	91 $\pm$ 4*	115 $\pm$ 8*	100 $\pm$ 4*
Leaf width, mm	28 $\pm$ 2	44 $\pm$ 3*	37 $\pm$ 2*
SLA, mm <sup>2</sup>	1810 $\pm$ 145	3580 $\pm$ 470*	2510 $\pm$ 150*
LMA, g/m <sup>2</sup>	45	55	52

Note: \* indicates statistically significant differences between the sites ( $p \leq 0.05$ ); means  $\pm$  SE ( $n = 10-12$ ).

The largest values of SLA were registered in the dampest and coldest season (2009). Similar data have been observed for *P. major* plants growing in different light conditions [17]. Plants typically respond to shade by producing leaves with less mass per unit area [18, 19]. Leaves with high LMA are usually thick and dense and have low values of specific leaf area (SLA). A plant with such leaves grows more slowly than high-SLA plants [20]. Studies have shown many LMA values are positively correlated with relative growth rate and maximum rate of photosynthesis [21]. Our data showed that the greatest values of the LMA corresponded mostly to cool and wet conditions (2009). Therefore, both, different levels of light during the year, the weather conditions can impact the linear dimensions of *P. media* leaves, their area, and mass.

### Lipid peroxidation

The intensity of lipid peroxidation is one of the functional characteristics of plant cells [22]. The content of MDA, an indicator of peroxidation of lipids (Table 3), in the plantain leaves varied widely from 3.5–30  $\mu\text{mol g}^{-1}$  dry weight.

**TABLE III**

The Content of Malondialdehyde (MDA,  $\mu\text{mol g}^{-1}$  dry weight) in the Leaves of *P. media* Plants Growing in Open (S1) and Shaded (S2) sites

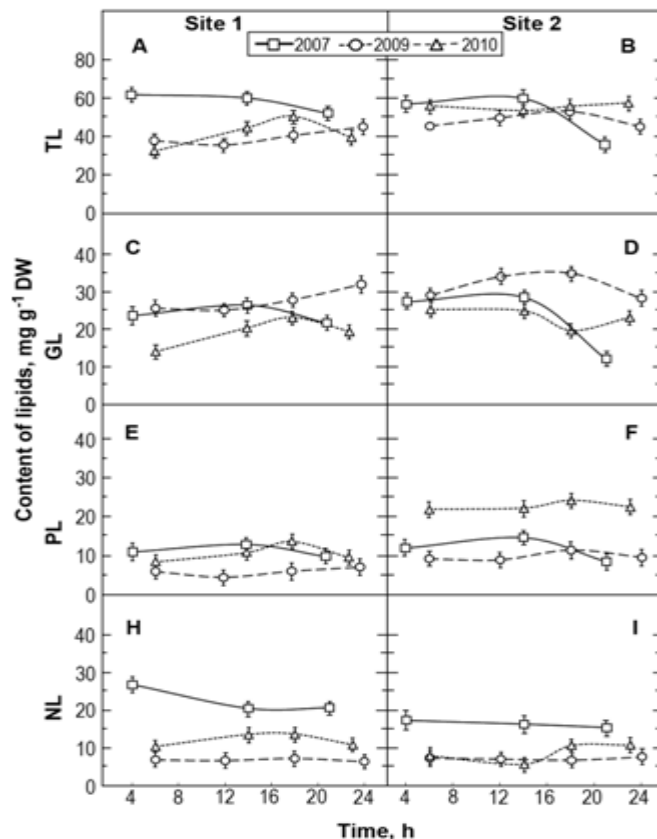
	Time of day, h					
	Site 1			Site 2		
	4 - 6	12 - 14	21 - 23	4 - 6	12 - 14	21 - 23
TL	11.2±0.5	30.2±2.7	18.8±1.4	10.9±0.6	22.1±1.7	13.2±1.7
GL	NA	3.3±0.3	5.8±0.6	NA	3.6±0.5	5.0±0.5
PL	15.0±1.4	20.7±3.3	20.3±4.4	10.0±2.3	15.7±2.7	9.3±1.5

Note: NA - not measured.

In the warm years (2007 and 2010), S1 plants had a higher MDA content in their leaves compared to S2 plants; in the cold, low-sunlight, and rainy year (2009), the level of MDA was equally low in leaves of both S1 and S2 plants. In the warmer season of 2007, an increase in MDA was observed in the afternoon compared to morning hours. In the warm and dry 2010 season, lipid peroxidation was revealed only in the leaves of S2 plants. In the leaves of S1 plants, a high MDA level was maintained until late in the evening. The differences in the content of MDA between S1 and S2 plants were larger in the warm season with sunny days.

### Content of lipids

The morphometric parameters' change of leaves occurred when lipid composition was modified. The content of TL was 1.3–1.5 - fold higher in dry and warm years (2007 and 2010) than in cool and rainy ones (2009) (Fig. 2A and B). The greatest differences in TL between plants at S1 and 2 occurred in 2007.



**Fig. 2** Daily changes in lipid contents in the leaves of *Plantago media* plants in open (A, C, E, H) and shaded (B, D, F, I) sites. TL – total lipids; GL – glycolipids; PL – phospholipids; NL – non-polar lipids. For Figs. 2, 3, values shown are means ± SE from triplicate experiments., and the asterisk indicates a significant difference at  $p \leq 0.05$ .

The major components of TL (> 75%) were polar GL and PL. Leaf concentrations of GL were higher in 2009 in comparison to other years. Also the concentration of GL was higher in shaded S2 plants during the day compared to S1 plants (Fig. 2C and D). The lowest level of GL was observed when there was the most stable number of hours of sunshine, for instance in 2010.

The PL amounted to about 25% of total lipids. As seen in Fig. 2, the year-related climatic conditions affected the PL content. In the cold and rainy 2009 year, PL concentration in leaves was lower than that in the other (drier and warmer) years (Fig.2 E and F). During all years, the leaf content of PL in S2 plants was higher than that in S1 ones. The differences were larger in the dry and warm season of 2010. However, variations in daily flow of total PL content were less significant in comparison with GL.

The NL are referred to as storage lipids, and their content is comparable to that of PL (Fig. 2H and I). In 2007 and 2010, the content of NL in S1 plants was higher than that in S2 plants. Plants of both groups accumulated the largest quantities of NL in their leaves in 2007, when the season was warm and amount of precipitation was sufficient. In the colder and rainy 2009 year, the leaves accumulated less NL.

### Fatty acid composition

About 20 FA were identified in the leaves of *P. media*, with C<sub>16</sub> and C<sub>18</sub> acids comprising more than 90%. The leaves of the plantain plants had a relatively low FA content, containing some hydrocarbon chains shorter than 16 carbons (< 5%) and less FA with hydrocarbon chains longer than 20 carbons (< 2%). The main component of unsaturated FA (USFA) was linolenic acid (C<sub>18:3</sub>; 33–58% of total FA) and predominant saturation FA (SFA) was palmitic acid (C<sub>16:0</sub>; 18–26%). After integrating the data of all three years, the major FA can be depicted in a series based on their content in the following order: C<sub>18:3</sub> > C<sub>16:0</sub> > C<sub>18:2</sub> > C<sub>18:1</sub> ≥ C<sub>18:0</sub>.

The differences in the FA contents between S1 and S2 plants depend on the environmental conditions. So, in 2009 (Figure 3C, D) the levels of trienoic FA were higher in S1 and S2 plants compared to those in 2007 (Figure 3A, B) and 2010 (by 10–20%) (Fig. 3E, F). The lowest content of monoenic FA was registered in 2009 (about 5% of total FA). The highest content of USFA was observed in the leaves of plants in the cold 2009 year.

### DISCUSSION

The results of our work show that variation of weather and microclimate conditions affects morphological and

biochemical features of leaves of *P. media* plants growing in different light condition of northeast Russia (South Timan). Plants on the open site formed smaller leaves with a high LMA. Laminas of shaded plants were larger and had a low LMA value. The LMA variability of plantain leaves seems to relate to differences in their anatomy and chemical composition.

Our data also show the effects of environmental on leaf content of MDA, an indicator of lipid peroxidation. In S1 plants, the level of lipid peroxidation was higher than in S2 ones. The differences in the MDA contents between S1 and S2 plants were larger in the warm season with sunny days. This can indicate a stronger effect of reactive oxygen species on the lipid components of cellular membranes in the leaf tissues of plants growing in high amounts of light and warm air temperature. At high light intensities, there is a higher probability of electrons in the electron transport chain to leak onto oxygen molecules, forming free radicals [23]. In the damp and cold season of 2009, when illumination of plants was low, the differences in the MDA contents between S1 and S2 plants almost completely disappeared. A more pronounced daily dynamic of NL and MDA in 2007 and 2010 may indicate a more intensive lipid exchange in warm, dry years. Perhaps, the storage and metabolically important NL, especially triacylglycerides, were used by mesophyll cells to repair the membrane lipids damaged in the process of lipid peroxidation.

2007; C, D – 2009; E, F – 2010; M – monoenic; D – dienic; T – trienic; SFA – saturation fatty acids.

The more light and warm temperature *P. media* plants received, the less GL was found in their leaves. Since GL are the main lipids of the thylakoid membranes, which contain photosystems with light-harvesting pigment-protein complexes, they are probably maintained at a certain ratio to chlorophylls. This observation may indicate a change in the number of chloroplasts and their structure, such as changes in the number and size of grana of chloroplasts that affected the change in the photosynthetic intensity. Plant leaves contained less PL in the cool year (2009) than in warm and dry years. The greatest changes in the composition of the main PL occurred in 2010. The content of PL and NL were more dependent on light conditions than the content of the GL.

Modification of the FA pool is considered to play a key role in the adaptation of organisms to environmental conditions [24]. Data in the literature indicate that changes in the composition and content of FA have an evident adaptive character; they adjust physiologically vital properties of cellular membranes, primarily fluidity [8, 25]. We found that high rate of light (S1) and sunny days (2007, 2010) lead to increased content of monoenoic FA. On the other hand, in a cold year (2009) noted the largest content of trienoic FA. As a result, ratios of the SFA and USFAs varied.

Using a mathematical method, we determined the degree of influence of individual factors on the composition and content of lipids (Table 4).

TABLE IV

Correlation between the content of lipids in the leaves of *Plantago media* plants and ecologic factors

Lipids	Spearman's correlation coefficient					
	Temperature, °C		Precipitation		Duration of sun shining, h	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
TL	-	-0.05	-	0.34	0.78*	0.34
GL	0.70*	0.48*	0.68*	0.68*	0.68*	0.68*
PL	0.72*	0.31	0.75*	0.79*	0.75*	0.79*
NL	0.94*	0.75*	0.54*	-0.20	0.54*	0.20
SFA	0.72*	0.75*	0.59*	0.44*	0.59*	0.44*
USFA	-	-	0.57*	0.61*	0.57*	0.61*

Note: the asterisk indicates a significant difference at  $p \leq 0.05$ .

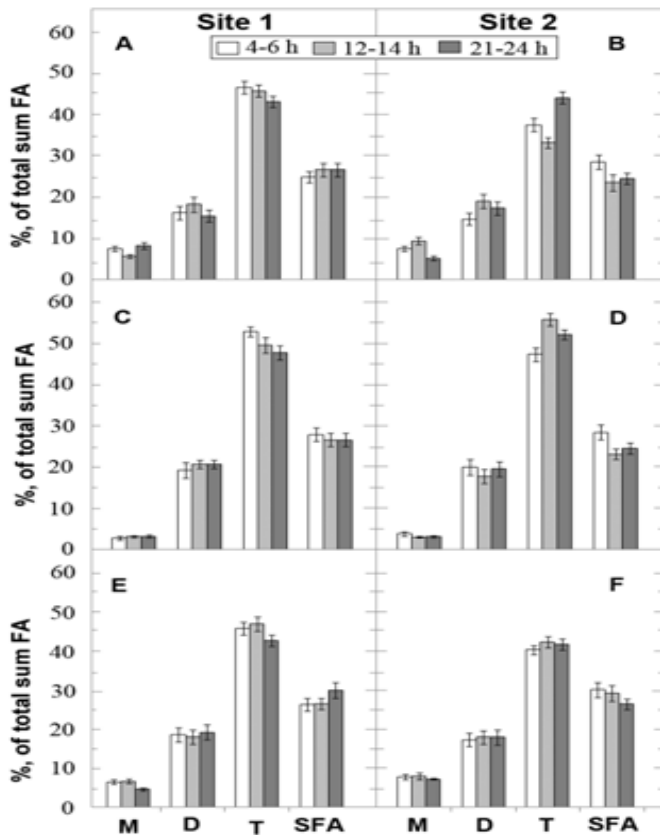


Fig. 3: Daily changes in main fatty acids contents in the leaves of *P. media* plants in open and shaded sites. A, B –

As the duration of sunshine increased, the content of total lipids increased ( $r = 0.78$ ). At the same time, an

increase in temperature resulted in a decrease of their content ( $r = -0.7$ ), especially for plants in high-sunshine habitats. Concentration of GL in leaves of shaded plants increased with increasing precipitation but decreased with increasing temperature and the duration of sunshine. The same effect is exerted by rainfall on PL content. Weather factors had a statistically significant but opposite effect on the contents of FA. Amount of SFA increased with increasing temperature and the duration of sunshine. Precipitation contributed to the accumulation of USFA.

## CONCLUSIONS

Thus, the changes in lipid and FA composition have been studied in *P. media* leaves in response to light conditions in northeastern Russia (South Timan). The content of lipids in the leaves depended on weather and microclimate conditions. In general, our results suggest that the variability of lipid content, as well as morphological variability of leaves are functional features that define ecological plasticity of *P. media*. Apparently, due to their ecological plasticity, *P. media* plants can occupy different ecotopes, and, as a result, they have a wide geographic distribution.

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