Physiological research on some species of the genus *Plantago* L. from Dobrogea region

Ivan Mihaela Aurelia

1Alexandru Ioan Cuza University of Iasi, Faculty of Biology

*Corresponding author. Email: michelin_alina@yahoo.com

Abstract  *Plantago* genus comprises 35 species, found in European area: in Romania vegetate 17 species, all of them spontaneous. Three species (*P. major* L., *P. media* L. and *P. lanceolata* L.) are cultivated for their medicinal properties, recognized both in modern, as well in traditional medicine. Due to their similar chemical composition, these three species play mostly multiple and convergent pharmacological functions. The present paper aims to study physiological comparative aspects regarding *P. lanceolata* L., *P. coronopus* L. and *P. maritima* L. from Dobrogea, in order to establish their value as indicator species for saline environments. The degree of hydration in foliar tissues has been gravimetrically determined, and photosynthetic pigments determined by spectrophotometric assay. The analyses concerning processes of photosynthesis and transpiration were performed with LCI portable system on field. the LCI portable system uses infrared rays for the determinations, in this way the plant is not harmed in any way. Statistical analyses of the results has been conducted, using calculation of correlations. The comparative analyses of the biochemical and functional parameters show the existence of positive correlations, suggesting that these parameters are closely linked.

Key words

photosynthesis, transpiration, foliar pigments, LCI system

Abreviations used in graphic representations: *veg.* – vegetative period; *inf.* – flowering period.

The genus *Plantago* includes about 260 plant species on the European continent, some of which are used in pharmaceuticals, cosmetics and food. Intense use in traditional medicine and modern species that belong to the genus *Plantago* is a consequence of the remarkable variety of curative properties: astringent, expectorant, antimicrobial, anti-inflammatory and diuretic [8, 17, 21]. In addition, some *Plantago* species included in the diet, being consumed as fresh in salads, soups, side dishes or being used for making teas. Seeds of some species can be boiled and used as a source of starch, or after grinding, can be added to flour for making bread or cakes of sorts [7, 8].

The ethnopharmacological importance [9, 15] of the *Plantago lanceolata* L., *Plantago coronopus* L. and *Plantago maritima* L. species collected from Dobrogea led to the initiation of a physiological study relating to regarding the activity that represents the purpose of this research. We hypothesized that there might be interspecific differences in physiological processes in the species taken into study.

Materials and Methods

The physiological investigations were performed on individuals belonging to species *Plantago lanceolata* L., *Plantago maritima* L. and *Plantago coronopus* L. spontaneous in Dobrogea. *Plantago coronopus* L. which vegetates in two habitats located at different distances from the Black Sea shore: a habitat represented by the seaside itself (shorthand notation Z I) and a habitat located at approximately 1000 m distance from the shore (shorthand notation Z II). The determinations have covered the vegetation period of 2012, being achieved in two different ontogenetic moments (vegetative and flowering).

For the physiological processes (photosynthesis, respiration, foliar transpiration), and for the determination of CO₂ in the substomatal cavity was used the portable system for in vivo measuring Lci (ABC BioScientific Ltd, Hoddesdon, Great Britain). The device has a non-destructive system for measuring physiological processes on the leaf, in this way it doesn’t appear any stressful factor on the foliar surface, which could have a negative role on the increase expression of these processes. LCI is designed to carry out precise measurements of physiological parameters, by controlling the leaf chamber environment. The registered parameters, are calculated automatically based on recognized formulas.

The water content and the dry matter content were gravimetrically determined by maintaining, with plant material bringing to constant weight by drying at
105°C, the results were calculated percentage [1]. The chlorophyll pigments were extracted from the leaves using acetone solution 85% solvent [2].

The concentration of foliar pigments was determined by the spectrophotometric method Mayer-Bertenrath [2]. For the determination of the absorption spectra of assimilatory pigments was used an extract of pigments in acetone 85%. Extinctions leaf extracts were read at UV-VIS spectrophotometer at characteristic wavelengths for maximum absorbance (663, 645 and 472 nm).

The Pearson correlation was used for statistical analyses to determine the relations between biochemical and functional parameters. This coefficient of the correlation shows how strong are the relationships between biochemical and physiological processes at the species taken into study.

The correlation coefficient (r) can take values from 0 to +1. If the correlation coefficient r is close to ± 1, this means a very strong correlation and if r > 0 this means a positive relationship [22]. Statistical data were processed and represented graphically in EXCEL (Windows 2007).

Results and Discussions

The content of chlorophyll pigments registered a large range of values comprised between 0,347-0,684 mg pigments/g fresh material for chlorophyll a and between 0,130-0,239 mg pigments/g fresh material for chlorophyll b (Fig. 1). In the vegetative/flowering phenophase the ratio between chlorophyll a and chlorophyll b is higher than 3. Exception to this statement is observed in vegetative phenophase of the Plantago maritima, where the chlorophyll a / chlorophyll b ratio is 2,87 and in the flowering phenophase of the Plantago lanceolata, the chlorophyll a / chlorophyll b ratio is 2,98 (Fig 2). The proportion chlorophyll a/b has values comprised between 2,86 - 3,10 which indicates a specific pace of biosynthesis of the two types of chlorophylls at every species [19, 18]. The proportion chlorophyll a/b is a parameter that could be an expression of interspecific differences and adaptability of plants to the condition of luminosity [14].

Some authors quoted by Masarovičová E., and Eliáš P., (1980) showed that one of the indicators of the photosynthetic capacity of plants is the quantity of chlorophyll [6]. Also according to the study made by
Gabrielsen (1948), the intensity of photosynthesis increases simultaneous with the increasing of chlorophyll content [3].

For the analyzed species, there was found a significant positive correlation between the intensity of photosynthesis and chlorophyllian pigments content (Fig. 3), the correlation coefficient value showed small variations in both analyzed phenophases. The values of this index are between 0.931 (for Plantago lanceolata) and 0.985 (for Plantago maritima) in the vegetative stage; the values for flowering phenophase register.

The highest variations of the water content can be noticed in leaves because the leaves has the biggest contact surface with the atmosphere [14]. Water can control the temperature of the plant tissue, protecting them in this way from reaching to very high values of their temperature, in co-relation with excessive temperatures [5, 23, 12]. The watercontents register values between 80.44% – 87.90% in leaves. In the leaves the highest values of water contents have been recorded at the species Plantago coronopus (Z II). In the climatic conditions in June 2012, to the investigated species, were found in the dynamic different degrees of hydration of leaf tissue (Fig 4).
The dry matter contains mineral elements and the organic substances from the analysed organs and reflects the biomass accumulation [14]. In our case, the dry matter content registers values between 12.1 and 19.5 g % in leaves. Has been registered that during the flowering period at P. lanceolata and P. maritima a high progressive dry matter content to the detriment of hydration during vegetative period, where the foliar tissues are very hydrated (Fig. 4). At the species Plantago coronopus during the vegetative period the high dry matter content to the detriment of hydration can be observed at all the species P. coronopus ZII.

The analysis of leaf dry matter content, performed in vegetative and flowering phenophase to the investigated species revealed its inversely proportional variation compared with the degree of hydration foliar (Fig 4).

Water content of the leaves is one of the most important indices of the vital activity of plants, between the degree of saturation of water and leaf photosynthetic capacity of the plant being established an approach [16; 19]. The value limits between the water content in the leaves of the investigate species are between: 80.47% (P. coronopus Z I) and 87.91% (P. coronopus Z II); the values were correlated with the intensity of the photosynthesis process (Fig. 5).

Another operational parameter on which we focus in our research was the photosynthesis process, recorded in vivo, in the subsequent development of the two captured moments of their ontogenetic cycle.

Referring to this aspect, we can say that, according to general data the dynamic process of the photosynthesis is correlated with the investigated species and the phenophase, the substomatal CO₂ content and the foliar chlorophyll pigments content.

The results obtained by us on this line confirms the statements presented above, species on which we focus, present in our opinion an intense plant metabolism in both analyzed life moments (Fig.6.).
Some authors established a correlation between internal concentration of CO$_2$ (Ci) and photosynthesis [4; 12; 19]. In the vegetative phenophase at _Plantago coronopus_ ZII species, the increased photosynthesis is directly proportional to the amount of substomatal CO$_2$ and can be explained by stomatal opening that releases a large amount of CO$_2$ (Fig. 7).

Increasing of the substomatal CO$_2$ (Ci) is correlate with the photosynthesis rate, but at _Plantago lanceolata_ in the vegetative period, the increasing of the concentration of the Ci which not led to the increasing of the photosynthesis. This aspect can been explained by the closing of stomata, this could take place in some condition of increased humidity, sun exposition, and plant species [1]. The decreasing CO$_2$ concentration from the substomatic cavity, as well is consumed in assimilation process [13].

Water is necessary to maintain an intense transpiration, that occurs during the process of photosynthesis [5, 16]. The photosynthesis and transpiration intensity increase with the degree of hydration of the plant. The increasing of photosynthesis rate, transpiration rate, respiration rate has been explained by the increased of the substomatal CO$_2$ concentrations.

High water content of leaf acts by stomata opening which results in a good supply with CO$_2$ and an intensification of photosynthesis and transpiration [16, 13]. The exception is _Plantago maritima_ which had the lowest intensity of the transpiration process, was characterized by a high water content in leaves, and the explanation could be related to reduction of stomatal opening (Fig. 7).

The average values obtained from the undertaken investigations during the ontogenetic cycle of the _Plantago_ genus species taken into study, allows us to consider, first that the respiration intensity varies in strict correlation with the phenophase and the degree of the foliar hydration (Fig. 8).
Since there was expressed the hypothesis that transpiration, being a phenomenon of active transport of water, requires the consumption of the energy supplied by respiration [10], we tried to analyze whether certain correlations are established between the intensity dynamic of the respiration and transpiration processes (depending on the ontogenetic stage of the plant individual), and their water status (water content at the foliar level) (Fig. 9.)

We observe the establishment of a positive correlation between the respiratory intensity and transpiration intensity, thereby confirming with the literature data [16]. According to the most of the results thus obtained, the respiration intensity influences the water elimination processes, promoting the transpiration [16; 20; 23].

Between the respiration and the transpiration intensity there is not a certain correlation report, the most taken into discussion parameter in literature to influence the established correlation is the type of plant, the species developing in time specific adaptive responses to the environment in which they grow [11; 16].
Conclusions

The results from the determination of water content in the leaf tissue present quantitative fluctuations based on phenophase and species. The highest values were registered at *Plantago coronopus* and *Plantago maritima* (85.14–87.91%) which indicates a higher water necessity of these species.

The water content and dry matter foliar is balanced in all species, values allowing us to indicate corresponding hydration foliar of the mesophyll, indicating a normal of metabolic processes of the plant.

Chlorophyll content of leaf pigments differ depending on vegetative stage, with elevated values, observing a dynamic content of chlorophyll pigments during the ontogenetic phenophases (vegetative stage and flowering stage). According to the literature [3], the intensity of photosynthesis registered a large range according to species and ontogenetic moments (vegetative and flowering) the maximum value has been registered in *Plantago coronopus* (3.39 mmol CO$_2$ m$^{-2}$s$^{-1}$) and a minimum value in *Plantago lanceolata* (0.80 mmol CO$_2$ m$^{-2}$s$^{-1}$).

Positive correlations obtained from statistical calculations between the transpiration, respiration and presence of water in leaf tissues indicate a dynamic intensity of the two physiological processes according with vegetative and flowering phenophase and content of leaf water. Water content values recorded allow the constructions of all metabolic reactions that involve the presence of water for the good development of the plant throughout the growing season.

References

5. Duca Maria, 2006, Fiziologia plantelor. Edit. Știința, Chișinău, Republica Moldova p. 49-52; 99-100;