INTRODUCTION

The non-halophytic medicinal species of the genus *Plantago* L. (recognized by *Romanian Pharmacopeia*), occupies an important place among cultivated species, which are generally sensitive to increased soil salinity. From the literature review we noted a lack of information on salinity tolerance of the genus *Plantago* L. species analyzed from the Romanian spontaneous flora (under natural field conditions), as well as the low amounts of physiological and biochemical data that can help in understanding and interpreting the mechanism of adaptation to soil salinity of the halophytes.

PERSONAL RESULTS

GOAL, OBJECTIVES, MATERIAL AND WORK METHODOLOGY

This thesis aims to deepen the knowledge about the adaptation strategies of halophyte species (*Plantago maritima* L. *Plantago schwarzenbergiana* Schur., *Plantago coronopus* L.) and some non-halophytic species (*Plantago lanceolata* L. and *Plantago media* L.), from SE and NE areas of Romania by revealing some morphological, anatomical, physiological and biochemical adaptations specific to thermo-hydric and salt stress.

A first important objective is the preparation of digital maps for geographical distribution of halophytic species of the genus *Plantago* L. studied within our country, as well as the study of dimensional variability of the foliar apparatus, analyses required to extend the existing experimental data on national level in this regard.

The overall objective of the physiological and biochemical research was to determine the intensity of fundamental physiological processes and the identification and determination of phytochemical compounds with antioxidant role from non-halophytic plants (recognized as medicinal plants by *Romanian Pharmacopoeia),* as well as from halophytic ones (recognized as pharmacological important plants in other countries *Pharmacopoeia*), in the perspective of their use for phytopharmaceutical purpose.

The usefulness of this research is to identify species that tolerates the best negative effects of soil salinity, drought and long periods of dryness, for possible introduction in culture as medicinal species and / or environmental improvement effect.

Material and research methodology

The morphological, anatomical, physiological and biochemical investigated plant material was represented by five species of wild *Plantago* L., analyzed in three moments corresponding to phenological phases of the ontogenetic cycle of the investigated species.

The plants belonging to *P. coronopus* species were analyzed in two areas: *P. coronopus* specimens noted as ZII were collected / analyzed from about 1000 m from the littoral area of the Black Sea area and those of the *P. coronopus* species noted as ZI, were collected / analyzed from the littoral area of the Black Sea.

- **Methods used in the chorology study -** geographical distribution of investigated species was performed using the **ArcView GIS 3.2** program.
- **Methods used in morphometric analysis of leaf area -** measuring leaf area and associated parameters was performed "in vivo" using AM-100 Area Meter compact portable device.
- **Methods used in histo-anatomical research -** The plant material was sectioned and prepared according to the conventional method used in histo-anatomy. Subsequently observations were performed and photomicrographs are taken using a NOVEX (Holland) photon microscopy.
- **Methods used in physiological research –** the intensities of photosynthesis, respiration and of light flux were analyzed using the ADC BioScientific LCi portable device. In parallel with the estimation of the physiological processes, the environmental temperature and humidity was recorded using the thermo-hygrometer Testo 625.
- **Methods used in biochemical research**

- **Determination of water and dry matter** was performed by the gravimetric method (Bodor *et al*., 1982).
- **Dosage of assimilating pigments** was performed according to the Mayer-Bertenrath method (with the modifications Ştirban and Fărcuş) (Boldor et al., 1982).
- **Superoxide dismutase activity determination** was carried out according to the Winterbourn, Hawkins, Brian and Carrell method (ARTENIE *et al*., 2008).
- **Determination of catalase activity** was carried out according to the Sinha method (ARTENIE *et al*., 2008).

- **Determination of peroxidase activity** was carried out according to the Gudkova L.V. & Degtiari R.G method (ARTENIE *et al*., 2008).
- **The quantitative determination of soluble proteins** was carried out according to the Bradford method (BRADFORD, 1976).
- **Determination of total polyphenol** - was carried out according to the WATERHOUSE, 2001 method.
- **Determination of flavonoids** was carried out according to the CHANG *et al*., 2002 method.
- **Determination of proline** was carried out according to the Bates (1973) method, modified and adapted accordingly (NAQVI *et al*., 2002)
- **Determination of antioxidant capacity** was carried out using the DPPH method according to the MISER-SALIHOGLU *et al*., (2013) method.

- **Methods used in soil research**
- **Determination of soil pH and soil salinity** was carried out according to the TEODORESCU-SOARE, (2011) method.

CHARACTERIZATION OF COLLECTION AREAS OF THE INVESTIGATED SPECIES

Soil reaction of the areas of the investigated species was situated in highly alkaline domain and was positively correlated with the electrical conductivity for each stationary.

According to the data from the Meteorological Registry at Sulina, Murighiol, Istria and Leţcani-Dumești station the research year 2012 was characterized by the following climatic conditions: high thermal regime, reduced precipitation, low humidity and a long duration of sunshine, compared to year of research 2013, which had optimal climatic parameters;

CHOROLOGY OF SOME *PLANTAGO* L. SPECIES, PLANTAGINACEAE FAMILY

The digital maps of halophytic species distribution from Romanian flora highlights a *predominant* distribution in the north-east of the country for *Plantago schwarzenbergiana*, a sporadic distribution for *Plantago coronopus* (only in sandy areas ± salinized from "Danube Delta" Biosphere Reserve), wich is a *rare species, partially threatened*, and for *Plantago maritima* a predominant distribution in the west (**Fig 1**; **Fig 2**), distributions correlated to, probably, in our opinion, with the presence of salty soil, the vicinity of the salt lakes and the presence solonetzs with sodium, sodium sulfate or sodium carbonate from the stationary of origin for the the analyzed plant material.

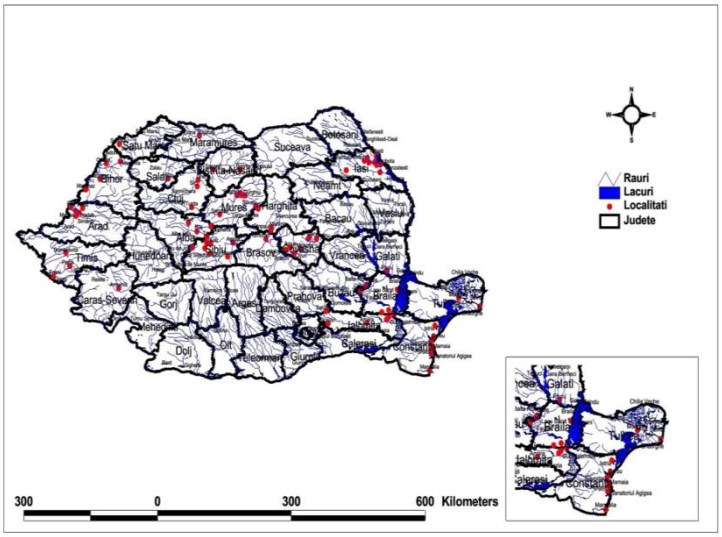

Fig. 1. Geographical distribution of the *Plantago maritima* L. species on the Romanian territory (original)

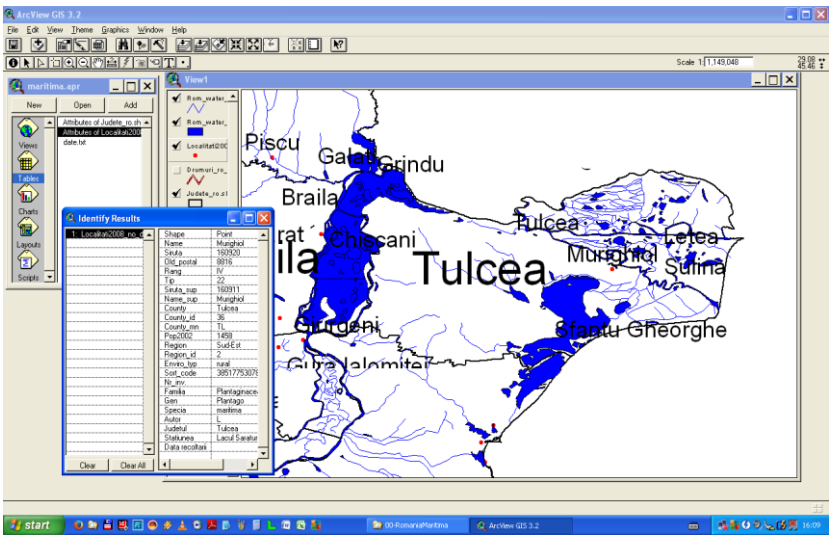

Fig. 2. Geographical distribution of the *Plantago maritima* L. species on the Romanian territory - detail from Dobrogea area (original)

RESEARCHES ON THE FOLIAGE DIMENSIONAL VARIABILITY IN SOME SPECIES OF THE *PLANTAGO* L. GENUS

The coefficient of variation of the total leaf area of the plants from Natural Reserve, Sărăturile din Valea Ilenei" recorded a maximum of 48.42%, and those from Dobrogea, a maximum of 85.75%, which reflects the specific ecological values of saline habitat of the areas of origin of biological material analyzed.

The analysis of covariance of the total leaf area and number of leaves from rosette recorded the lowest values for *Plantago maritima* (16.87) and the high values for *Plantago media* (973,93), respectively *Plantago lanceolata* from Murighiol Stationary (1160), differences associated with specific pedo-climatic conditions of the stationary of origin of the analyzed plants.

HISTO-ANATOMICAL INVESTIGATIONS OF THE VEGETATIVE ORGANS IN SOME SPECIES OF THE *PLANTAGO* L. GENUS

For the analyzed halophytic species (*Plantago coronopus*, *Plantago maritima*, *Plantago schwarzenbergiana*) the xeromorphic characters indicate the presence of specific adaptation to the habitats of origin: aerenchym in the root, tector hairs and/or secretory hairs, additional development of sclerenchyma, bifacial equifacial limb structure, mesophilic character of halo-succulent (**Fig 3**; **Fig 4**).

For the *Plantago coronopus* the rhizome structure was analyzed, organ which was not described so far in this species (according to the consulted literature).

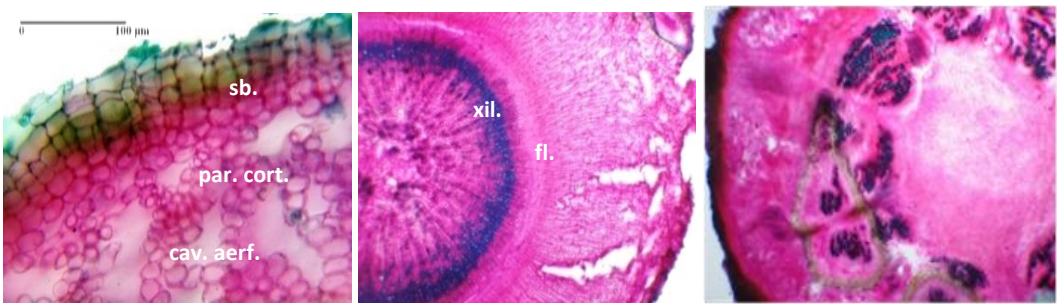

Fig. 3. Cross-section through A - main root in individuals of the species *P. coronopus* , B – the rhizome of the *Plantago coronopus* L., C - the rhizome of the *Plantago maritima* L. xil.- xylem; fl. –phloem; rzd.- root epidermis; sb. – suberin tissue; par. cort. – cortical parenchyma; cav. aerf. – air cavities; mdv. – marrow

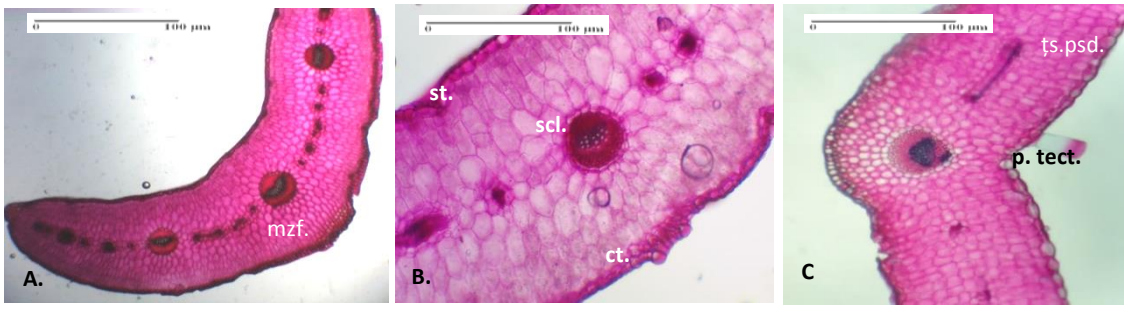

Fig. 4. Cross-section through the leaf lamina from individuals belonging to the species of A - *Plantago coronopus* L.; B - *Plantago maritima* L.; C - *Plantago schwarzenbergiana* Schur.; ct. – cuticula; st. - stomata; mzf. – mesophyll; col. – collenchyma; p.tect. – tector hair; ţs. psd. –palisadic tissue

Mesophilic structure provides succulent character for the species *P. coronopus*, *P. maritima* and *P. schwarzenbergiana* and helps the plant to better tolerate the dehydration and cope with water or saline stress.

ECOPHYSIOLOGICAL RESEARCH

- **Research on the dynamics of the foliar assimilating pigments content**

During the two years of research the results indicate a wide variation of the assimilating pigments content (**Table 1**), depending on the species, stage of development and climate peculiarities of two years and specific soil reaction of the Dobrogea stationary area.

Table 1. The content of assimilating pigments of the individuals belonging to *Plantago* L. genus collected from Dobrogea, during ontogenetic cycle of the years 2012 and 2013 (average values for the two years of analysis)

Species	Phenophase	Chlorophyll *a* (mg/g D.W.))	Chlorophyll *b* (mg/g D.W.))	Carotenoids (mg/g D.W.)	Total chlorophyll (mg/g D.W.)	Total assimilating pigments (mg/g D.W.)
P. maritima (Istria stationary)	vegetative	6.2119	2.3217	0.3022	8.5335	8.8358
	flowering	4.3426	1.9518	0.3953	6.2944	6.6897
	fructification	2.8800	1.6217	0.1735	4.5017	4.6752
P. lanceolata (Murighiol stationary)	vegetative	8.6057	3.0961	0.5719	11.7018	12.2737
	flowering	9.9625	3.5597	0.5447	13.5222	14.0669
	fructification	4.7857	1.8513	0.1145	6.6370	6.7515
P. coronopus (ZII Sulina stationary)	vegetative	9.9767	4.1619	0.4392	14.1386	14.5778
	flowering	4.8651	1.7464	0.3104	6.6114	6.9219
	fructification	5.9234	1.8801	0.3021	7.8035	8.1057
P. coronopus (ZI Sulina stationary)	vegetative	6.0260	2.7559	0.2471	8.7819	9.0290
	flowering	8.5143	3.7126	0.3763	12.2269	12.6032
	fructification	3.7450	1.0236	0.2942	4.7686	5.0629

As a whole, the total assimilating pigments show the highest values in the vegetative stage (*P. maritima*, *P. coronopus* from stationary ZII) or in the flowering stage (*P. lanceolata* and *P. coronopus* from ZI stationary).

- **Knowledge contributions to the photosynthesis process in *Plantago* L. species**

The dynamics of photosynthesis in studied plants shows a general downward trend over the ontogenetic cycle, the recorded values correlating positively with those of the total content of chlorophyll and the amount of photosynthetically active radiation, confirming the submitted data by Teramura & Strain (2011) and Jităreanu (2007).

- **The water content and dry matter of individuals of *Plantago* L. species**

The investigated specimens showed different degrees of foliar tissue hydration dynamic under climatic conditions in Dobrogea area for the years 2012 and 2013 in May - June, and October. The downward trend of the dry matter content from the vegetative phenophase to the flowering stage in the case of *P. coronopus* individuals from the Sulina stationary is due to, at least partially, the low level of relative humidity and low rainfall in 2012 compared to 2013.

- **Contributions to the knowledge of the foliar respiration process dynamics**

From the interpretation of experimental data for respiration intensity it appears that this physiological process variation correlates directly with humidity and atmospheric temperature,

measured near the foliage of analyzed individuals; thus, decreases in atmospheric humidity caused by the atmospheric temperature increase causes leaf respiration to intensify.

The results showing the respiration increase in the vegetative phenophase, confirms the studies made by Burzo *et al*., (1999) on several plant species considering normal to have wide variations in different taxa in conjunction with environmental factors.

- **Knowledge contributions of the leaf transpiration process dynamics**

The decrease in transpiration intensity during flowering phenophase in 2012 and 2013 for the *P. coronopus* and *P. maritima* is negatively correlated with P.A.R index and atmospheric temperature, as adaptation to drought conditions, related to the presence of xeromorphic characters, thus contributing to a decrease in transpiration rate and finally to reduce the loss of water from the leaf tissue.

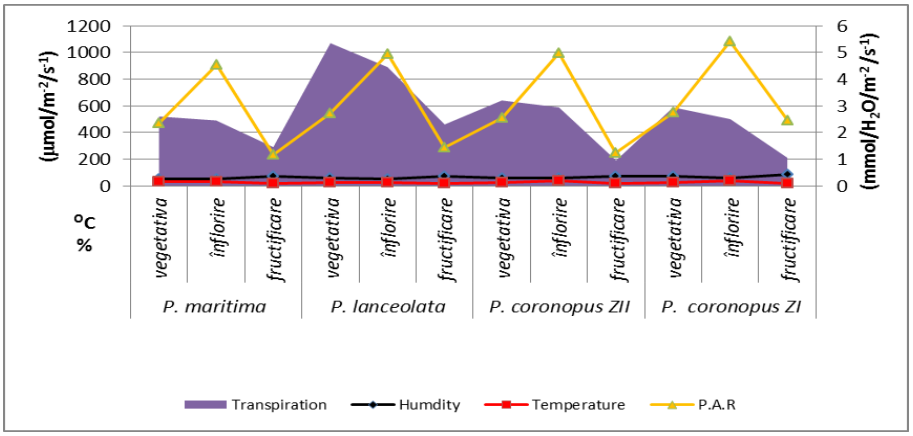

Fig. 5. Foliar transpiration intensity variation depending on environmental factors (humidity and atmospheric temperature) in individuals belonging to the *Plantago* L. genus from Dobrogea during the ontogenetic cycle of 2012

According to data from the literature the atmospheric moisture greatly influences the transpiration intensity: as the humidity increases, transpiration decreases and vice versa, the results obtained by us confirming practically the hypothesis launched by Burzo *et al*., 1999. In this regard, the combination of high air temperature with low humidity enhances water loss through transpiration (**Fig. 5**).

BIOCHEMICAL RESEARCH

- **Changes in proline content in individuals of a species belonging to the *Plantago* L. genus**

Results of research conducted on the contents of proline in individuals of the studied species in specific ecological conditions of 2012 (**Fig. 6**) from some Dobrogea stationary area, showed a high value of this compound during the two phenological phases, a situation that relate to climatic conditions and with some soil properties (soil reaction is strongly alkaline medium to high electrical conductivity).

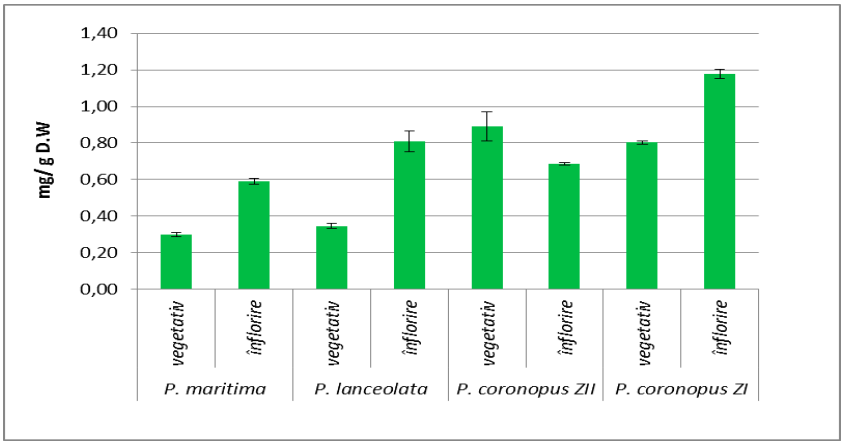

Fig. 6. Variation in proline content in leaf tissues from individuals belonging to the *Plantago* L. genus collected from Dobrogea in the vegetative and flowering phenophases of 2012

In individuals of the investigated halophytic species, the proline accumulation varies depending on the degree of their tolerance to action of the saline stress, in general, the proline being in a close interrelationship with the reaction and the salinity of the soil.

- **Variation of some foliar enzymes involved in oxidative stress in individuals belonging to the *Plantago* L. species**

Low activity of antioxidant enzymes in individuals of the studied species for 2013 compared to 2012 can be explained by the specific climatic conditions in 2013, characterized by a thermal regime subjected to short periods of drought alternating with periods of rich precipitation, relative slightly lower values of the air humidity and constant light intensities.

In 2012 in individuals of some analyzed species from Dobrogea stationarys (Sulina, Istria, Murighiol) the catalase activity was undetectable. This likely can be explained by the presence of small amounts of hydrogen peroxide, the role of this enzyme being taken over by the peroxidase and H_2O_2 at reduced concentrations which has a signaling role in the installation of stress conditions (APEL & Paper, 2004).

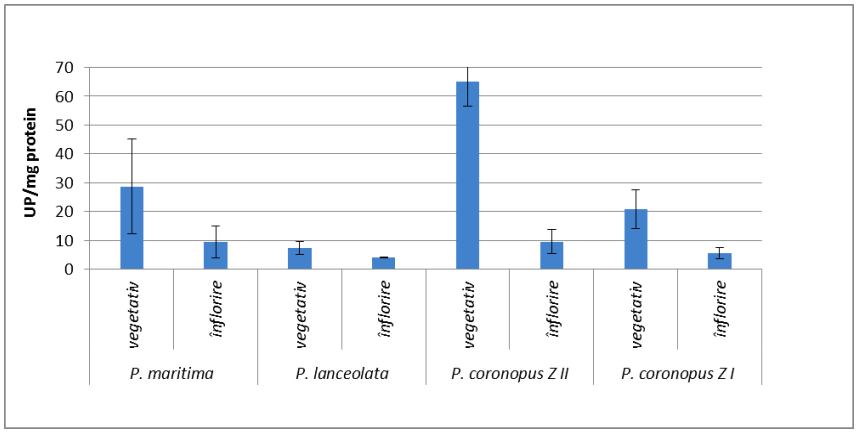

Fig. 7. Variation in peroxidase content in individuals belonging to the *Plantago* L. genus collected from Dobrogea in the vegetative and flowering phenophases of 2012

In the analyses that were carried on halophytes collected from Sulina, the peroxidase activity indicates a varied response depending on the species and collection stationary **(Fig. 7).**

Intensification of peroxidase activity in the investigated species may be a consequence of the formation of hydrogen peroxide in large quantities due to different levels of salinity in collection stationaries.

- **Total polyphenol content in individuals belonging to the *Plantago* L. species**

In the climatic conditions of 2012 year the analyzed plants species showed an intense buildup in polyphenolic compounds as specific physiological response to excessive climate conditions of that year, the investigated plants intensified their biosynthesis and accumulation of non-enzymatic compounds to protect their cell structures to harmful action of heat, hydric and saline stress.

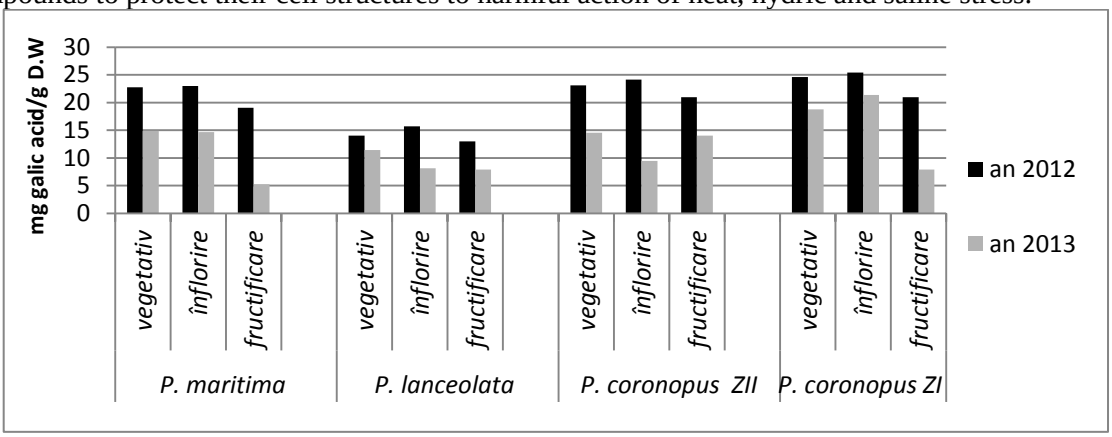

Fig. 8. Total polyphenol content in individuals belonging to the *Plantago* L. genus collected from Dobrogea in the vegetative and flowering phenophases of 2012 and 2013

In both years 2012-2013, *P. lanceolata* specimens had the lowest values of total polyphenols content, correlated with topoclimatic influences induced by Sărăturile Lake that tempers the drought and dryness, and the highest values were determined on specimens *P. coronopus* from ZI stationary (coastal area) correlated with negative effect of salt levigation (**Fig 8**).

• The content of flavonoids in individuals belonging to the *Plantago* L. species

Comparative analysis of results (**Fig. 9**) according to climatic conditions indicate that in the environmental conditions of 2012 the analyzed specimens had lower values of the content of flavonoids, compared with those in 2013, which were slightly higher.

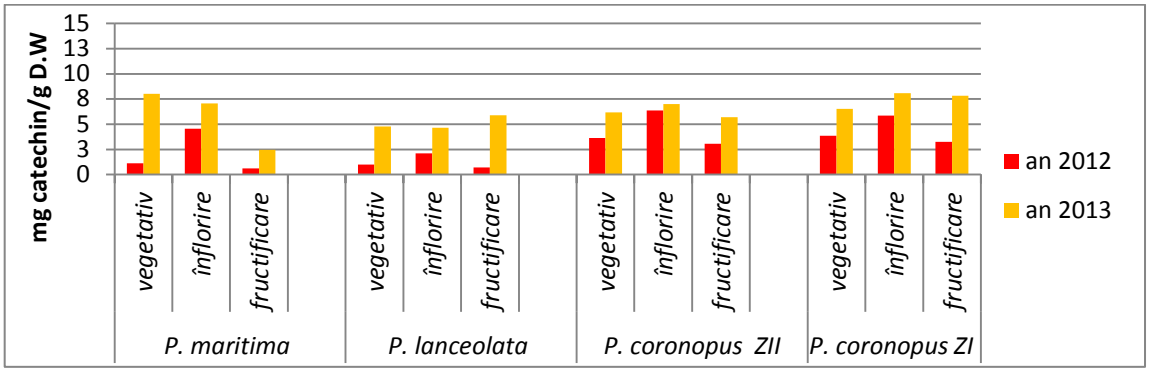

Fig. 9. Total flavonoids content in individuals belonging to the *Plantago* L. genus collected from Dobrogea in the vegetative and flowering phenophases of 2012 and 2013

The increase in the content of flavonoids in vegetative phenophase to flowering phenophase may be due to intensified UV radiation in summer, characterized by long periods of dryness and drought (DIXON & PAIVA, 1995), as well as adaptive response, protection of cellular structures and tissues against the negative effects of oxidative stress, results also highlighted by literature (CALDWELL *et al.*, 1981; CALDWELL *et al.*, 1983; NYBAKKEN *et al.*, 2004; TREUTTER, 2006).

From the interpretation of our own results, it is obvious that the species *P. coronopus* and *P. maritima* are the most valuable in terms of the content of biologically active compounds compared with the other species studied.

• Variation of antioxidant capacity in individuals belonging to the *Plantago* L. species

The results showed that the antioxidant activity of the extracts of *Plantago sp.* varied depending on the considered stationary within wide limits **(Table 2)**: in the flowering phenophase a lower activity was registered in *P. coronopus* extracts of steppe and semi-arid region of Dobrogea, compared with extracts of *P. lanceolata* collected from Ileana Valley stationary, which had a more intense antioxidant activity.

Table. 2 DPPH scavenging activities (as % inhibition) of methanolic extracts from the leaves of five *Plantago* species in vegetative and flowering stage (mean ± standard deviation, n = 5)

Collecting point area	Species	Growth stage	
		Vegetative stage (% inhibition)	Flowering stage (% inhibition)
Dobrogea	*P. maritima*	49.88±3.86	51.21±1.37
	P. lanceolata	42.41±1.16	40.29±4.85
	P. coronopus	71.73±6.52	21.56±1.95
Valea Ilenei	*P. media*	81.77±4.57	77.67±4.51
	P. lanceolata	90.86±3.13	94.17±0.36
	P. schwarzenbergiana	21.70±5.14	45.56±1.,64

Our results correlates with the data presented in the literature (GÁLVEZ *et al.*, 2005; BEARA *et al.*, 2009; Jankovic *et al.*, (2010), attesting that the vegetative extract obtained from species of *Plantago sp.* possesses a significant antioxidant activity.

CONCLUSIONS

- **Characteristics of ecological specificity of the local area**

- The year of research 2012 was characterized by the following climatic conditions: high thermal regime, reduced precipitation, low humidity and a long duration of sunshine, compared to year of research 2013, which had optimal climatic parameters;

- Soil reaction ranged in highly alkaline and was positively correlated with species-specific electrical conductivity and partially stationary.

- **Chorological research**

- The digital maps of halophytic species distribution from Romanian flora highlights a predominant distribution in the north-east of the country for *Plantago schwarzenbergiana*, a sporadic distribution for *Plantago coronopus* (rare species, partialy threatened), and for *Plantago maritima* a predominant distribution in the west.

- **Morphometric research**

- Biostatistics calculation results show that between total area and the foliar perimeter, and between the total area and the number of leaves a positive correlation is set, the correlation coefficient ***r*** shows that morphometric parameters of analyzed plants are moderate and directly proportional.

- **Histo-anatomical research**

- For the analyzed halophytic species (*Plantago coronopus*, *Plantago maritima*, *Plantago schwarzenbergiana*) the xeromorphic characters indicate the presence of specific adaptation to the origin of vegetation habitats (aerenchym at the root, tector hairs and/or secretory hairs, additional development of sclerenchyma, bifacial equifacial limb structure, mesophilic character of halo succulent).

- For the *Plantago coronopus* the rhizome structure was highlighted, which was not described so far (according to the consulted literature).

- **Eco-physiological research**

- The amount of assimilating pigments is positively related to the sunshine duration, atmospheric temperature and average rainfall in 2013 and negative with atmospheric humidity and precipitation for 2012.

- The dynamics of photosynthesis in individuals of the studied species shows a general downward trend during the ontogenetic cycle, the values recorded correlates positively with the total chlorophyll content; photosynthesis intensity values for 2012 were lower compared to those of 2013, which correlates with the joint action of pedoclimatic factors.

- Analysis of experimentally obtained data on the intensity of respiration depending on the specific climate of Dobrogea, highlights a higher values of vegetative and flowering phenophases of 2012, with the highest values in individuals of the *Plantago coronopus* species, phenomenon correlated with the tissues hydration and the values of air humidity.

- An increase in air temperature and P.A.R. indices in the flowering phenophase in 2012 and 2013 for all species was accompanied by decreases in the intensity of transpiration as a possible adaptation of the analyzed individuals in conditions of dryness of the vegetation environment, adaptation supported by certain specific xeromorphic characters presented by literature and confirmed by histo-anatomical research undertaken in this work.

- **Biochemical researches**

- The quantitative differences of proline content recorded during the two years of research to species representatives of the genus *Plantago* can be explained by variations in specific zonal pedological indicators such as electrical conductivity and soil reaction.

- The content of enzymes with antioxidant role in halophytic individuals of plantain from Istria and Sulina stationary correlates with the strength of their saline environment, biochemical reactions and physiological response is influenced by the collection areas (different habitats) and the different stages of development achieved by the plants during the investigated vegetative cycle.

- The total content of polyphenols registered high values in *Plantago* L. from Dobrogea areas especially in 2012, characterized by thermo-hydric conditions that imposed intensification of biosynthesis and accumulation of mentioned compounds to protect their cell structures. The species analyzed included (*Plantago lanceolata*, *Plantago media*) or not (*Plantago coronopus*, *Plantago schwarzenbergiana*, *Plantago maritima*) in *Romanian Pharmacopoeia* presented values of total content of polyphenolic compounds comparable and from this point of view we considered species with phyto-pharmaceutical potential.

- Regarding the concentration of flavonoids, we notice the dependence of species and pedo-climatic conditions specific to the origin of areas of the plant material investigated.

- According to performance criteria of anti-oxidants compounds biosynthesis evaluated by antioxidant capacity analyses, the *Plantago* species can be descendingly ordered as follows: *Plantago lanceolata, Plantago media, Plantago coronopus, Plantago maritima, Plantago schwarzenbergiana*.