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COMPUTATIONAL APPROACHES TO IDENTIFY REGULATORS OF STRESS INDUCIBLE GENES OF RAB FAMILY IN *S. LYCOPERSICUM*

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Keywords: gene expression; promoter; tomato; salinity stress; *cis*-regulatory elements; transcription factor
Abstract: Adverse environmental conditions with increasing soil salinization have become a major problem in realizing the potential yields of crops. Plants deploy various mechanisms to deal with salt stress facilitated by the expression of several stress related genes. One such group is the late embryogenesis abundant (LEA) protein gene family which has various functions including protection of cellular structures from dehydration. To investigate the molecular basis of high tolerance of Punjab Keshari cv of tomato, we performed a comprehensive analysis of the proximal promoters of candidate members *Rab1A, Rab1B, Rab1C, Rab11a* of *Rab* gene family which are essential components of stress response. Genes and protein structures were also studied to identify the active sites. The completion and enhanced annotations of tomato genome sequence has provided the opportunity for genome-wide in-depth analysis of gene expression. Analysis suggests presence of various common stress related *cis*-acting elements (CREs) in the putative promoters of members of this gene family. Our results strengthen the idea that plants are comprised of numerous genes with an exceptionally wide range of functionalities along with huge number of promoters and regulatory elements and more remains to be unravelled. With advances in bioinformatics, functional genomics will aid in understanding the molecular and physiological basis to improve the salinity tolerance for sustainable crop production.

INTRODUCTION

High salinity is a prevalent abiotic stress limiting the productivity and the geographical distribution of plants. During the past decade, our understanding of plant response to salt stress has been greatly improved by studying model plants. Salt tolerance is believed to be achieved by many different genes involved in different pathways, such as ion compartmentation, ion extrusion, ion selectivity, compatible solute synthesis and reactive oxygen species (ROS) scavenging (Blumwald et al, 2000; Zhu, 2003, 2016; Munns and Tester, 2008). Cultivated tomato, one of the most important vegetable crops in the world, is moderately sensitive to salinity. Abiotic stress is the negative impact of non-living factors on plants in a specific environment. Cold, salinity and drought are among the major abiotic stresses that adversely affect plant growth and productivity. In fact, these abiotic stresses represent the main cause of crop failure worldwide, leading to average yields of major crops. Stress signals are transmitted through the plasma membrane (many times due to hormones such as abscisic acid, cytokinins, or ethylene). The signal is transmitted within the cell by secondary messengers (e.g. ROS, Ca^{2+}, or IP molecules). The complex interplay between many different kinds of transcription factors (TFs) within the nucleus is responsible for changes in gene expression levels in response to a given form of stress. The expression of numerous plant genes is regulated by abiotic environmental stresses such as drought, high salinity and cold (Shinozaki, 2007).

Plants have developed several physiological and biochemical strategies to adapt or tolerate osmotic stress conditions and deal with salt injury. Reduction of sodium ions in the cytoplasm and the accumulation of compatible, low molecular weight protective compounds called osmolytes have been suggested as the two major mechanisms that underlie the adaptation or tolerance process (Hasegawa et al, 2015). In addition to such metabolic changes, a large set of plant genes commonly called *LEA* (Late Embryogenesis Abundant) are transcriptionally activated, which leads to the accumulation of novel proteins in the vegetative tissues of plants under osmotic stress (Skriver and Mundy, 1990). It is generally assumed that these stress induced LEA proteins might play a protective role in tolerance which is essential for plant survival during episodes of various stress conditions. However, direct and clear experimental evidence supporting the exact functions of LEA proteins is still lacking and the physiological roles of many stress-responsive genes remain largely unknown or poorly understood. Studies conducted with several indica varieties of rice show that the levels of Group 2 LEA proteins (also known as dehydrins) and Group 3 LEA proteins in roots were significantly higher or induced by abscisic acid (ABA) and salt stress only in salt tolerant varieties as compared to salt sensitive varieties (Moons et al, 1995). Our earlier communication (Roychowdhury et al, 2007) showed that *Rab16A* gene is expressed at a much higher and constitutive level in salt tolerant rice cultivar while the transcript is almost undetectable in salt sensitive variety even upon salt stress (Kim et al, 2012, 2015). This probably gives an indication of the involvement of Group 2 and Group 3 *LEA* genes in salt tolerance.

The elucidation of transcriptional regulation in plant genes is important area of research for plant scientists, following the mapping of various plant genomes, such as *A. thaliana*, *O. sativa*, *S. lycopersicum* and *Z. mays*. A variety of bioinformatic servers or databases of plant promoters have been established. The combinatorial interaction of TFs is

important in regulating the gene group that is associated with the same expression pattern. Therefore, a tool for detecting the co-regulation of TFs in a group of gene promoters is required. Investigations on TFs and their corresponding CREs in promoters have attracted much attention from researchers of gene regulation. However, defining all functional binding sites within an identified promoter is difficult, and the existence of some additional binding sites should be assumed (Wray, 2003).

In the present study, we have analyzed GTPases on the basis of sequence, motif identification, protein sequences, and active site prediction. Such computational analyses provide insight into the structure and function of target biomolecules. These results may aid to build up a view of these Rab proteins and their role in plant stress response. Here we report the putative promoters of 4*Rab* genes that contain stress-responsive CREs which may be responsible for regulating the expression of these genes under abiotic stress conditions.

Binding of TFs to CREs or transcription factor binding sites (TFBSs) are involved in transcriptional regulation of gene expression. Discovering *cis*-regulatory elements has been an important research challenge for some years (FitzGerald, 2004; Hernandez-garcia, 2014). With the availability of genome sequences, the computational approach provides an alternate low-cost method to find CREs that can effectively deal with a large number of genes (Bussemaker, 2000; Cora`, 2005). In recent years, many computational approaches have been proposed to find putative CREs. To evaluate the accuracy, computationally discovered CREs are compared with the known TFBSs from public databases or published literatures. This type of evaluation, however, does not validate or associate the putative CREs with biological functions. Another disadvantage of the other studies is that they did not investigate the relationships between the putative CREs. The expression of a gene is usually not regulated by a single TF, but by clusters of TFs that might bind to different CREs. Therefore by exploring the combinatorial regulation of gene expression, one can obtain a better understanding of the complex gene regulation machinery. This study will endeavour to determine the coexistence of putative CREs and will also take advantage of phylogenetic footprinting to improve the prediction accuracy in the search of CREs.

MATERIALS AND METHODS

Biocomputation: Methodology

The selection of the DNA sequences for the identification of Transcription Factor Binding Sites (TFBS) is driven by the typical location of the binding sites.The detailed information of stress-related *cis*-element sequences and annotation is found in Table 1/ 2, whereas the position and abundance of all *cis*-elements predicted to localize in promoter regions of targeted *Rab* genes are shown in Figure 3. Based on our previous transcriptomic study where *Rab* genes were found upregulated in tomato plant under salinity stress, all complete mRNA coding sequences of tomato *Rab* genes (Table1 and Fig.1) were collected from the RefSeq database of NCBI (http://www.ncbi.nlm.nih.gov). Constructed gene structures with noncoding and coding regions have been shown in Figure 1. In order to recognize the upstream promoter region, nucleotide sequences of 1.0 Kbp extending 5′ from the genes' translation start site were identified (https://solgenomics.net). Genome sequences and gene coordinates stored at The Institute for Genomic Research (TIGR), Sol Genomic Network (SGN), NCBI were used for the analyses. 1.0 Kbp of the 5′ upstream promoter region of each gene was scanned for the presence of *cis*-acting regulatory elements involved in abiotic stress signalling pathways using PLACE, PlantPan and Plant CARE program (www. Plant CARE. Com/encyclopedia). A number of 4 *Rab* genes (*Rab1A/1B/1C and Rab11a*) were nominated for the network study based on containing the most varied motifs involved in abiotic stress tolerance. In order to identify the varied CREs in the putative promoter region and analyze co-expressed genes, the MEME and PlantPan web tools were used. The domain architectures of the deduced proteins (Fig. 3) were identified based upon their sequence alignment using various tools like SMART, MAFFT, MUSCLE and ClustalX/ClustalW.

Table 1. List of genes under study with annotations.

Gene name *Solanum lycopersicum*	**Accssn. No. of NCBI**	**Sizes of the cDNAs base pair**	**Source tomato sp. & Annotation**
Rab1A	U38464	611	LA1221, Small GTP binding protein
Rab1B	U38465	611	LA1221, Small GTP binding protein
Rab1C	U38466	611	LA1221, Small GTP binding protein
Rab11a	AJ245570	656	Ailsa Craig, Rab11GTPase putative role in secretion in cell wall

Table 2. List of regulatory motifs with consensus sequences. Motif logo generated using MEME (http://meme-suite.org/) Nucleotides: A-Adenine; C-Cytosine; G-Guanine; T-Thymine; M-A or C; R-A or G;W-A or T; S-C or G; Y-C or T; K -G or T; B -C., G or T; D -A, G or T; H -A, C or T; V -A, C or G; N –Any

***cis*-Regulatory Elements**	**Consensus Sequence**	**Motif logo**
ABRE	MACGYGB	
CE1	SSBCACCSV	
LTRE	CCGAC	
WRKY	WTGACH	
MYB	CNGTTR	
MYC	CANNTG	
ELRE	TTGACC	
DPB	ACACNNG	
GATA	WGATAR	

Analysis by PLACE Database (PLACE) (Higo et al, 1999) and PLANTPAN 2.0

Characterisation of 1 kb upstream sequences: Normally, putative regulatory regions have been located in within 500 bp and 1 kb upstream of the transcription or translation start point (Goda, 2004; Thijs et al, 2001). Therefore, considering that the analysis of intergenic regions revealed a median length of 1 kbp, the computational prediction of TFBSs was made using 1 kbp long sequences upstream of the start of a coding region. The default length of 1 kb was applied in all cases, including intergenic regions smaller than 1 kb, because it was not discarded that some coding sequences exert regulatory actions on a neighboring gene, moreover if the intergenic sequence of the neighboring gene is too short.

Identifying cis-regulatory elements in the plant genome: Many databases are there having collections of numerous TFs and found useful for the prediction of TFBSs in the promoter regions of plants. For instance, PLACE (Higo, 1999) is a database that collects various *cis*- and *trans*- acting regulatory DNA elements. TRANSFAC (Matys, 2006) is a database of TFs, including genomic binding sites and DNA-binding profiles. JASPAR (Bryne, 2008) is an open-access database of annotated, high-quality, matrix-based TFBS profiles for multicellular eukaryotes. Athena (O'Connor, 2005) is a database, which contains 30,067 predicted *Arabidopsis* promoter sequences and consensus sequences for 105 previously characterized TFBSs and provides analysis on over-represented TFBSs occurring in multiple promoters. PlnTFDB (Riano-Pachon, 2007) is an integrative plant transcription factor database that provides a web interface to access large sets of TFs of several plant species. AGRIS (Davuluri, 2003) contains an *Arabidopsis thaliana* transcription factor database (*At*TFDB) consisting of approximately 1,770 *Arabidopsis* TFs and their sequences (protein and DNA) grouped into around 50 families

with information on available mutants in the corresponding genes. AGRIS (Davuluri, 2003) integrates a variety of tools to determine TFs and their putative binding sites on all genes to reconstruct transcriptional regulatory networks in *Arabidopsis*. DATF (Guo, 2005) stores information on 3D structural templates, EST expression, TFBSs and nuclear location signals (NLSs) of known and predicted *Arabidopsis* transcription factors. PlantCARE (Lescot, 2002) is a database of plant CREs and a portal to tools for the *in silico* analysis of promoter sequences.

Access to PlantPAN is via a web interface, freely available to all interested users, at http://PlantPAN.mbc.nctu.edu.tw. CREs published in PLACE and AGRIS were downloaded. The visual representation of the motif logos was constructed (Table 2 and 3). There are so many recent techniques being used for analysis of stress response in plants. Transcriptome is the set of all mRNAs/transcripts produced under a given set of conditions. Transcriptomic data (Roy, 2015) was taken from our previous work for this study. Transcriptomics is always considered as a step next to genomics in the study of biological systems.

RESULTS AND DISCUSSIONS

Upstream and protein sequences of 4 Rabs:

To better understand plant stress responses, transcript profiling experiments have been successfully employed for many different abiotic and biotic stresses. Based on our previous differential expression of 10 stress responsive genes, we carried out comprehensive searches for the stress related CREs in the upstream regions of 4 genes (*Rab1A, Rab1B, Rab1C and Rab11a*). To fully understand the role of the 4 genes under study in tomato plants, it is therefore essential to characterize these genes. Studying the structure of genes of interest is crucial in biology and can provide important clues concerning gene evolution. Accordingly, we analyzed the promoters of the up-regulated genes to identify potential TF-binding motifs important for activating stress resistance. We also selected elements as more abundant CREs in promoter regions of these genes without mention of common CREs such as CAAT-box and TATA-box. The both of them have fundamental role in initiation of transcription process and are found in all the promoter regions of genes.

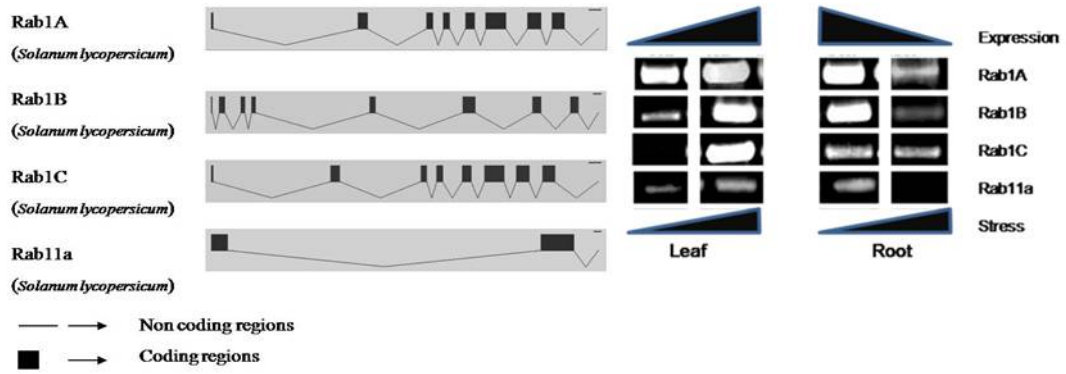

Figure 1. A schematic representation of four candidate genes with coding exon and noncoding intron. The expression profile at the right side showing expression pattern of 4 *Rab* genes(*Rab1A, Rab1B, Rab1C* and *Rab11a*) in tomato(var. Punjab Keshari) under salt stress.

Genes of LEA proteins have been identified in many plant species, and at least six different groups of LEA proteins have been defined on the basis of expression pattern and sequence. Although a series of Group 2 *LEA* genes have been isolated and their accumulation

correlated with exogenous stress (Roychowdhury et al, 2007), The Gr2 proteins, also called dehydrin or RAB (responsive to ABA) proteins (Close et al, 1989). Group 2 proteins are characterized by up to three sequence motifs, known as the K-domain (lysine-rich), the Y-domain (DEYGNP) and the S-segment (poly-serine stutter). In our previous findings (Roy, 2015) all the 4 *Rabs* were up regulated (higher expression detected in cv Punjab Keshari than cv Pusa Ruby of tomato) in salt treated tomato leaves only (down regulated in treated roots). It is known that protein functions are closely related to their structures. Predicted amino acid sequences (Fig. 2) of the Rab protein were aligned using multiple sequence alignment software (MUSCLE). The degree of similarity was lower for the Rab1A than of Rab1B and Rab1C plant GTP-binding proteins; Similarly, lower sequence identities were observed between 11a and 1A/B/C. The four regions (Fig. 2 boxed area) required for nucleotide binding (Bednarek, 1994), characteristic of Rab proteins in sequence and spacing are present in these Rab proteins. These sequence segments, GDQSVGKTS, WDTAG , NKTD and ETSA were found identical. The effector region (Fig.2 SF1-SF4) for the genes above, sequences are completely alike. All information about gene structure, motif distribution, and protein size of *Rab* genes under study support the conserved *Rab* genes in the same groups shown in Figure 2.

Increasing evidence indicates that genes with the similar expression patterns may contain the same regulatory elements in their promoters (Wittkopp et al, 2012; Uygun et al, 2016). In general, signalling networks involve *cis*-elements working with their cognate TFs. The tomato genome was sequenced as the cornerstone of an International Solanaceae Genome Initiative, a project that aims to develop the family Solanaceae as a model for systems biology for understanding plant adaptation and diversification. The tomato genome comprises approximately 950 Mb of DNA. The sequencing of the tomato genome and sequencing of the wild relative were achieved and published in the SGN database (http://solgenomics.net/tomato/). The putative promoter regions of the 4 genes under study retrieved from SGN showed several putative environment stimulus responsive CREs when scanned through PlantPan/PLACE. The detailed information of stress-related cis-element sequences and annotation is found in Table1 and 2, and the position and abundance of all CREs predicted to localize in promoter regions of tomato *Rab* genes are shown in Fig 3. We found surprising differences in the numbers of *cis*-elements in their upstream regions. The evaluated CREs represented a subset of 49 *cis*-elements retrieved from the databases PLACE, SOL Genomics and AGRIS corresponded varying oligonucleotides. This study reports TFBSs correspond to nine different consensus oligonucleotides including ABRE, WBOX, CRTDRE, MYB, MYC, DPB, GATA, LTRE, ELRE.

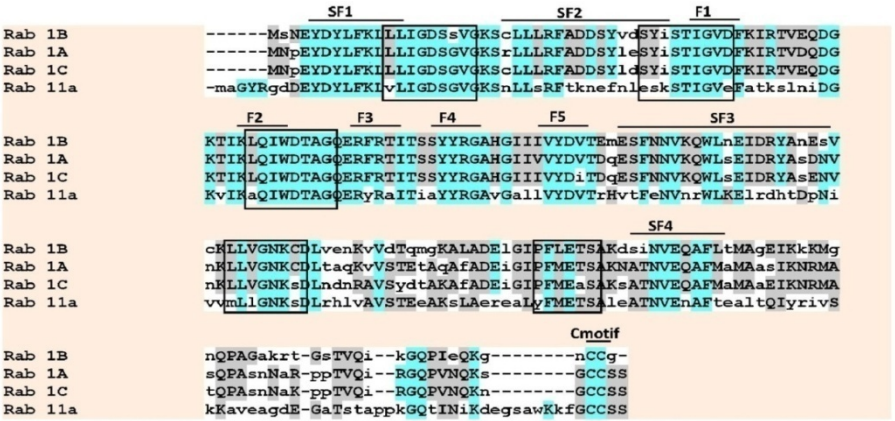

Figure 2. Multiple Sequence Alignment of Rab1A, Rab1B, Rab1C and Rab11a with MUSCLE software. Similar residues are coloured as the most conserved one (according to BLOSUM62). Average BLOSUM62 score: Max: 3.0 Mid: 1.5 Low: 0.5

GDP/GTP binding domains (G1–G5) are boxed which are important for guanine nucleotide interactions and GTP hydrolysis. Other five Rab family-specific regions (F1-F5) conserved for the Rab family and the four Rab subfamily specific regions (SF1-SF4), which are suggested to be recognized by Rab subfamily effectors. A Cys–Cys motif (C motif) at the carboxyl-terminal of 4 Rabs is essential for prenylation, a crucial modification necessary for its action as a molecular switch regulating intracellular vesicular transport and correct association with membranes.

The CREs elements present in the putative upstream region are described below (Fig.3 and Table 3):

ABRE: ABRE (GCCACGTGCA): ABA response complex consisting of ABRE element and a novel coupling element CE1 (TGCCACCGG) was sufficient for high-level ABA induction. The interaction between these 2 elements determined the specificity in ABA regulated gene expression as detected from barley (Shen and Ho, 1995). In our upstream analysis, ABRE elements were found in 5' flanking region between position –900 and –200 from transcription start site in *Rab* genes except in *Rab11a*.

LTRE: (CCGAC): is a low temperature responsive element (LTRE) found in *cor15a* gene in *Arabidopsis thaliana* . This element involved in cold induction of *BN115* gene from the winter *Brassica napus* and light signalling mediated by phytochrome for cold or drought induced gene expression in *Arabidopsis thaliana* (Jiang et al, 1996). In our analytical study it was identified as a single occurence in the flanking region of *Rab1B* gene only at position in between -900 to –1000 from transcription start site. ABRE (Abscisic Acid Responsive Element) and DRE/LTRE (for Dehydration Responsive Element/Low Temperature Responsive Element) are the two major *cis*-acting elements involved in the regulation of gene expression in response to osmotic stress in ABA-independent and ABA-dependent signalling pathways respectively (Yamaguchi-Shinozaki, 2005).

WBOX/WRKY: (T/A)TGAC(T/A): The WRKY domain forms a unique wedge-shaped structure that enters perpendicularly in the major groove of the DNA strand. WRKY protein domains interact with the (T/A)TGAC(T/A) *cis*-element, also called the W-box. In our study this group of *cis*-elements were noticed scattered in all the 4 genes with rich number. (TTTGACT) was found in the Parsley WRKY3 gene promoter that required for elicitor responsiveness. WB box and WC box constitute a palindrome WRKY1 protein binding site that play an important role in the

regulation of early defence response gene (Eulgem et al, 2000). They are recognized specifically by salicylic acid (SA)-induced WRKY DNA binding proteins.

MYC: (CANNTG): MYC recognition site found in the promoters of the dehydration–responsive gene (Abe et al, 2003). 2 to 6 of this element was observed in the upstream sequences of the candidate genes in the 5’ flanking region between position –200 and –1100 from transcription start site.

MYB: (CNGTTR): Plant MYB proteins ATMYB1 and ATMYB2, both isolated from *Arabidopsis*; ATMYB2 is involved in regulation of genes that are responsive to water stress in *Arabidopsis*. The maize cl gene was the first plant transcription factor described that encoded a MYB protein, and is involved in the regulation of anthocyanin biosynthesis in seed development (Paz-Ares et al, 1987). Our analysis shows presence of MYB mostly in *Rab1C* and few in *Rab1B* too.

Elicitor-responsive element (ElRE): It is W-box-like (TTGACC) (Rushton et al, 1996). V K Srivastava reported (2014), ELRECOREPCRP1 a well defined component of promoter region responsible for defence signalling with defined regulatory elements in pathogen- and wound induced signalling. Here this element was noticed in *Rab11a* only.

DPB: DPB (ACACNNG): is a novel class of bZIP TFs. DPBF-1 and 2 binding core sequences were found in carrot (*Daucus carota*) *Dc3* gene promoter. Dc3 expression is normally embryo specific and also can be induced by ABA (Kim et al, 1997). Our findings exhibit lesser of such CREs in *Rab1A, Rab1B, Rab1C* in between the region –700 to –800 whereas none was detected in *Rab11a*.

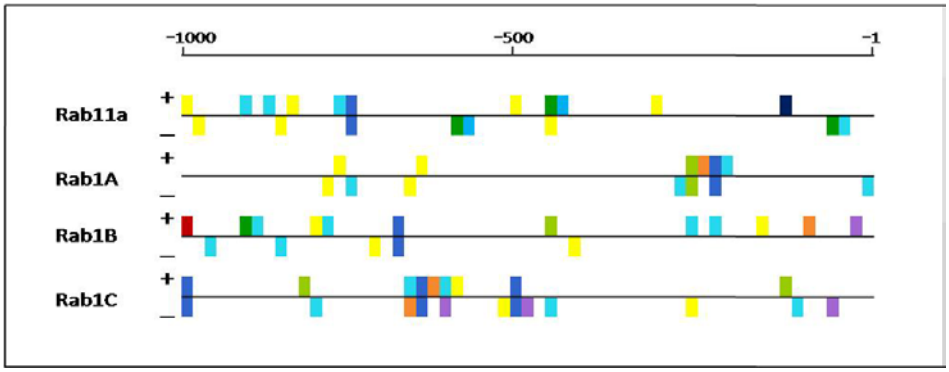

Figure 3. Cis-regulatory elements: **ABRE-, CE1-, LTRE-, WRKY-, MYB-, MYC-, ELRE-, DPB-, GATA-**
Positions of stress-responsive predictive *cis*-elements in the putative promoters of Rab family genes (tomato) with respect to start codon (ATG). The 1000 bp putative promoter regions of corresponding *Rab* genes were used for analysis, using colour code. Nine selected stress responsive *cis*-elements are: ABA responsive element (ABRE), WRKY binding site (W-box), CE1, LTRE, DPB, ELRE, MYC, MYB and GATA

GATA Box: (ATGATAAGG, WGATAR): Based on sequence analysis, Grob and Stuber (1987) identified a sequence motif 5’ -ATGATAAGG-3’ that is present in many *LHC* and *rbcS* genes from a number of plant species. Presence of core GATA motif in the set of relative modules in the promoters of many light regulated genes has been reported. Also, GATA motifs are known

to be often associated with other LREs including G-boxes. Plant GATA factors typically contain a single zinc finger. All the upstream sequences here show predominant presence of this motif.

CE1: (TGCCACCGG): ABRE alone is not sufficient, and another *cis*-element known as "coupling element (CE)" is required for full range ABA-regulation of gene expression. CE1 is necessary for the ABA-regulation of *HVA22* gene (Shen, 1996). In *RD29A* gene, DRE (Dehydration-responsive element, TACCGACAT) functions as a coupling element to ABRE (Narusaka, 2003). This CRE was only identified in the upstream region of *Rab11a* only at –150 site.

Variance and co-occurences of CREs: Considerable variation in the number of CREs exists across the upstream regions of *Rab1A, Rab1B* and *Rab1C.* The ABA-responsive element (ABRE; PyACGTG/TC) is a well-studied CRE involved in ABA-induced gene expressions (Fujita, 2011; Hattori, 2002)**.** ABRE-binding Protein/factors (AREB/ABF) have positive effects on the osmotic stress tolerance of plants (Kang, 2002; Kim, 2004a,b; Yoshida, 2015). Presence of ABREs in the promoters of the genes under study indicated a role for ABA-signalling in the regulation of their expressions. Absence of ABRE in Rab11a may supported by the reports which say that, one exception to this is the MYB- and MYC-regulated RD22 gene, which lacks any ABREs (Abe, 1997). However, all of these arrangements allow for regulation by varying combinations of TFs whose identities are determined by the availability of specific regulators and binding sites. In the upstream analysis of our selected *Rab* genes the most frequently found elements are namely MYB, MYC, GATA and WBOX. A rather large MYB family, reported from different plants, plays a variety of key roles in the regulation of gene expression, and is also related to transcriptional responses to hormones during seed development and germination. For example, the maize *C1* gene regulates the expression of genes that are involved in the biosynthesis of anthocyanin pigments in the aleurone (Cone et al, 1986; Paz-Ares et al, 1986, 1987; Hattori et al, 1992). Putative MYB binding sites involved in the up-regulation of genes in plants overexpressing AtMYB2 (Abe, 1997), and regulatory sequences involved in the regulation by cold stress (Hannah, 2005). Interestingly, the MYC and MYB consensus sequences were reported in the *rd22* promoter (*Arabidopsis*), which do not contain any typical ABRE recognition site (Yamaguchi-Shinozaki and Shinozaki, 1993). However, both AtMYC2 and AtMYB2 genes are induced by drought and ABA treatment, suggesting that AtMYB2 may regulate cooperatively with AtMYC2 in another regulatory system other than the ABRE-bZIP regulatory system in the ABA signalling pathway in vegetative tissues and seeds under drought and salt stress (Iwasaki et al, 1995; Abe et al, 1997, 2003). It is worth mentioning that an important element like CRT/DRE was found totally missing in the upstream region of tomato *Rab* genes (*Rab11a, Rab1A, Rab1B, Rab1C*).

Table 3. Co-occurrence of transcription factor binding sites in gene group of *Rab1A,Rab1B, Rab1C* and *Rab11a* of *Solanum lycopersicum*. 1kbp Upstream sequences of the genes were scanned and *cis*-regulatory elements along with their frequency were searched from different databases like TRNSFAC/PLACE/PLANTPAN/PLANTTFDB. Elements from both the strands of the upstream sequences were considered for comparison.

List of *cis*-elements	Rab1A	Rab1B	Rab1C	Rab11a
ABRE	2	1	2	-
CE1	-	-	-	1
DPB	1	1	2	-
ELRE	-	1	-	3
GATA	4	4	3	7
LTRE	-	-	1	-
MYB	-	1	3	-
MYC	2	2	6	2
WRKY	4	6	5	6

Rab genes under study were found simultaneously up regulated in salt treated leaves and down regulated in treated roots and as reported in our earlier report (Roy, 2015). Because co-expressed genes tend to behave similarly, they are expected to be co regulated. Under the simplifying assumption that this co regulation occurs at the transcriptional level, co-expressed genes should contain similar CREs in their promoter regions. As a consequence, these yet unknown CREs will be statistically overrepresented in the intergenic regions of the co expressed genes in comparison with their frequent occurrence in a set of unrelated sequences. Co-occurrence of TFBSs means that these CREs do not act alone but in networks, along with many other elements within the promoter (Nguyen, 2006). In Table 3, presentation shows co-occurrence of *cis*-elements like ABRE, ACGT, WBOX, WRKY, MYB/MYC and GATA. The underlying assumption here is that since proteins which all take part in the same biochemical process or pathway are basically to a large degree localized in the same cell compartment, and that since many of these genes are active at just about the same time, then this means that all or most of the genes should be regulated by common regulatory factors, and therefore most or all contain similar CREs (Walhout, 2006). Here the statistically significant co-occurrence of motifs with each other is what is used to define the regulation of a set of genes, which usually make up a genetic regulatory network. In this way, even those motifs can be detected which have a low occurrence, if they co-occur relatively many times along with another motif. For example, the program RiCES (Rice *Cis*-Element Searcher) uses likelihood statistics in order to discover significant relationships between pairs of motifs (Doi, 2008). A number of scientists have studied synergistic effects of pairs of motifs acting in concert with each other in order to increase the level of expression of genes that they co-occur in, compared to gene expression where only one of the motifs are present. These include studies of the ABRE and DRE elements (Zhang, 2005; Nakashima et al, 2014).

CONCLUSIONS

Present computational work is based on simple assumption that genes with similar expression profiles are considered “co-expressed” as well as co-regulated. Four *Rab* genes in the

current study were found up-regulated in tomato plant (cv. Punjab Keshari) under salinity stress in our previous comparative transcriptional experiment. These four candidate genes found to contain similar CREs in their putative promoter regions. Conserved domains in the amino acid sequences show their functional and structural conservation during evolution. Various stress related putative CREs including ABA, salt, cold and dehydration were commonly identified with variable frequencies. Two known TF binding motifs, the W-box and the GATA-box, were found to be significantly enriched in putative promoters of these up-regulated genes. The CREs under study show their highest abundance in the upstream region of *Rab1C*. This observation is in agreement with our previous data demonstrating highest accumulation of this transcript in leaves and roots of tomato plant under severe salt stress. Number of CREs can be critically important in attraction of cognate TFs indicating that more copies of motifs led to greater promoter activity.

This information may aid to manipulate the cell function through preparation of synthetic promoters using different motifs. Such knowledge is also important in understanding the cross-talks between distinctive signalling pathways and the underlying regulatory machinery of cellular stress response.

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PRELIMINARY ASPECTS ON THE PHYTOTOXICITY OF SOME *THYMUS* SPP. AQUEOUS EXTRACTS

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Abstract: Phytotoxic natural compounds enable the development of alternative biocontrol products, therefore increasing numbers of species are screened for identification of such substances. The current paper assesses the phytotoxic potential of aqueous extracts of three *Thymus* species: *T. comosus*, *T. dacicus* and *T. praecox* against *Raphanus sativus* and *Brassica oleracea* test species. The germination percentage, speed of germination (SG) and accumulated speed of germination (AS) were significantly lowered in both test species, especially by the aqueous macerates tested. Plantlet growth was reduced by macerates treatments in a significant manner, in *R. sativus*, while in *B. oleracea* plantlets growth was significantly reduced by only one macerate and by some of the hydrosols prepared from flowering plants. Observed phytotoxic activity can be assigned to the presence of bioactive secondary compounds in tested extracts, rather than to physico-chemical factors, as indicated by spectrophotometric, pH and conductivity analyses.

INTRODUCTION

Plant secondary metabolites reflect the necessities to defend against attacks from pathogen or predatory organisms as well as against the damaging effects of abiotic factors (excessive light radiation, extreme temperatures, salinity etc.) (Bennett & Wallsgrove, 1994; Wink, 2003; Kennedy & Wightman, 2011). Also the biosynthesis of such compounds may offer an advantage when competing with other plants for resources (Jilani et al., 2008).

Presently over 100.000 plant secondary metabolism products are known, broadly classified in alkaloids, sikhimic acid derivatives, terpenoids etc. (Wink, 2009). Among bioactivities of these compounds, the inhibition of growth of neighbouring plants more recently generated interest, such interactions pertaining to the allelopathic phenomenon (Kruse , 2000). The phytotoxic activity of such substances enables the development of natural herbicidal products (Bajwa, 2014). Known examples of this type of compounds are sorgoleone, momilactones A and B, artemisinin (Dayan et al., 1999), leptospermone (Dayan & Duke, 2014), 1,8-cineol and1,4-cineol (Soltys et al., 2013), which affect the germination, growth, photosynthetic rate, plant transpiration, etc. through metabolic pathway alterations (Lorenzo et al., 2013).

Phytotoxic compounds occur widely within the plant kingdom, being encountered in various families such as Asteraceae, Poaceae, Amaranthaceae, Lamiaceae etc. Within the *Thymus* genus, of identified bioactive compounds, thymol and carvacrol are the most significant ones, followed by p-cymene, y-terpinene, borneol, terpinene-4-ol și 1,8 cineol (Maksimović et al., 2008; Stahl-Biskup, 2011). Also, a high number of polyphenols, especially rosmarinic acid, chlorogenic and caffeic acid, along epicatechin and ferrulic acid etc. were identified within the species of the genus (Boros et al., 2010).

Studies regarding the phytotoxic activity of species belonging to *Thymus* genus (Lamiaceae family) revealed the prominent role played by terpenoidic compounds. Terpenoids synthesized by these plants are known to have a certain influence on neighbouring plants development, with evolutionary adaptations being identified due to interactions with *Thymus* plants. Moreover, different chemotypes of *T. vulgaris* may generate different responses from interacting plants, further emphasizing the major role of the chemical origin of the compounds in allelopathy (Thorpe et al., 2011). It is assumed that such compounds reach the soil as a result of rain, which leaches the volatile compounds out of glandulary structures found on leaves and stem surfaces. Meanwhile, shedded leaves further lead to the accumulation of bioactive compounds in the substrate. Once in the soil, these substances may generate phytotoxicity either in a raw state or as a result of microbial transformations (Linhart et al., 2014). Under natural conditions, allelopathic activity was identified in several *Thymus* species such as *T. vulgaris*, *T. pulegioides* and *T. serpyllum* (Thorpe et al., 2011), activity supposedly assigned to phenolic and nonphenolic monoterpenes synthesized in these plants (Callaway, 2010). Phytotoxic activity was proven in soil experiments for some *T. vulgaris* chemotypes (Linhart et al., 2014) as well as *in vitro* for various types of extracts (aqueous, alcoholic, etheric) or for volatile oils of *T. capitatus* and *T. vulgaris* (Hemada & El-Darier, 2011), *T. numidicus* (Ben El Hadj Ali et al., 2014), *T. serpyllum* (Yan, 2008), *T. vulgaris* (Arouiee et al., 2010), *T. kotschyanus* (Safari et al., 2010; Farajollahi et al., 2012; Gholinejad et al., 2012, Soliman & Zatout, 2014) and *T. caramanicus* (Bagheri & Arjomand Tajadini, 2011). For other species of the genus such studies are not available, but one can assume the presence of secondary

metabolites with similar modes of action due to phylogenetic proximity and of evolutionary acquired traits (Imatomi et al., 2013).

In the Romanian spontaneous flora, 17 *Thymus* species are found in all altitudinal levels, of which *T. dacicus* and *T. comosus* may be encountered in grasslands and rocky meadows, while *T. praecox* inhabits crystalline rocks habitats (Ciocarlan, 2009).

The current paper focuses on testing aqueous extracts (macerates and aqueous fractions of hydrodistillation) of spontaneous *Thymus* species (*T. dacicus, T. comosus* , *T. praecox*) plants from Romanian flora, aiming to assess the phytotoxic potential of aforementioned species.

MATERIAL AND METHODS

Material

Plant material was represented by aerial parts of *Thymus comosus* Heuff. ex Griseb. & Schenk, *Thymus dacicus* Borbás and *Thymus praecox* ssp. *polytrichus* (A. Kern. ex Borbás) Jalas. plants, collected in both vegetative as well as flowering development phases. Plant material was harvested from Lotrului Valley, Vâlcea County (*T. dacicus*), Jina, Sibiu County (*T. comosus*) and Parâng Mountains, Gorj County, alt. 950 m (*T. praecox*), Romania. Prior to the extraction, the plants were dried in ambiental conditions (22-24° C temperatures), protected from direct sunlight. The identity of the species was established by prof. PhD Ştefan N., "Alexandru Ioan Cuza" University, Iaşi, Romania. Vouchers were deposited for each species at the Faculty of Biology Herbarium, "Alexandru Ioan Cuza" University, Iași, Romania.

Extract preparation

Generally, for phytotoxic activity testing, water is preferred as the extraction media, as it has virtually no toxicity, it implies low costs and the extracts require no further processing for use. Two methods were employed for extract preparation: water maceration (5 g plant material in 95 ml tap water) under continuous mixing on a magnetic stirrer, for 4 hours, at room temperature, the extracts subsequently being filtered using Whatman no.1 paper; hydrodistillation (according to European Pharmacopoeia) using a NeoClevenger type apparatus (with water as solvent in a 4:1 ratio to the amount of plant material) for 3 hours, collecting the resulted aqueous fraction (hydrosol). The aqueous fractions contain a series of volatile compounds as a result of the contact of the distilled water in the capillary of the apparatus with the volatile oils.

Phytotoxic activity testing

For the phytotoxic assessment of the extracts, the inhibition of germination and of initial plantlet growth were evaluated in *Raphanus sativus* L. var. *sativus*, Rodica cultivar and *Brassica oleracea* L. var. *capitata* f. *alba* DC, Dittmarscher cultivar, obtained from local seed retailers. The germination and root and stem elongation are frequently used parameters in assessing phytotoxic activity (Hoagland & Williams, 2004).Initially, 25 seeds of each species were placed on filter paper in Petri dishes (9 cm diameter). The filter paper was moistened with 3 ml of extract or, respectively, distilled water in control plates. Seed germination and plantlet growth was performed using a Snijders Scientific ECD 109E growth chamber with a 12/12 h photoperiod, at a 22-24°C temperature.

Testing was made in 3 replicates for each extract and, respectively, controls. The number of germinated seeds was recorded at 24, 48, 72 h after the placement of seeds on the wet filter paper. Root and stem lengths were measured at 18 plantlets for each experimental variant, after 72 h from the initiation of the experiment. The filter paper was maintained moist during the experiment by adding the required quantities of water or extract.

Qualitative analysis of extracts

Obtained extracts were spectrophotometrically evaluated for the presence of chemical compounds. For each aqueous macerate, respectively, hydrosol, absorption spectra were obtained within the 190-1100 nm range, using a Beckman DU730 spectrophotometer and plastic cuvettes with a light path length of 10 mm. Also, pH and electrical conductivity were measured for each extract using a Consort C532 multimeter.

Statistical analysis

The values recorded for seed germination and plantlet growth are expressed as mean values ± standard errors. The statistical significance of the degree of inhibition was calculated using ANOVA ($p=0,01$) and post-hoc Dunnett test by means of GraphPad Prism 6.0 software.

The extracts treatment effects on seed germination was assessed calculating the: germination percentage (GP), speed of germination (S), speed of accumulated germination (AS) and coefficient of the rate of germination (CRG). The equations of these indices are described in papers concerning the effects of allelopathic compounds on test plants, considering that they adequately reflect the course of the germination process (Chiapusio et al., 1997; Anjum & Bajwa, 2005; Islam & Kato-Noguchi, 2014).

RESULTS AND DISCUSSIONS

The influence of the treatments on germination

In the case of *Raphanus sativus* seeds, the germination percentages were significantly lowered, compared to the control, by all aqueous macerates (80,96% – 95,25%) (Table 1) and hydrosols (84,21% - 94,75%) (Table 2) treatments after 24 hours from the beginning of the experiment. Significantly lower germination percentages were recorded after 72 hours only in the case of three aqueous macerates (*T. praecox* anthesis, *T. dacicus* vegetative and *T. dacicus* anthesis). The speed of germination (SG) and the accumulated speed of germination (AS) were lowered more by the treatments with aqueous macerates than the ones with hydrosols comparing with the controls. The coefficient of the rate of germination was the least affected germination index following the treatments (Figure 1).

Table 1. Germination percentages in *Raphanus sativus* seeds (control and aqueous extract treatments, means ± standard error, a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

	***T. praecox* vegetative**	***T. praecox* anthesis**	***T. dacicus* vegetative**	***T. dacicus* anthesis**	**Control**
24 h	5.33^{d}±2.31	1.33^{d}±2.31	1.33^{d}±2.31	1.33^{d}±2.31	28±4
48 h	28^{b}±8	26.67^{b}±4.62	22.67^{c}±2.31	18.67^{c}±11.55	56±4
72 h	41.33±6.11	34.67^{a}±4.62	30.67^{b}±15.14	38.67^{a}±4.62	60±6.93

Table 2. Germination percentages in *Raphanus sativus* seeds (control and hydrosol treatments; means ± standard error; a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

	***T. praecox* vegetative**	***T. praecox* anthesis**	***T. dacicus* vegetative**	***T. dacicus* anthesis**	***T. comosus* vegetative**	***T. comosus* anthesis**	**Control**
24 h	1.33^{c}±2.31	14.67±8.33	4^{b}±4	2.67^{b}±2.31	2.67^{b}±4.62	4^{b}±4	25.33±10.07
48 h	65.33±12.22	50.67±18.9	56±17.44	56±16	64±17.44	74.67±10.07	70.67±6.11
72 h	72±14.42	50.67±16.17	64±16	70.67±12.86	72±10.58	80±13.86	81.33±8.33

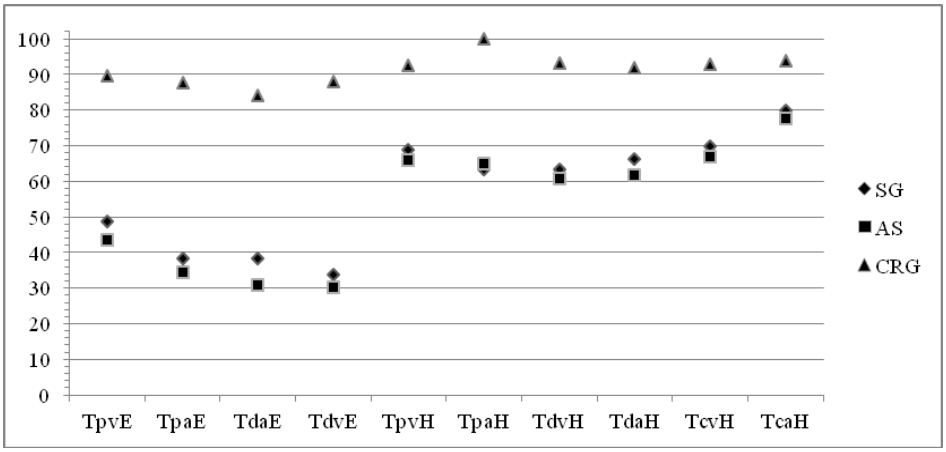

Figure 1. Speed of germination (SG), accumulated speed of germination (AS) and coefficient of the rate of germination (CRG) for *R. sativus* seeds treated (% from control) (T.p. – *Thymus praecox*; T. d. – *Thymus dacicus*; T. c. – *Thymus comosus*; v – vegetative phase; a – anthesis phase; E – aqueous macerate; H – hydrosol)

For *Brassica oleracea* seeds, the germination percentages were significantly reduced only following the treatments with aqueous macerates (Table 3), although lower values were recorded in the case of some hydrosols (Table 4), without being statistically significant. After 72 hours from the beginning of the experiment, the inhibition of germination was significant for the same three macerates which reduced the germination in *Raphanus sativus* seeds after the same time interval. The speed of germination (SG) and the accumulated speed of germination (AS) presented lower values compared to the controls in the case of all aqueous macerates. Only the hydrosols obtained from plants in the anthesis stage induced a reduction of these two indices, while hydrosols from vegetative stage plants stimulated germination. The coefficient of the rate of germination was, as in the case of radish seeds, the least sensible index (Figure 2).

Table 3. Germination percentages in *Brassica oleracea* seeds (control and aqueous extract treatments; means ± standard error; a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

	***T. praecox* vegetative**	***T. praecox* anthesis**	***T. dacicus* vegetative**	***T. dacicus* anthesis**	**Control**
24 h	0±0	1,33±2,31	0±0	0±0	0±0
48 h	17,33^{b}±16,17	22,67^{a}±22,03	13,33^{b}±4,62	22,67^{a}±4,62	62,67±12,86
72 h	53,33±8,33	38,67^{a}±22,03	33,33^{b}±16,17	45,33^{a}±6,11	81,33±10,07

Table 4. Germination percentages in *Brassica oleracea* seeds (control and hydrosol treatments; means ± standard error)

	***T. praecox* vegetative**	***T. praecox* anthesis**	***T. dacicus* vegetative**	***T. dacicus* anthesis**	***T. comosus* vegetative**	***T. comosus* anthesis**	**Control**
24 h	0±0	0±0	0±0	0±0	0±0	0±0	0±0
48 h	53.33±11.55	30.67±10.07	60±4	24±41.57	68±8	16±16	41.33±11.55
72 h	72±8	60±12	73.33±12.86	54.67±36.07	88±4	56±20	64±6.93

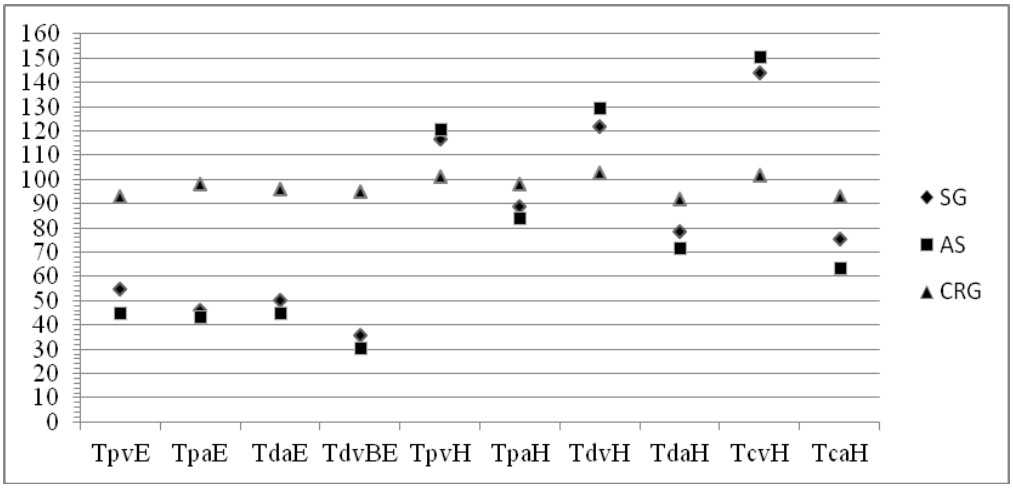

Figure 2. Speed of germination (SG), accumulated speed of germination (AS) and coefficient of the rate of germination (CRG) for *B. oleracea* seeds treated (% from control) (T.p. – *Thymus praecox*; T. d. – *Thymus dacicus*; T. c. – *Thymus comosus*; v – vegetative phase; a – anthesis phase; E – aqueous macerate; H – hydrosol)

The influence of the treatments on plantlet growth

For *Raphanus sativus* plantlets, a significant reduction in root and stem elongation was induced by all the treatments with aqueous macerates (Table 5 (a)), the*T. dacicus* extracts being generally more powerful than *T. praecox* ones. The hydrosol treatments did not significantly reduce the growth of radish plantlets, instead, the hydrosols obtained from vegetative stage plants stimulated the stem elongation (Table 5 (b)).

In the case of *Brassica oleracea* plantlets, a significant reduction of growth was observed only for *T. dacicus* aqueous macerate (Table 5 (a))., which reduced root length, the *T. dacicus*

anthesis hydrosol, which reduced stem length and *T. comosus* anthesis hydrosol, that reduced both root and stem elongation (Table 5 (b)).

Table 5 (a). Root and stem length (cm) in control and aqueous macerates treatments (means ± standard error; a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

Aqueous macerate	***T. praecox* vegetative**	***T. praecox* anthesis**	***T. dacicus* vegetative**	***T. dacicus* anthesis**	Control
R. sativus root length	**9.06^{b}±0.83**	**8.94^{b}±1.25**	**6.61^{d}±0.68**	**8.61^{b}±0.88**	14.33±1.62
R. sativus stem length	**4.44^{d}±0.40**	**5.72^{c}±0.81**	**4.28^{d}±0.47**	**4.50^{d}±0.36**	9.11±0.80
B. oleracea root length	5.94±0.66	5.22±0.56	4.33±0.55	**3.56^{c}±0.57**	6.94±0.64
B. oleracea stem length	2.83±0.29	3.00±0.33	2.61±0.29	2.39±0.20	3.06±0.26

Table 5 (b). Root and stem length (cm) in control and hydrosols treatments (means ± standard error; a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

Hydrosol	*T. praecox* vegetative	*T. praecox* anthesis	*T. dacicus* vegetative	*T. dacicus* anthesis	*T. comosus* vegetative	*T. comosus* anthesis	Control
R. sativus root length	17.22±5.71	13.50±5.85	14.67±5.59	13.61±4.00	18.06±6.78	17.17±5.59	17.72±6.13
R. sativus stem length	**10.11^{d}±1.68**	7.83±1.76	**9.33^{b}±1.91**	8.33±1.91	**10.39^{d}±1.72**	8.00±1.68	7.44±1.69
B. oleracea root length	7.89±2.19	7.06±2.56	9.11±4.20	5.00±2.43	10.94±3.98	**4.00^{c}±1.82**	8.22±3.81
B. oleracea stem length	3.11±0.83	3.11±0.96	3.44±1.20	**2.56^{c}±1.89**	3.33±1.09	**1.39^{d}±1.29**	4.22±1.26

Physico-chemical characteristics of the extracts

The pH values of the aqueous macerates indicate a more acid character compared to the initial extractive media (water). For hydrosols, the pH had higher values (alkaline character) than the distilled water used for extraction. Conductivity values show an increased concentration of mineral elements compared to the initial extraction media, in both aqueous macerates and hydrosols, with higher values for the macerates (Table 6).

Table 6. Conductivity and pH values for extraction media and extracts (T.W. – tap water; D.W. – distilled water; T.p. – *Thymus praecox*; T. d. – *Thymus dacicus*; T. c. – *Thymus comosus*; v – vegetative phase; a – anthesis phase; E – aqueous macerate; H – hydrosol)

Solution	T.W.	D.W.	TPvE	TPaE	TDvE	TDaE	TPvH	TPaH	TDvH	TDaH	TCvH	TCaH
pH	7.70	6.46	6.61	6.48	6.48	6.29	9.49	8.30	9.29	8.80	7.26	7.02
Conductivity (mS)	0.36	0.01	1.55	2.55	1.59	1.72	0.17	0.13	0.24	0.09	0.06	0.04

The absorption spectra indicate the presence of different types of compounds in the tested extracts (Figure 3, 4). The high values of absorbances in the 240-260 nm region possibly signify

phenolic compounds which usually have an absorption maxima (K bands) at 254 nm. Meanwhile, peaks in the 270-280 nm region are characteristic to different types of ethers. Also, the presence of polycyclic aromatic compounds may be assumed from the peaks in 320-350 nm area. However, one must take into account the polarity of the solvent used (water) which usually induces an absorbance shift towards higher wavelengths (red shift) of $\pi \rightarrow \pi^*$ transitions and towards lower wavelengths (blue shift) of $n \rightarrow \pi^*$ (Kalsi, 2004; Pretsch et al., 2009; Kaye & Laby, 2005). The recorded absorbances indicate a higher concentration of phenolic compounds in the macerates than in hydrosols.

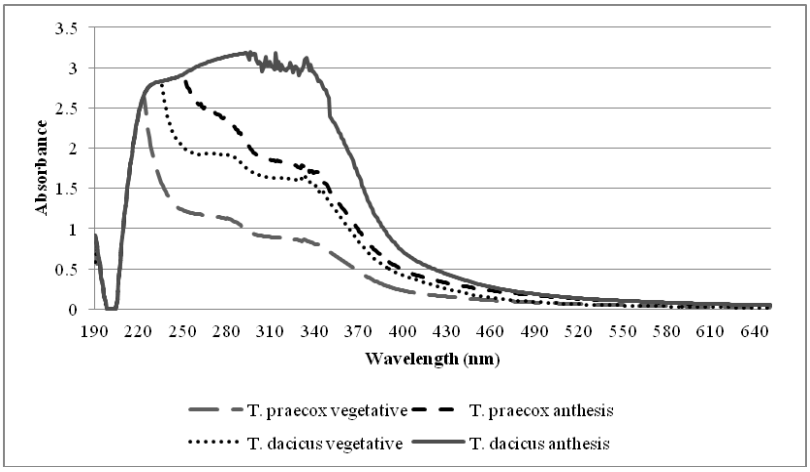

Figure 3. Absorbance spectra of aqueous macerates

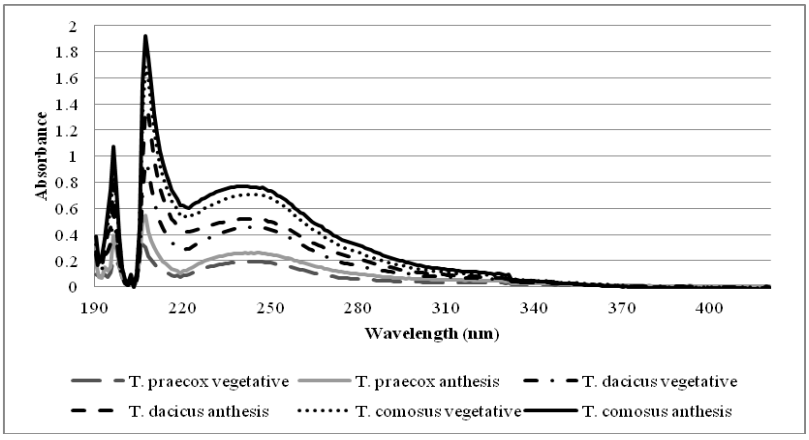

Figure 4. Absorbance spectra of hydrosols

The inhibitory effects of treatments with various extracts on germination and plantlet growth of tested species might be assigned to the compounds present in these extracts, presumably

phenolics and terpenoids, as observed from the spectrophotometric analyses.

The phytotoxic activity is known for phenolic compounds, which determine the inhibition of germination in 10^{-3} to 10^{-5} M concentrations (Williams & Hoagland, 1982). Such phytotoxicity is induced by various mechanisms such as alterations in membrane permeability, nutrient uptake reduction, inhibition of cell division and celular elongation, lowering of photosynthetic and respiration rates, alterations in enzymatic activities, endogenous phytohormones and proteic synthesis rate reduction (Li et al., 2010). Similarly, terpenoids are potent inhibitors of the germination and growth of plants, with more powerful effects in the case of monoterpenes and sesquiterpenes (Macias et al., 1999). Terpenoids inhibitory activities are due to mitochondrial respiration inhibition, plasmatic membrane permeability alteration, inhibition of chlorophyll and hormone synthesis etc., however, the modes of action being known for relatively few compounds (Duke & Oliva, 2004).

Testing for phytotoxic activity must account for pH and conductivity of extracts in order to discriminate between effects of actual compounds and those of different values of osmolarities and acidities than normal, physiological ones present in cells and tissues of test plants (Hoagland & Williams, 2004; Dayan & Duke, 2005).

Optimal pH values for germination of some cultivated species are between 6.5 – 6.7, with no significant effects of higher values, but with inhibition occurring at values below 6.5 (Pérez-Fernández et al., 2006; Deska et al., 2011). For the tested *Thymus* extracts, the values of the pH ranged between 6.29 – 6.61 (aqueous macerates) and 7.02 – 9.49 (hydrosols), allowing thus the assumption that this parameter did not significantly influence the germination process.

The conductivity of solutions is an indicator of ionic concentration, a factor that may lead to an inhibitory effect due to a hyper or hypotonic media. The conductivity values of the tested *Thymus* extracts varied between 0.24 mS (hydrosols) and 2.55 mS (aqueous macerates), which are lower than inhibitory values of 4 – 8 mS as determined for some cultivated plant species (Pendleton & Meyer, 1990; Mer et al., 2000).

The degree of phytotoxicity varies among the tested extracts. Regarding the aqueous macerates, in both *Raphanus sativus* and *Brassica oleracea* seeds, the most pronounced inhibitory effect on the germination process is observed for the extracts with the highest amounts of compounds as seen in the corresponding absorption spectra. On the other hand, the most active hydrosols concerning inhibition of plantlet growth of both test species are those obtained from vegetative stage plants of the three *Thymus* species. The inhibitory effect of hydrosols on organ elongation is in an inverse relation with the amount of compounds observed in the spectrophotograms, suggesting thus that the nature and not the amount of compounds determines the phytotoxicity. The variation of the chemical composition of volatile oils depending on the ontogenetic phase is known in many plants, including *Thymus* species where the anthesis is accompanied by a richer compound variety and concentration (Jordan et al., 2006; Gallaso et al., 2014). Generally, different compounds are attributed different phytotoxicities, for *Thymus* species, the carvacrol and thymol being considered the most active substances (Tarayre et al., 1995; Morales, 2002).

The effectiveness of the treatments varied with the type of extract used, generally the germination process being inhibited to a larger extent by the aqueous macerates compared to the hydrosols. The same observation stands also for the growth of *Raphanus sativus* plantlets, where some hydrosols even determined a stimulatory effect.

Several studies underlined the allelopathic activity of some Thymus species in natural environments and demonstrated such effects under controlled conditions, as germination and plant

growth was inhibited by several extracts or by individual terpenoidic compounds (Vokou et al., 2003; Angelini et al., 2003; Hemada & El-Darier, 2011; Ben El Hadj Ali et al., 2014). The obtained results reveal similar activities for the *T. dacicus*, *T. comosus*, *T. praecox* species, complementing previous studies.

CONCLUSIONS

The tested extracts exert a certain degree of phytotoxicity towards the two test species used. A higher inhibitory effect on germination (for both test species) was identified for the aqueous macerates (5% concentration). The growth of the plantlets was significantly reduced by the aqueous macerates for *Raphanus sativus*, while *Brassica oleracea* was less sensible to the treatments. Considering the pH and conductivity of the tested extracts, the inhibitory effects on germination and growth might be attributed to the compounds present rather than to the physico-chemical properties of the treatments. Because there are relatively few results concerning the phytotoxicity of the investigated *Thymus* species, further testing of various concentrations of extracts is required.

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INFLUENCE OF PRESERVING BY FREEZING ON SOME BIOCHEMICAL PARAMETERS IN FRUITS OF *RIBES NIGRUM* L. AND *RIBES RUBRUM* L.

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Keyword: *R. nigrum, R. rubrum,* anthocyanins, polyphenols, antioxidant capacity
Abstract: *R. nigrum* and *R. rubrum* are species with remarkable potential of production. The quality and quantity of the chemical compounds present in the fruit collected from species of plants in the genus Ribes are influenced by genetic qualities, climatic conditions, procedures of cultivation, the way of harvesting and depositing the fruit etc. The results of our study show the fact that, in fresh state, the fruit harvested from *Ribes nigrum* present antioxidant capacity and content of anthocyanins superior to the fruit harvest from *Ribes rubrum,* while the content of water has slightly lower values in the *Ribes rubrum*. The analyses carried out after maintaining the biologic material to be analysed for nine months in the freezer underlines modifications of the biochemical parameters investigates. Thus, the content of total polyphenols was reduced by 16.03% in *Ribes rubrum* and by 17.09% in *Ribes nigrum.* The contents of anthocyanins and dry substance as well as the antioxidant capacity have registered values superior to those evaluated for the fruit in fresh state.

INTRODUCTION

Ribes nigrum L. (black currant) and *Ribes rubrum* L. (red currant) (Grosulariaceae family) are species of bushes found in culture on all the continents, mainly in the temperate area of the northern hemisphere. They grow spontaneously in Europe and Asia (Grădinaru and Istrate, 2004).

Currently, at international level and in Romania, there are concerns regarding the cultivation, research and valuing the fruit from the species mentioned for nutritional and therapeutic purposes.

The fruit of red currant and black currant are used fresh, frozen or processed in various forms (juice, syrup, compote, marmalade, jam, jelly, wine, liquor etc. (Pârvu, 2002).

The bioactive compounds in the fruit of red and black currant (simple carbohydrates, fibres, vitamins, phenolic compounds, organic acids, mineral salts etc.) (Maldin and Mladin 1992; Cionea et al., 2009; Milivojević et al., 2009; Nour et al., 2011; Cosmulescu et al., 2015) exhibit an extremely diverse biological activity, which confers them therapeutic value, being recommended for a balanced nutrition (Maldin and Mladin 1992; Pârvu, 2002; Tabart et al., 2012; Khoo et al., 2012; Burzo, 2015). The fruit of *R. nigrum* and *Ribes rubrum* are known as sourced of bioactive polyphenols (Moyer et al., 2002, Milivojevic et al., 2010, Vakula et al., 2015). The polyphenolic compounds (phenolic acids, flavonoids, anthocyanins, tannins) are secondary metabolites, synthesized in plastids and stored in vacuoles (Burzo, 2015) with a role in plants protection. The polyphenolic compounds have beneficial effects for the human health, with vital role in preventing and improving some cardiovascular or neuronal diseases, cancer, diabetes, eye diseases etc. These effects are due to their antioxidant, antibacterial, antiviral, anti-inflammatory and proliferative, cardio tonic properties etc. (Gosh and Konishi, 2007; Milivojevic et al., 2010; Tabart et al., 2012; Subash et al., 2014; Vakula et al., 2015).

The red and blue colours of the fruits of *Ribes nigrum* and *Ribes rubrum* respectively are due to the presence of anthocyanins, group of pigments soluble in water, located in vacuoles in form of glycosides, which present antioxidant potential (Bakowska- Barczak et Kolodziejczyk, 2011) and can be used as natural dyes in food industry (Nour et al., 2011).

The fruit of red and black currants are very perishable. Preserving them by various procedures modifies their content of bioactive compounds. One of the preserving methods the most used is freezing. The fruits of red currant and black currant are well suitable to freezing, the period to preserve them at the temperature of -18^{o}C being between 10 and 14 months (Pârvu, 2002; http://posdru.afiprofamilia.ro/).

In the literature in the field, there are data regarding the effects of depositing by freezing on some bioactive compounds (polyphenols, anthocians etc.) and the antioxidant capacity in various fruit of berry type (Ancos et al., 2000; Ścibisz et al., 2007; Poiană et al., 2010) or in *Ribes nigrum* (Bakowska-Barczak et Kolodziejczyk, 2011; Oancea et al., 2014) but, regarding the *Ribes rubrum*, there is little information.

This paper has as purpose to underline some possible modifications of some biochemical parameters (water content, dry substance content, concentration of mineral elements, concentration of anthocians, concentration of total polyphenols and antioxidant capacity) in fruit of *Ribes nigrum* (black currant) and *Ribes rubrum* (red currant) at biological maturity, after preserving them by maintaining in the freezer, at a certain temperature, for a certain period of time.

MATERIAL AND METHOD

The plant material to be analysed is represented by fruit, harvested at biological maturity from specimens of *Ribes nigrum* and *Ribes rubrum*, coming from a particular crop (Vlădeni, Botoşani County).

The biochemical indicators by the experimental model were evaluated at fresh fruit (water contents, dry substance, total polyphenols, anthocians and antioxidant capacity) and frozen at the temperature of -18°C for nine months (apart from the indicators mentioned it was also evaluated the content of total mineral elements).

The dry matter and water contents are determined by the gravimetric method. This basically consists of evaluating the indicator by keeping the biological material at a temperature of 105°C to constant weight. The results are expressed in g of dry matter per 100 g of freshly analysed material. By difference, the amount of water contained in the biological material to be analysed is evaluated (Boldor şi colab., 1983).

The total mineral elements content was determined by assessing the calcinated residues at 550ºC. Keeping the sample to be analysed at the calcination temperature leads to the loss of organic substances and some of the volatile mineral substances. The results, representing the average of three consecutive determinations, are expressed in g of calcined residue/100 g of dry analysed material (Mănescu et al., 1978; Boldor et.al., 1983).

The content of total polyphenols was determined by the spectrophotometric method Folin - Ciocâlteu (Singleton and Rosi, 1965). The dose of total polyphenols is based mainly on the property of the compounds in this class to react in alkaline environment with the reagent Folin-Ciocâlteu, leading to a compound of blue colour. Color intensity evaluation is done at 765nm. The concentration of total polyphenols of the extracts is calculated by means of a calibration curve set in parallel and in the same conditions as the extracts, using a control solution of gallic acid. The results obtained, average of three parallel determinations, are expressed in mg/g equivalent of gallic acid.

Determination of anthocians content was done by the extraction of the biologic material to be analysed with an acidic alcoholic solution and measuring the absorption at the specific wave length of 515nm (Fuleki and Francis, 1968).

The free radical scavenging activity of the lyophilized powder was determined using the stable radical DPPH (2,4-dinitrophenyl-1-picryl hydrazyl) method as previously described (Seal, 2012). 200 μL of the tested sample were placed in test tubes and 2 mL of freshly prepared DPPH solution (60 μM) in methanol was added in each test tube and mixed. 30 minutes later, the absorbance was measured at 517 nm (Shimadzu UV-1700 spectrophotometer). The capability to scavenge the DPPH radical was calculated, using the following equation:

DPPHscavenged(%)=[(Ac−At)/Ac]×100; Ac - absorbance of the control and At - absorbance of the sample.

For the antioxidant activity we used a 3 mg/mL methanol extract solution. The results are the average of three determinations.

RESULTS AND DISCUSSIONS

The analysis of the experimental results regarding the content of water done on fresh fruit, harvested at biologic maturity from the species *Ribes nigrum* and *Ribes rubrum* respectively, indicates valued of 81.63 % and 84.68 % respectively) (table I), which confirms the data present in the literature in the field (Gherghi et al., 1973; Ekholm et al., 2007). Water, essential for the normal development of the metabolic processes, assures succulence and sweetness to the fruit. Different authors reported for other species values ranging from 80% to 90% (Mladin et Mladin, 1992; Ancos et al., 2000; Ekholm et al. 2007).

The dry matter content of fruits of the two species (table 1) is consistent with the data presented by Nour et al. (2011) in different species of *Ribes nigrum* (17.94- 23.17%) and *Ribes rubrum* (15.12 – 17.54%).

Regarding the total polyphenol content, we again notice the superiority of the black currant fruits that are over 3,5 times higher than those calculated for the red currants (table I).

Table I. Biochemical indicators for fruits harvested at biological maturity from *Ribes nigrum* and *Ribes rubrum*

The analyzed indicator (average value ± standard deviation; n=3)	Species	
	Ribes nigrum	*Ribes rubrum*

The water content (g %)	81.63±0.75	84.68±0.19
The dry matter content (g%)	18.36±0.75	15.31±0.19
The content of total polyphenols (mg/g)	30.67±0.05	8.8 ±0.15
The content of anthocians (mg%)	875.86±6.04	58.44±1.05
The antioxidant capacity (%)	65.7±1.13	72.7±0.42

Regarding the content of anthocians, our experimental results indicate the fact that the value of the indicator is about 15 times higher in the case of the fruit harvested from the species *Ribes nigrum*, comparing with those harvested from the species *Ribes rubrum* (table I).

In developing the experimental model, it was necessary to apply dilutions to the vegetal extracts carried out in order to determine the antioxidant capacity both in the case of the fruit of *Ribes rubrum* and in those of *Ribes nigrum*. The values mentioned in table I are for extracts that contain 5.04 mg vegetal material/ml for red currant and 1 mg vegetal material/ml for black currant. For this reason, the antioxidant capacity of the fruits harvested from Ribes nigrum is about five times higher than that harvested from the Ribes rubrum species.

The results presented in the literature in the field are in compliance with the data obtained in this study (Moyer et al., 2002; Pantelidis et al., 2007; Milivojević et al., 2010; Nour et al., 2011; Anisimovienė et al., 2013; Burzo, 2015). Some authors do not support the existence of a correlation between the content of anthocians and total polyphenols with antioxidant activity in the fruit of berry type (Moyer et al., 2002; Anisimovienė et al., 2013), but they mention that in the case of these fruits, anthocians and other compounds (tannins, stilbene, protoanthocyanidins, phenolic acids) participate in order to get the antioxidant capacity.

The research carried out by (Borges et al., 2010) underline the fact that the anthocians contribute to the increase of the total antioxidant capacity in proportion of about 73 % in the case of fruit of *Ribes nigrum* and 21% in the fruit of *Ribes rubrum.* In order to get the antioxidant activity, vitamin C also participates in proportion of 18% in *Ribes nigrum* and 47.5% in *Ribes rubrum*, but also other chemical compounds.

The results obtained after the determination of some biochemical indicators on fruit harvested from *Ribes nigrum* and *Ribes rubrum*, preserved by freezing at -18^{o}C for nine months, are presented in table II. The analysis of the results regarding the water content underlines the fact that the process of preservation by freezing led to the reduction of the value of the indicator by 1.01 % in the fruit of red gooseberry and by 4.26% in the fruit of black gooseberry. The content of dry substance presents amplitudes opposite to those presented in relation with the content of water (table II).

Decreasing of water content and implicitly, increasing othe dry smatter content of the frozen fruits of *Ribes rubrum* and *Ribes nigrum* is due to the formation of ice crystals which cause the alteration of the cytoplasm and the breaking of the cell walls and as a result some of the water content is released (Burzo, 1986).

Tabel II. Biochemical indicators for fruit harvested at biological maturity from *Ribes nigrum* and *Ribes rubrum*, preserved by freezing

The analyzed indicator (average value± standard deviation; n=3)	Species	
	Ribes nigrum	*Ribes rubrum*
The water content (g%)	78.16±0.89	83.53±0.58
- % compared to the fresh fruits	-4.26 %	-1.01%

The dry matter content (g%)	21.83±0.89	16.64±0.58
The content of total mineral elements (g%)	12.71	10.67
The content of total polyphenols (mg/g)	25.43±0.75	7.39±0.08
- % compared to the fresh fruits	-17.09	-16.03
The content of anthocians (mg%)	1093.6±2.95	81.68±0.25
+ % compared to the fresh fruits	+24.86	+40.07
The antioxidant capacity (%)	83.51	79.22
+ % compared to the fresh fruits	+27.10 %	+8.96

Regarding the total mineral content evaluated after freezing, we find values of about 16% more of the fruits harvested from the *Ribes nigrum* species compared to those harvested from the *Ribes rubrum* species (Table II). It is mentioned in the literature that the mineral elements present in red currant and black currant fruits are potassium, calcium, magnesium, phosphorus, sodium, chlorine, manganese, iron, zinc and copper (Ekholm et al., 2007; Nour et al., 2011).

The content of total polyphenols analyzed after the time when the fruits were preserved by freezing recorded a decrease of 16.03% in the fruits harvested from *Ribes rubrum* and 17.09% respectively in the ones harvested from *Ribes nigrum* (Table II). The results obtained by us are consistent with data present in the literature (Bakowska - Barczak et Kolodziejczyk, 2011). This behaviour may be explained by the slowing down of enzymatic processes due to the conservation and retardation conditions found and reported in the literature of fruit belonging to other fruit tree species such as *Rubus idaeus*, *Rubus fruticosus* or *Vaccinium myrtillus* (Ancos et al. 2000; Poiană et al., 2010).

By comparison with the fresh fruit, we notice an obvious increase of the content of anthocians after maintaining the biologic material to analyse at the temperature of -18^{o}C for nine months. Our results indicate values 24.86% and 40.07% higher in *Ribes nigrum* and *Ribes rubrum* respectively (table II). In the literature it is mentioned such a behaviour also at other intervals of time of preservation by freezing of the Ribes fruits (Oancea et al., 2014), but in the case of the fruit harvested from *Ribes nigrum* and maintained at the temperature of -20^{o}C for 9 months other authors notice the decease of the content of anthocians (Bakowska- Barczak and Kolodziejczyk, 2011). Changes in anthocyanins content during the period of preservation by freezing were reported also in fruit harvested from other species of shrubs with edible fruits, such as *Rubus idaeus*, *Rubus fruticosus*, *Lonicera caerulea*, *Vaccinium myrtillus* (Ancos et al., 2000; Poiană et al., 2010, Olteanu Z. et al., 2013).

Stability of anthocyanin content in fruit tissues during the freezing process depends on several factors, including the chemical composition of the fruit, the pH value, the organic acid content or the carbohydrate concentration.

Determination of antioxidant capacity after keeping fruits at -18^{o}C for nine months indicates the increase of the value of the investigated indicator by 27.10% in *Ribes nigrum* and by 8.96 in *Ribes rubrum* (tab. II). There is data in the literature showing that the temperature of freezing does not influence negatively the antioxidant capacity of the fruit harvested from certain bushes (Bakowska- Barczak and Kolodziejczyk, 2011).

These seemingly contradictory data on antioxidant capacity show us the multiple influences that the indicator can bear. It is genotype, variety, location, cultivation technique, maturation degree, season, storage conditions, processing conditions etc. (Skrankova et al., 2015).

CONCLUSIONS

The fruits and leaves of *Ribes nigrum* and *Ribes rubrum* are sources of biologically active compounds with depurative, sudorific, vitaminizing, remineralizing, healing, anti-rheumatic, antioxidant properties etc.

Analysis of fresh fruits from these species highlights high concentrations of total polyphenols and anthocyanins as well as important antioxidant activity. By comparison, the fruits harvested from *Ribes nigrum* have higher antioxidant capacity, higher anthocyanins content and slightly lower water content than those harvested from *Ribes rubrum*.

The conservation of black and red currants at -18 ° C for nine months caused, on the one hand, a slight reduction of total polyphenols and water and, on the other hand, the increase in anthocyanin content, dry matter content and antioxidant capacity.

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ENDOMETRIAL CANCER. A REVIEW AND EVALUATION OF RISK FACTORS.

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Keywords: endometrial cancer, age at diagnosis, risk factors, tamoxifen, treatment
Abstract: Uterine body cancer represents the uncontrolled and chaotic growth of some abnormal cells from the womb lining, being thus included in the category of gynaecologic cancers. The main risk factors for endometrial cancer are: ageing, nutritional imbalances that lead to obesity, diabetes, high blood pressure, nulliparity. This article is made up of a retrospective study for incidence of the endometrial cancer. We had reviewed the literature and created evidence-based practice recommendations for diagnosis and treatment. This review examines: risk factors, diagnostic and metastatic evaluation.

INTRODUCTION

Anatomy: The endometrium is the inner lining of the uterus and has both functional and basal layers. The functional layer is hormonally sensitive and is shed in a cyclical pattern during menstruation in reproductive-age women. Both estrogen and progesterone are necessary to maintain a normal endometrial lining. However, factors that lead to an excess of estrogen, including obesity and anovulation, lead to an increase in the deposition of the endometrial lining (Jayaraman M. et al., 2017). These changes may lead to endometrial hyperplasia, and, in some cases, endometrial cancer. Whatever the cause, a thickened lining will lead to sloughing of the endometrial tissue through the endometrial canal and into the vagina. As a result, heavy menstrual bleeding or bleeding after menopause are often the initial signs of endometrial cancer. This symptom tends to happen early in the disease course, allowing for identification of the disease at an early stage for most women (Ferlay J. et al., 2013).

Endometrial cancer is the sixth most frequent cancer in women worldwide, with about 290,000 new cases and 74,000 deaths in 2008 (Andreano A. et al., 2014). It is the most common gynaecological malignancy in western countries with 49,560 estimated new cases in 2013 in the USA and 98,900 in 2012 in Europe (Ferlay J. et al., 2013); (Sala E. et al., 2013). Well-known negative prognostic factors are high tumour grade, deep myometrial invasion (≥50% myometrial thickness), lymphovascular space invasion, non-endometrioid histology, and cervical stroma involvement (Ferlay J. et al., 2013). Among those, the most important single morphological prognostic feature is depth of myometrial invasion with a one-half cut-off, which divides the current stage I of the International Federation of Gynecology and Obstetrics (FIGO) staging system into Ia and Ib (Kisu I. et al., 2013); (Sala E. et al., 2013). The purpose of this document is to review the risks and benefits of current treatment options and optimize treatment for women with endometrial cancer.

Epidemiology

In the United States, endometrial cancer will be diagnosed in an estimated 52,630 women in 2014,with 8590 succumbing to their disease. Most endometrial cancers are diagnosed at an early stage (75%), and the reported survival rate is 75% (Burke WM. et al., 2014). The mean age of diagnosis in the United States is 60 years. Caucasian women have a 2.88% lifetime risk of developing uterine cancer compared with a 1.69% risk for African - American women. African-American women are more likely to have non-endometrioid, high-grade tumors and a more advanced stage of disease at the time of diagnosis compared with Caucasian women who have similar demographics (Siegel R. et al., 2013). Endometrial cancer usually appears at elderly women, the peak frequency being at ages of 55-65 years old, but it can be met at the young woman (<40 years old). In Romania, endometrial cancer is on the fourth place, among gynaecologic cancers, and seventh place, as a number of deaths through cancer. Endometrial cancer is a disease characteristic for the postmenopausal period, the majority of the female patients of the studied lot, having the age between 51-60 years old (Valu MV, Toma O., 2017). Prognosis depends on patient age, histological type, grade, tumour size, depth of myometrial invasion, the presence of cervical stroma invasion, and lymph-node metastases (Epstein E. et al., 2014). Most women are diagnosed at stage I, in which prognosis is generally good, and the 5-year survival is 88% (Antonsen SL. et al., 2013); (Mascilini F. et al., 2013). The prognosis is worse for women with high-risk cancer (i.e. deep myometrial invasion, cervical stroma invasion, or high-grade disease see below for an explanation), as these women are at increased risk of lymph-node metastases (Thomsen HS. et al., 2013). Most cases of endometrial cancer cannot be prevented, but there are some things that may lower your risk of

developing this disease. One way to lower endometrial cancer risk is to do what you can to change your risk factors when ever possible. For example, women who are overweight or obese have up to 3½ times the risk of getting endometrial cancer compared with women at a healthy weight. Getting to and maintaining a healthy weight is one way to lower the risk of this cancer. Studies have also linked higher levels of physical activity to lower risks of endometrial cancer, so engaging in regular physical activity (exercise) may also be a way to help lower endometrial cancer risk (Bendifallah S. et al., 2015); (Creutzberg CL. et al., 2015). An active lifestyle can help you stay at a healthy weight, as well as lower the risk of high blood pressure and diabetes (other risk factors for endometrial cancer). Estrogen to treat the symptoms of menopause is available in many different forms like pills, skin patches, shots, creams, and vaginal rings. Progestins (progesterone-like drugs) can reduce the risk of endometrial cancer in women taking estrogen therapy, but this combination increases the risk of breast cancer. Getting proper treatment of pre-cancerous disorders of the endometrium is another way to lower the risk of endometrial cancer. Most endometrial cancers develop over a period of years (Creutzberg CL. et al., 2015). Many are known to follow and possibly start from less serious abnormalities of the endometrium called endometrial hyperplasia. Some cases of hyperplasia will go away without treatment, but it sometimes needs to be treated with hormones or even surgery. Treatment with progestins and a dilation and curettage (D&C) or hysterectomy can prevent hyperplasia from becoming cancerous. Abnormal vaginal bleeding is the most common symptom of endometrial pre-cancers and cancers, and it needs to be reported and evaluated right away. Women with hereditary nonpolyposis colon cancer (HNPCC or Lynch syndrome) have a very high risk of endometrial cancer. A woman with HNPCC may choose to have her uterus removed (a hysterectomy) after she has finished having children to prevent endometrial cancer. One study found that none of 61 women with HNPCC who had prophylactic (preventive) hysterectomies was later found to have endometrial cancer, while 1/3 of the women who didn't have the surgery were diagnosed with endometrial cancer over the next 7 years (Jayaraman M. et al., 2017).

Risk factors

We do not yet know exactly what causes most cases of endometrial cancer, but we do know certain risk factors, particularly hormone imbalance, for this type of cancer. A great deal of research is going on to learn more about the disease. We know that most endometrial cancer cells contain estrogen and/or progesterone receptors on their surfaces. Somehow, interaction of these receptors with their hormones leads to increased growth of the endometrium. This can mark the beginning of cancer. The increased growth can become more and more abnormal until it develops into a cancer. (Jayaraman M. et al., 2017). Prolonged unopposed estrogen exposure is associated with most type I endometrial cancers. Estrogen replacement therapy prescribed to control menopausal symptoms increases the risk of developing endometrial cancer by 2- to 20-fold, with an increasing risk correlating with the duration of use. Concomitant administration of progestins continuously or intermittently (10 to 15 days/month) significantly reduces this increased risk of cancer (Busch EL. et al., 2017). Exposure to unopposed endogenous estrogen, as occurs in chronic anovulation (polycystic ovary syndrome), with estrogen-producing tumors, and with excessive peripheral conversion of androgens to estrone in adipose tissue, is also associated with an increased risk for developing endometrial hyperplasia and cancer. Tamoxifen, a selective estrogen receptor modulator, acts as an estrogen antagonist in breast tissues and an agonist in bone and endometrial tissues. Tamoxifen use is associated with a 6- to 8-fold increase in the incidence of endometrial cancer (Guinney J. et al., 2015); (Mutter GL. et al., 2014); (Zaino RJ. et al., 2014). The obesity epidemic in the Romania may have a profound impact on the incidence of endometrial cancer seen this country. The profound increased incidence of endometrial cancer associated with obesity may be explained by higher endogenous estrogen production via aromatization in adipose tissues. Additionally, premenopausal obese women are more likely to suffer from chronic anovulation (Mutter GL. et al., 2014). Diabetes mellitus is associated with an increased risk for endometrial cancer that may be related to concurrent obesity, although an independent association between diabetes and endometrial cancer has been reported. Hypertension has been epidemiologically associated with an increased risk of endometrial cancer, but whether hypertension represents an independent risk factor or the association is confounded by the presence of medical comorbidities, such as diabetes and obesity, is unclear (Zaino RJ. et al., 2014). Age also represents an important risk factor for developing endometrial cancer. Most women are diagnosed after menopause, with only 15% diagnosed before the age of 50 years and only 5% before 40 years of age. Younger women who develop endometrial cancer are more likely to be obese and nulliparous and have well-differentiated endometrioid histology and lower-stage disease than older women (Guinney J. et al., 2015). Reproductive characteristics associated with increased risk of endometrial cancer include nulliparity, infertility, early age of menarche, and late age of menopause (Allot EH. et al., 2016); (Cheang MC. et al., 2015). Importantly, the use of combination oral contraceptive pills, depot medroxyprogesterone acetate, and progesterone secreting intra-uterine devices reduces the risk of developing endometrial cancer. Smoking has also been associated with a reduced risk for endometrial cancer, especially in postmenopausal women (Allot EH. et al., 2016). Genetic disorders can also cause endometrial cancer. Overall, hereditary causes contribute to 2–10% of endometrial cancer cases. Lynch syndrome, an autosomal dominant genetic disorder that mainly causes colorectal cancer, also causes endometrial cancer, especially before menopause. Women with Lynch syndrome have a 40–60% risk of developing

endometrial cancer, higher than their risk of developing colorectal (bowel) or ovarian cancer (Jayaraman M. et al., 2017). Ovarian and endometrial cancer develop simultaneously in 20% of people. Endometrial cancer nearly always develops before colon cancer, on average, 11 years before. Carcinogenesis in Lynch syndrome comes from a mutation in MLH1 and/or MLH2: genes that participate in the process of mismatch repair, which allows a cell to correct mistakes in the DNA (Guinney J. et al., 2015). Other genes mutated in Lynch syndrome include MSH2, MSH6, and PMS2, which are also mismatch repair genes. Women with Lynch syndrome represent 2–3% of endometrial cancer cases; some sources place this as high as 5%. Depending on the gene mutation, women with Lynch syndrome have different risks of endometrial cancer. With MLH1 mutations, the risk is 54%; with MSH2, 21%; and with MSH6, 16%. Women with a family history of endometrial cancer are at higher risk. Two genes most commonly associated with some other women's cancers, BRCA1 and BRCA2, do not cause endometrial cancer (Mutter GL. et al., 2014). There is an apparent link with these genes but it is attributable to the use of tamoxifen, a drug that itself can cause endometrial cancer, in breast and ovarian cancers. The inherited genetic condition Cowden syndrome can also cause endometrial cancer. Women with this disorder have a 5–10% lifetime risk of developing endometrial cancer, compared to the 2–3% risk for unaffected women (Allot EH. et al., 2016). The International Federation of Gynecology and Obstetrics (FIGO) classification system is most often used to stage endometrial carcinoma (Table 1), and the staging remains surgical as the treatment is most often surgical.

Table 1

FIGO 2010 classification of carcinoma of the endometrium.
IA. Tumour confined to the uterus, no or <50% myometrial invasion
IB. Tumour confined to the uterus, 50% myometrial invasion
II. Cervical stromal invasion, but not beyond the uterus
IIIA. Tumour invades serosa or adnexa
IIIB. Vaginal, parametrial involvement, or both
IIIC1. Pelvic-lymph node involvement
IIIC2. Para-aortic lymph-node involvement
IVA. Tumour invasion of bladder, bowel mucosa, or both
IVB. Distant metastases, including abdominal metastases, inguinal lymph nodes, or both

FIGO, International Federation of Gynecology and Obstetrics (2010).

Clinical presentation and diagnostic assessment

Abnormal uterine bleeding - sometimes associated with vaginal discharge and pyometra is the most frequent symptom of endometrial cancer and is noted in about 90% of patients (usually during menopause). Patients with advanced disease might have symptoms similar to those of advanced ovarian cancer, such as abdominal or pelvic pain and abdominal distension (Ferlay J. et al., 2015). Disease can easily be diagnosed on the basis of office-based pipelle sampling or other techniques. The histological information provided by endometrial biopsy is sufficient for preoperative assessment and planning. However, pipelle sampling can be infeasible in some postmenopausal women because of cervical stenosis (Siegel RL. et al., 2015); (Trabert B. et al., 2015); (Hussein YR. et al., 2015). When histological findings from an endometrial biopsy are insufficient to confirm diagnosis, cervical dilation and curettage is recommended, although this investigation necessitates anaesthesia and has been associated with disease underestimation (Ferlay J. et al., 2015). A biopsy under hysteroscopy remains the gold standard for diagnosis of endometrial cancer and yields higher accuracy than does blind dilation and curettage. Results of some studies suggested a higher incidence of malignant peritoneal cytology at the time of hysterectomy in patients who underwent previous hysteroscopy than in those who did not, but no evidence supports an association between diagnostic hysteroscopy and worse prognosis (Hussein YR. et al., 2015). Thus, the standard strategy for investigation of abnormal uterine bleeding is pelvic ultrasonography with an endometrial biopsy in cases of increased endometrial thickness and a hysteroscopy when diagnosis is uncertain. A review of 13 studies showed that, in menopausal women, an endometrial thickness cutoff of 5 mm on ultrasonography had sensitivity of 90% and specificity of 54% compared with 98% and 35%, respectively, when the cutoff was reduced to 3 mm (Siegel RL. et al., 2015).

Treatment and survival

Uterine cancers are usually treated with surgery, radiation therapy, hormone therapy, and/or chemotherapy, depending on stage of disease and histologic type (Jagsi R. et al., 2014). Surgery alone, consisting of hysterectomy (often along with bilateral salpingo-oopherectomy), is used to treat 72% of patients with early-stage disease. Approximately 22% of early-stage disease is high-risk disease and is treated with radiation either alone, or in combination with chemotherapy, in addition to surgery. The majority (64%) of women with advanced disease undergo surgery followed by radiation therapy and/or chemotherapy. Clinical trials are currently assessing the most appropriate regimen of radiation therapy and chemotherapy for women with metastatic or recurrent uterine cancer (James JA. et al., 2015). Most cancers of the uterine corpus (68%) are diagnosed at an early stage, usually because of postmenopausal bleeding (Todo Y. et al., 2015). The 1-year and 5-year relative survival rates for patients with cancer of the uterine corpus are 92.1% and 81.5%, respectively. The 5-year survival rate is 95.3% for localized disease, 67.5% for regional disease, and 16.9% for distant-stage disease. The overall 5-year survival for white women (84%) is 23% higher than that for black women (61%) (Deura I. et al., 2015); (Biglia N. et al., 2015); (Rowlands IJ et al., 2015). Higher body weight adversely affects endometrial cancer survival, whereas physical activity is associated with improved survival (Hopp EE. et al., 2015); (Hareyama H. et al., 2015). Any hysterectomy causes infertility. Bilateral oophorectomy will cause menopause in premenopausal women, which can lead to symptoms such as hot flashes, night sweats, vaginal dryness, and osteoporosis (Ferrandina G. et al., 2014). Long-term side effects of radiation therapy for uterine cancer can include bladder and bowel dysfunction, as well as vaginal dryness and stenosis. Sexual problems are commonly reported among uterine cancer survivors (Herling SF. et al., 2015); (Beesley VL. et al., 2015). Pelvic lymphadenectomy can lead to lower extremity lymphedema, particularly for women who also receive radiation therapy (Bae HS. et al., 2016); (Mitra D. et al., 2016); (Mendivil AA. et al., 2016). The “gold standard” treatment for endometrial cancer is completely staged surgery, followed by radiation or chemotherapy, based on the final pathological surgical stage and requirements. In the primary treatment of endometrial cancers, hormones are rarely taken into consideration after primary surgery (Bendifallah S. et al., 2015); (Creutzberg CL. et al., 2015). Primary treatment with hormones to preserve fertility in younger women with endometrial cancer is an attractive option, and many successful cases have been reported, although the majority of them finally received definite therapy, including total hysterectomy (Beesley VL. et al., 2015). The role of hormone therapy is often delayed in recurrent disease; response rates to progestins and tamoxifen or aromatase inhibitors in advanced/recurrent endometrial cancers are approximately 15-20% and nearly ≤10%, respectively (Creutzberg CL. et al., 2015).

Surgery: Total hysterectomy and removal of both tubes and ovaries is the standard treatment for apparent stage I endometrial cancer and is effective in most cases. Alternatives to primary hysterectomy in women who want to preserve their fertility have been comprehensively reviewed. Hysterectomy and adnexectomy can be done with minimally invasive techniques (laparoscopy or robot-assisted surgery), vaginally, or laparotomically (Bradford LS. et al., 2015). The safety of laparoscopy has been shown in randomized clinical trials and is associated with shorter hospital stays and fewer postoperative complications than laparotomy. Survival rates seem similar, which should be confirmed by completed trials (eg, NCT00096408). Laparoscopic or robotic approaches should be avoided in cases of bulky uterine malignant disease that might necessite morcellation, because morcellation can lead to tumour spillage, increasing local or peritoneal recurrence and thereby affecting survival (Oza A. et al., 2015); (Slomovitz BM. et al., 2015). Although simple total hysterectomy is sufficient for most women, radical hysterectomy is sometimes done in cases of gross cervical invasion or when uncertainty exists about whether the primary tumour is endocervical or endometrial in origin. Surgical staging for endometrial cancer includes careful assessment of the peritoneal surfaces (Matulonis U. et al., 2015). Omental and peritoneal biopsies are commonly done in high-risk disease. Estimated cumulative risk of endometrial cancer is 0-96%; the corresponding mortality risk is 0-23% and mortality-to-incidence ratio is 0-24 - lower than that of breast cancer (0-32), ovarian cancer (0-63), and uterine cervical cancer (0-55). Most endometrial cancers (75%) are diagnosed at an early stage (FIGO stages I or II): 5 year overall survival ranges from 74% to 91%; for FIGO stage III, 5 year overall survival is 57–66%, and for FIGO stage IV disease is 20–26%, 5 year disease survival is estimated at 90% in patients without lymph node metastasis, 60–70% in those with pelvic lymph node metastasis, and 30–40% in those with paraaortic lymph node metastasis (Howitt BE. et al., 2015); (Moir-Meyer GL. et al., 2015). However, a substantial proportion of patients with endometrial cancer die from other health conditions as these patients often have several comorbidities (Coleman RL. et al., 2015). Survival is dependent on other predictive factors, such as the tumour grade, age, comorbidities, tumour diameter, American Society of Anesthesiologists score, lymphovascular space involvement, and postoperative complications at 30 days (Slomovitz BM. et al., 2015). Among the various nomograms predicting survival, two have been validated externally. The first to be published consists of five simple criteria (age at diagnosis, negative lymph nodes, FIGO stage, final histological grade, and histological subtype). The second was validated in randomly assigned patients from the PORTEC 1 and PORTEC 2 trials and showed that age, tumour grade, and lymphovascular space involvement were highly predictive for all outcomes (Bradford LS. et al., 2015).

CONCLUSIONS

We searched Pubmed and Embase for studies on body mass index and the risk of endometrial cancer, published from 2013 to 2017. Data were independently extracted and analyzed using random or fixed effects meta-analysis depending on the degree of heterogeneity. The findings from this meta-analysis strongly support that the conditions of EBW (excess body weight), overweight, and obesity are all associated with an increased risk of endometrial cancer. Also, the strength of the association increases with increasing BMI (body mass index - ≥30kg m²). The findings from this meta-analysis strongly support that the conditions of EBW, overweight, and obesity are all associated with an increased risk of endometrial cancer. Also, the strength of the association increases with increasing BMI.

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IN MEMORIAM
DR. VALENTINA JURCĂ
1931 – 2017

În ziua de 29 iulie 2017, aripa morții s-a rotit din nou asupra buchetului de cadre didactice pensionare de la Facultatea de Biologie a Universității „Alexandru Ioan Cuza” din Iași, făcând să înceteze a mai bate inima uneia dintre distinsele noastre colege, Valentina Jurcă, șef de lucrări doctor la disciplinele de Biochimie și Chimie Generală.

Născută la 26 ianuarie 1931, în comuna Coșcodeni, regiunea Bălți (din R.S.S. Moldovenească), Valentina Jurcă a urmat școala primară în comuna natală (1939 – 1943), apoi studiile gimnaziale și liceale la Liceul de Fete din Tecuci, între anii 1943 – 1951. După absolvirea liceului, s-a înscris la Facultatea de Chimie a Universității „Alexandru Ioan Cuza” din Iași, unde și-a luat licența în anul 1955.

Remarcându-se în anii de studenție prin deosebita pregătire de specialitate și prin pasiune pentru cercetarea științifică, Valentina Jurcă a fost numită la 15 noiembrie 1955, șef de laborator la Catedra de Tehnologia Substanțelor Anorganice de la Facultatea de Chimie Industrială a Institutului Politehnic „Gh. Asachi” din Iași. La 1 februarie 1957, Valentina Jurcă s-a transferat pe postul de asistent suplinitor la disciplina Chimie Biologică de la Facultatea de Chimie a Universității „Alexandru Ioan Cuza” din Iași, unde a fost avansată asistent în anul 1958. Ca asistent la disciplină de Chimie biologică devine colaboratoarea apropiată a regretatei profesor dr. Elisabeta Văscăuțeanu (1897 – 1989), titularul de atunci al acestei discipline. Trebuie să subliniem că în acea vreme Chimia biologică era disciplină obligatorie în planul de învățământ de la Facultatea de Științe Naturale-Geografie și numai disciplină facultativă la Facultatea de Chimie. În calitate de asistent, Valentina Jurcă a dovedit o mare putere de muncă, pasiune pentru Chimia biologică, atașament față de laboratorul în care

IN MEMORIAM
DR. (PhD) VALENTINA JURCĂ
1931 – 2017

On 29 July 2017, the wing of death was rotated once again over the bouquet of teachers retirement from the Faculty of Biology of “Alexandru Ioan Cuza” University of Iași City, halting the heart beats of one of our most distinguished peers, Valentina Jurcă, Senior Lecturer PhD of Biochemistry and General Chemistry.

Born on 26 January 1931, in Coșcodeni commune, Bălți District (of the Moldavian Soviet Socialist Republic), Valentina Jurcă attended elementary education in her home locality (1939 – 1943), then middle school and secondary education with Liceul de Fete din Tecuci (*Girls’ High School of Tecuci*), from 1943 to 1951. After graduating secondary education, she enrolled in the Faculty of Chemistry of “Alexandru Ioan Cuza” University of Iași City, where she was awarded the Bachelor of Science in 1955.

As during her period of undergraduate studies, she remarked herself through her outstanding speciality training and passion for scientific research, Valentina Jurcă was appointed on 15 November 1955, as head of laboratory of the Department for the Technology of Inorganic Substances of the Faculty of Industrial Chemistry of “Gh. Asachi” Polytechnic Institute of Iași City. On 1 February 1957, Valentina Jurcă transferred on the position of junior assistant at the discipline of Biological Chemistry of the Faculty of Chemistry of “Alexandru Ioan Cuza” University of Iași City, where she was promoted to assistant in 1958. As assistant for the discipline of Biological Chemistry she intimately cooperates with the regretted Professor PhD Elisabeta Văscăuțeanu (1897 – 1989), then tenured professor of this course. We must point out in that time, Biological Chemistry was compulsory course within the curriculum of the Faculty of Natural Sciences – Geography and only facultative course with

lucra, interes pentru continua perfecționare, remarcându-se ca un bun cadru didactic, preocupat de desfășurarea unei activități eficiente cu studenții.

La 1 octombrie 1961 a fost promovată șef de lucrări suplinitor la disciplina de Chimie biologică din Catedra de Chimie Fizică și Chimie Generală, iar la 15 februarie 1969 a fost titularizată ca șef de lucrări la aceeași disciplină, Catedra de Chimie Generală de la Facultatea de Chimie a Universității „Alexandru Ioan Cuza” din Iași.

În a doua jumătate a anului 1969, Valentina Jurcă obține titlul de doctor în chimie, specialitatea Biochimie, în urma susținerii cu succes a tezei de doctorat „Contribuții privind studiul metabolismului fosforat la pești(*Salmo gairdnerii*), în funcție de unii factori de mediu și hrană”, elaborată sub conducerea științifică a academicianului Eugen Macovschi de la Universitatea din București. Teza de doctorat aduce contribuții importante referitoare la diferite aspecte ale metabolismul fosforului și proteinelor la păstrăvul curcubeu, aceste aspecte având implicații în ameliorarea condițiilor de creștere a speciei de pește menționată.

De la data de 15 septembrie 1974, în urma transferării Facultății de Chimie de la Universitatea „Alexandru Ioan Cuza” la Institutul Politehnic din Iași, Colectivul de Chimie și Biochimie, din care făcea parte și Valentina Jurcă, a trecut la Facultatea de Biologie-Geografie. Toți membrii micului Colectiv de Chimie și Biochimie veniți de la Facultatea de Chimie s-au integrat rapid și eficient în colectivul Catedrei de Biologie Animală de la Facultatea de Biologie-Geografie-Geologie, remarcându-se prin punctualitate, responsabilitate, conștiinciozitate și devotament față de noul loc de muncă.

În calitate de asistent și șef de lucrări, Valentina Jurcă a condus lucrări practice de laborator la disciplina de Chimie biologică cu studenții din anul V de la Facultatea de

the Faculty of Chemistry. As assistant, Valentina Jurcă worked hard and ardently for Biological Chemistry, developed affection for the laboratory she worked in, was keen on further training, remarking herself as proficient professor, preoccupied with efficiently working with students.

On 1 October 1961 Valentina Jurcă was promoted as junior lecturer in the Biological Chemistry discipline at the Department of Physical Chemistry and General Chemistry, and on 15 February 1969 she was appointed as Senior Lecturer for the same course, the Department of General Chemistry of the Faculty of Chemistry of “Alexandru Ioan Cuza” University of Iaşi.

In the second half of 1969, Valentina Jurcă earns the PhD of Science in Chemistry, speciality Biochemistry, following the successful defence of the doctoral thesis “Contributions on the study of the phosphate metabolism of fish (*Salmo gairdnerii*), depending on a series of factors of environment and food”, drafted under the scientific supervision of the Academician Eugen Macovschi of the University of Bucharest. The doctoral thesis brings important contributions with regard to the various aspects of phosphate and protein metabolism at the rainbow trout, such aspects having impact on the improvement of the growth conditions of the fish species mentioned.

As of 15 September 1974, following the transfer of the Faculty of Chemistry from “Alexandru Ioan Cuza” University of Iași to the Polytechnic Institute of Iași City, the teaching staff of Chemistry and Biochemistry of which Valentina Jurcă was equally part of, was included in the Faculty of Biology – Geography. All members of the small staff of Chemistry and Biochemistry coming from the Faculty of Chemistry rapidly and efficiently integrated with the staff of the Department of Animal Biology of the Faculty of Biology – Geography – Geology, remarking themselves

Chimie, de asemenea, din anii II și III de la Facultatea de Științe Naturale-Geografie și de la Secția de Științe Naturale și Agricole a Institutului Pedagogic de 3 ani. Totodată a predat cursul de C*himie biologică* studenţilor de la Secţia fără frecvenţă a Facultăţii de Biologie-Geografie, precum și cursul de B*iochimie* pentru studenţii din anul al II-lea de la Secţia de Ştiinţe Naturale și Agricole. După pensionarea profesoarei dr. Elena Budeanu (1916 – 1992), începând din anul universitar 1976 – 1977 Valentina Jurcă a predat cursul de *Chimie generală* studenților din anul I de la specializarea Biologie a Facultății de Biologie-Geografie-Geologie. A onorat acest curs peste o decadă, până la 1 septembrie 1988, când a ieșit la pensie, după o îndelungată și meritorie activitate didactică și științifică.

Cursurile predate de Valentina Jurcă se distingeau printr-o ținută științifică aleasă, prin claritate și documentare, fiind audiate cu interes de numeroasele serii de studenții naturaliști și biologi, contribuind la formarea lor ca specialiști.

Pentru a veni în sprijinul pregătirii studenților, Valentina Jurcă a elaborat și a multiplicat la Centrul de Multiplicare al Universității „Alexandru Ioan Cuza” următoarele manuale : în anul 1973, în calitate de singur autor, un manual de *Lucrări practice de chimie biologică* ; în anul 1984, împreună cu prof. dr. Elena Budeanu și șef de lucrări dr. Elvira Tănase, un manual de *Lucrări practice de chimie generală*, iar apoi ca autor unic *Cursul de Chimie Generală*, în trei volume : Volumul I (1978) ; Volumul II (1980) și Volumul III (1982).

Paralel cu activitatea didactică, axată pe conducerea lucrărilor de laborator și predarea diferitelor cursuri, Valentina Jurcă a antrenat și a inițiat studenții naturaliști și biologi în munca de cercetare științifică în cadrul cercurilor științifice studențești de Chimie biologică și de Chimie generală. Unele din temele cercetate de studenți în cadrul acestei forme de activitate, sub directa

through punctuality, responsibility, consciousness and devotion as opposed to the new workplace.

As assistant and senior lecturer, Valentina Jurcă supervised practical laboratory works at the course of Biological Chemistry with 5th year students of the Faculty of Chemistry, but also with students of the 2nd and 3rd years of the Faculty of Natural Sciences – Geography and of the Department of Natural and Agricultural Sciences of the 3-year Pedagogic Institute. Moreover, she taught the course of *Biological Chemistry* to students of the distance learning department of the Faculty of Biology – Geography, as well as the course of *Biochemistry* to 2nd year students of the Department of Natural and Agricultural Sciences. After the retirement of Professor PhD Elena Budeanu (1916 – 1992), as of the academic year 1976 – 1977 Valentina Jurcă taught the course of *General Chemistry* to 1st year students of the speciality of Biology of the Faculty of Biology – Geography – Geology. She was in charge with this lecture for more than a decade, when she retired, following a long and illustrious teaching and scientific activity.

The lectures taught by Valentina Jurcă distinguished themselves through an outstanding scientific allure, through clarity and documentation, being listened to with interest by the numerous generations of naturalist and biology students, contributing to their formation as specialists.

To support the students' training, Valentina Jurcă drafted and multiplied at the Multiplication Centre of "Alexandru Ioan Cuza" University the following textbooks: in 1973, as single author, a textbook *Lucrări practice de chimie biologică (Practical Works of Chemical Biology)*; in 1984, together with Professor PhD Elena Budeanu and Senior Lecturer PhD Elvira Tănase, a textbook *Lucrări practice de chimie generală (Practical Works of General Chemistry)*, and then as single author *Cursul de Chimie*

îndrumare a șefei de lucrări dr. Valentina Jurcă, au fost finalizate în valoroase lucrări de diplomă sau teze de licență.

În activitatea de cercetare științifică, Valentina Jurcă a abordat teme fundamentale și aplicative din domeniul biochimiei, reușind să elaboreze și să publice 42 de lucrări științifice originale în diferite reviste de specialitate. La multe din lucrările publicate, Valentina Jurcă este unic sau prim autor. Pe lângă lucrările științifice publicate, Valentina Jurcă este autor al unui brevet de invenţie. Unele din temele de cercetare ştiinţifică au fost realizate pe bază de contract cu diferiţi beneficiari.

Între temele științifice cercetate de Valentina Jurcă se înscriu :

- studiul complecșilor formați de unii alcaloizi sterici (chinina, chinidina

și cinconina) cu cloruri de cobalt și cupru ;

- sinteza unor compuși ai acidului nicotinic cu acidul fosforic și a

sărurilor acidului ascorbic cu diferiți alcaloizi și 5-nitro-2-furaldehidsemicarbazona având influență asupra unor germeni patogeni (*Salmonella pullorum*) de la puii de găină ;

- investigarea efectelor produse de unele erbicide asupra diferitelor

procese fiziologie și asupra activității unor enzime la plante ;

- cercetarea dinamicii proteinelor, calciului și fosforului în sângele

crapului și păstrăvului curcubeu în dependență de condițiile de creștere și dezvoltare ; în domeniul biochimiei animalelor se înscrie și urmărirea metabolismului fosforului, calciului și carotenoidelor la găini și în ouăle lor în legătură cu condițiile de viață ;

- studiul influenței unor ioni de metale alcaline (litiu, sodiu și potasiu),

cu rol în tratamentul bolilor maniaco-depresive, asupra activității unor enzime *in*

Generală (the Course of General Chemistry), in three volumes: 1st Volume (1978) ; 2nd Volume (1980) and 3rd Volume (1982).

In parallel with the scientific activity, focused on the supervision of laboratory works and teaching of various lectures, Valentina Jurcă trained and initiated naturalist and biology students in the research work within the student scientific groups of Biological Chemistry and General Chemistry. Some of the topics researched by students within this form of activity, under the direct supervision of Senior Lecturer PhD Valentina Jurcă, turned into valuable diploma projects and graduation theses.

In the scientific research activity, Valentina Jurcă approached fundamental and applicative topics of the field of Biochemistry, managing to draft and publishes 42 original scientific papers in various speciality journals. For most of the published papers, Valentina Jurcă is single or first author. Apart from the scientific papers published, Valentina Jurcă is author of a patent. Some of the scientific research topics were conducted based on contracts with various beneficiaries.

Amongst the scientific topics researched by Valentina Jurcă one may count:

- the study of the complexes formed by a some steric alkaloids (quinine,

quinidine and cinchonine) with cobalt and copper chlorides;

- the synthesis of a series of compounds of the nicotinic acid with the

phosphoric acid and of the salts of the ascorbic acid with various alkaloids and 5-nitro-2-furaldehidsemicarbazona influencing some pathogenic germs (*Salmonella pullorum*) of the chicken;

- the investigation of the effects produced by a series of herbicides on

the various physiological processes and on the activity of a series of enzymes in plants;

vitro și din diferite țesuturi animale și sânge uman.

Valentina Jurcă a efectuat cercetarea științifică în cadrul Catedrei de Chimie Fizică și Chimie Generală și în cadrul Catedrei de Chimie Generală de la Facultatea de Chimie, iar după 1974 în cadrul Catedrei de Biologie Animală de la Facultatea de Biologie-Geografie-Geologie.

Valentina Jurcă s-a remarcat nu numai ca dascăl și cercetător științific, dar și ca un cadru didactic care a desfășurat o lăudabilă activitate socială și ca îndrumător generos al studenților cu care a lucrat.

Plecarea șefei de lucrări dr. Valentina Jurcă în împărăția neagră a morții este regretată de conducerea Facultății de Biologie, de colegi, de foști studenți, de toți cei care au cunoscut-o și au prețuit-o.

Prof. Univ. Emeritus Dr. Vlad ARTENIE
Academician Prof. Univ. Dr. Constantin TOMA
Facultatea de Biologie, Universitatea „Alexandru Ioan Cuza” Iași, ROMÂNIA

- the research of the dynamics of proteins, calcium and phosphorous in

the blood of the carp and rainbow trout depending on growth and development conditions; in the field of Animal Biochemistry one may equally count the monitoring of phosphor, calcium and carotene metabolism in hens and their eggs in relation to life conditions;

- the study of the influence of a series of ions of alkaline metals (lithium,

sodium and potassium), with role in the treatment of a maniacal – depressive diseases, on the activity of a series of enzymes *in vitro* and from the various animal tissues and human blood.

Valentina Jurcă carried out the scientific research within the Department of Physical Chemistry and General Chemistry and within the Department of General Chemistry of the Faculty of Chemistry, and after 1974 within the Department of Animal Biology of the Faculty of Biology – Geography – Geology.

Valentina Jurcă did not remark herself only as teacher and scientific researcher, but also as member of the teaching staff conducting a fruitful social activity and as generous supervisor for the students she worked with.

The departure of Senior Lecturer PhD Valentina Jurcă for the black kingdom of death is regretted by the management of the Faculty of Biology, his colleagues, ex-students, by all those who met and cherished her.

Prof. Univ. Emeritus PhD Vlad ARTENIE
Academician Prof. Univ. PhD Constantin TOMA
Faculty of Biology, „Alexandru Ioan Cuza” University, Iași, ROMANIA

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