



**Plovdiv University "Paisii Hilendarski"**

### **Annotation**

**of the materials under art. 65 (1) of the Regulations for development of academic staff of PU "Paisii Hilendarski", including self-evaluation of overall contributions and input**

**of ch. assist. prof. Elena Dimitrova Apostolova-Kuzova, PhD**

Department of Plant physiology and molecular biology, Faculty of Biology, Plovdiv University "P. Hilendarski"

Participant in the competition for the academic position "Associate Professor" in the field of higher education **4. Natural Sciences, Mathematics and Informatics**, professional field **4.3. Biological sciences**, scientific specialty **Molecular Biology**, announced in the State Gazette, **issue 99/20.11.2020**

## **I. Compliance with the conditions for holding the academic position of "Associate Professor" under Art. 65. (1) of the regulations for development of the academic staff of PU "P. Hilendarski "**

In 2007 I obtained my PhD at Agrarian University – Plovdiv, department “Genetics and Plant breeding”. Since 2011 I have been teaching/lecturing at Plovdiv University “P. Hilendarski” and I am part of the team of the Department of Plant Physiology and Molecular Biology. I have been holding the academic position "Chief Assistant Professor" since 2012 in Molecular Biology.

**To participate in the competition for the academic position "Associate Professor" in the scientific specialty Molecular Biology, I hereby present a total of 29 scientific works that have not been used in the procedures for obtaining the educational and scientific degree "Doctor" and for the academic position of "Assistant Professor". They are classified into the following groups (in accordance to the list of scientific papers):**

- ✓14 publications in journals with impact factor;
- ✓10 publications in scientific journals without impact factor;
- ✓3 book chapters;
- ✓2 textbooks.

**Total Impact Factor: 26,991**

**Total SJR: 11,867**

**Total number of citations: 153**

**All scientific papers submitted for review are co-authored, 24 of them are written in English, 2 are written in Bulgarian. I am a leading author in 10 of the presented scientific articles.**

### **SCIENTIFIC PUBLICATIONS IN JOURNALS WITH IMPACT FACTOR**

1. **Apostolova, E.**, Hadjieva, N., Ivanova, D.P., Yahubyan, G., Baev, V., Gozmanova, M. (2021) MicroRNA expression dynamics reshape the cultivar-specific response of pepper (*Capsicum annuum* L.) to potato spindle tuber viroid (PSTVd) infection. *Scientia Horticulturae*, 278, art. no. 109845. **Q1; SJR - 0,838; IF - 2,769**

**Abstract:** Potato spindle tuber viroid (PSTVd) is one of the smallest pathogenic RNAs infecting plants. It harbors a circular single-stranded RNA genome, which, owing to its considerable self-complementarity, can adopt a specific rod-shaped secondary structure. PSTVd pathogenicity is a complex phenomenon mediated by the sequence- and structure-specific characteristics of the viroid strain, host species-specific genomic traits, and interactions between PSTVd and its host in a dynamic environment. Viroid-responsive miRNAs and their potential role as regulatory determinants in the pathogenicity of PSTVd have been studied in two Bulgarian pepper (*Capsicum annuum* L.) cultivars:

Djulunska shipka (DS) and Kurtovska kapia (KK). In these cultivars, conserved miRNAs such as can-miR397, can-miR398, can-miR408, and can-miR482 display cultivar-specific expression upon PSTVd infection at 28 days post-inoculation (dpi), as shown by sRNA-seq and validated by RT-qPCR. Unlike in KK, the level of can-miR397a-5p was significantly reduced in PSTVd-infected DS, and the expression of its corresponding target gene, *Laccase-like 4*, was proportionately upregulated at 43 dpi, suggesting that this miRNA/mRNA circuit is implicated in the susceptibility of DS to PSTVd. These results advance our understanding of the cultivar-specific regulation of miRNAs associated with PSTVd response in pepper, and extend the functional role of miRNAs in regulatory networks that operate in the PSTVd–pepper pathosystem.

2. Gecheva G., Mollov I., Yahubyan G., Gozmanova M., **Apostolova E.**, Vasileva T., Nikolova M., Dimitrova-Dyulgerova I., Radoukova T. Can Biomarkers Respond Upon Freshwater Pollution? — A Moss-Bag Approach. *Biology*. 2021; 10(1):3. Q1; SJR - 1,739; IF - 3,796

**Abstract:** Moss-bags were applied to study the effect of contamination in three standing water bodies in Bulgaria (Kardzhali, Studen Kladenets and Zhrebchevo Reservoirs), the first two with old industrial contamination and the last polluted with short-chain chlorinated paraffins (SCCPs). *Fontinalis antipyretica* Hedw. collected from the background (unpolluted) site was placed in cages for a period of 30 days. The present study examined whether inorganic and organic pollution detected with moss-bags resulted in corresponding differences in molecular, chemical and micromorphological markers. Suppressed large subunit of ribulose-1,5-bisphosphate carboxylase (*rbcL*) expression was assessed in moss-bags from two of the reservoirs, contaminated with heavy metals. There was a decrease of the total phenolic content (TPC) in the moss-bags, which provides a basis for further studies of the chemical content of aquatic mosses. *Fontinalis antipyretica* also showed a response through leaf micromorphological characteristics. In the all three reservoirs, an increase of the twig leaf cell number was recorded ( $p \leq 0.01$  for Kardzhali and  $p \leq 0.001$  for Studen Kladenets and Zhrebchevo reservoirs), as well as of the stem leaf cell number in Zhrebchevo Reservoir ( $p \leq 0.001$ ). On the contrary, the width of the cells decreased in the studied anthropogenically impacted reservoirs. All three studied groups of biomarkers (molecular, chemical and micromorphological) appeared to be sensitive to freshwater pollution. The results achieved indicated that *rbcL* gene expression, TPC, cell number and size are promising biomonitoring tools.

3. Raycheva, T., Stoyanov K., Naimov S., **Apostolova-Kuzova E.** "Crocus adamioides (Iridaceae) in Bulgarian flora." *Acta Botanica Croatica* 80, br. 1 (2021): 0-0. Q3; SJR - 0,289; IF - 1,051

**Abstract:** *Crocus adamioides* Kernd. et Pasche, as recently known, was originally treated as *C. biflorus* Mill. subsp. *adamii* B. Mathew in the flora of Bulgaria by Mathew (1982) and verified by Rukšāns (2017). The taxon was afterwards described as a separate species (Kerndorff et al. 2012), with the holotype collected in Kırklareli Province, European Turkey. The species was for the first time mapped in two floristic regions of Bulgaria. The diagnostic characters based on the general morphology and leaf anatomy were defined from the natural populations of the species and compared to the type specimen and relevant data from the literature. The phylogenetic position of the species was clarified by sequencing of the internal transcribed spacer region (ITS: ITS1 + 5.SsrDNA + ITS2) and comparison of the obtained sequence with those annotated in NCBI. A phylogenetic tree was built using Bayesian

phylogeny. Results have shown highest phylogenetic similarity with *C. adamioides* from Turkey. The closest relative *C. ranjeloviciorum* Kernd., Pasche, Harpke et Raca remains in proximity. Our morphological, anatomical and molecular analyses have revealed that the Bulgarian population shows a peculiar combination of characters specific to *C. adamioides*.

4. **Apostolova E.**, M. Gozmanova, L. Nacheva, Z. Ivanova, V. Toneva, I. Minkov, V. Baev, and G. Yahubyan. (2020) MicroRNA profiling of the resurrection plants *Haberlea rhodopensis* unveils essential regulators of survival under severe drought. *Biologia Plantarum*, 64, pp. 541-550. Q1; SJR - 0,564; IF - 1,601

**Abstract:** Small RNAs (sRNAs) are essential components of gene-regulatory networks, which guide plant development and tune it to environmental challenges. Though the past years have witnessed evidence on sRNA importance for stress response, there is scarce data on their involvement in resurrection plant survival under severe drought. *Haberlea rhodopensis* is an angiosperm resurrection species, whose vegetative tissues can tolerate desiccation and recover upon rehydration. In this study, high-throughput sequencing sRNAs indicated a higher complexity of the sRNA population, especially of a 24 nt sRNA category, in the desiccated vegetative tissue of *H. rhodopensis* compared to unstressed tissues. The cross-species discovery was performed to predict 77 mature microRNAs (miRNAs), most of which were assigned to 23 high-confidence conserved miRNA families in the leaf tissue. Several members of the miR156/157, miR166, and miR399 families were found to be desiccation-responsive. The miR156/157 family members were found up-regulated upon dehydration and down-regulated upon rehydration, while the miR166 and miR399 family members followed an opposite trend of expression. A probable miR156/157 target, orthologous to the SQUAMOSA promoter binding protein- like, was reconstructed in *H. rhodopensis* based on genomic data available for this species and the closely related *Boea hygrometrica*. Reverse transcription quantitative PCR analysis confirmed the expression profile of hrh-miR156a-5p and hrh-miR157-5p established by sRNA sequencing and revealed an inverse expression pattern between these miRNAs and their targets in the desiccated tissue. Our study suggests that the miR156/157 and miR399 families are essential for plant survival under severe drought due to their ability to control plant development and growth by modulating transcription factor expression.

5. Naydenov M, Gospodinova N, **Apostolova E.** Anachkov N, Baev V, Gozmanova M, Minkov I, Yahubyan G. (2018) Transposon-associated polymorphisms of stress-responsive gene promoters in selected accessions of *Arabidopsis thaliana*. *Acta Biochim Pol.* 65(3):391-396. Q2; SJR - 0,576; IF - 1,626

**Abstract:** Genetic diversity caused by transposable element movement can play an important role in plant adaptation to local environments. Regarding genes, transposon-induced alleles were mostly related to gene bodies and a few of them to promoter regions. In this study, promoter regions of 9 stress-related genes were searched for transposable element insertions in 12 natural accessions of *Arabidopsis thaliana*. The promoter screening was performed via PCR amplification with primers designed to flank transposable element insertions in the promoter regions of the reference accession Col-0. Transposable element-associated insertion/deletion (indel) polymorphisms were identified in 7 of the 12 promoter loci across studied accessions that can be developed further as molecular markers. The transposable element absence in the promoter regions of orthologous genes in *A. lyrata* indicated that the insertion

of these transposable elements in *A. thaliana* lineage had occurred after its divergence from *A. lyrata*. Sequence analysis of the promoter regions of CML41 (Calmodulin-like protein 41) and CHAP (chaperone protein dnaJ-related) confirmed the indel polymorphic sites in four accessions – Col-0, Wassilewskija, Shahdara, and Pirin. The observed indel polymorphism of the CHAP promoter region was associated with specific gene expression profiles in the different accessions grown at a normal and elevated temperature in a plant growth chamber. The collected data can be a starting point for gene expression profiling studies under conditions resembling the natural habitats of accessions.

6. Iliev I, Yahubyan G, Marhova M, Apostolova E, Gozmanova M, Gecheva G, Kostadinova S, Ivanova A, Baev V.: Metagenomic profiling of the microbial freshwater communities in two Bulgarian reservoirs. *J Basic Microbiol.* 2017; 9999:1–11. Q2; SJR - 0,545; IF - 1,580  
Cited by: 8

**Abstract:** Microorganisms inhabiting freshwater environments are an integral part of the aquatic ecosystems. Very few data are available regarding the profiles of the microbial communities in the reservoirs in Bulgaria, despite their key role in the biogeochemical processes. In the present study, we provide the first comprehensive metagenomic analysis on the planktonic bacterial diversity of two large and economically important Bulgarian reservoirs (Batak and Tsankov Kamak) using next-generation sequencing of 16S ribosomal RNA gene (16S rRNA). Analysis of the metagenomic amplicon datasets, including quality filtering, clustering of Operational Taxonomic Units and taxonomy assignment revealed that 78.45% of the microbial communities between the two reservoirs were overlapping. The diversity (H) and Pielou's evenness (J) indices declined along the longitudinal axis of both reservoirs. The estimated values for the Shannon diversity index are typically observed in oligotrophic lakes. The microbial communities of both reservoirs were dominated by *Proteobacteria*, followed by *Actinobacteria* and *Bacteroidetes* all comprising over 95% of the relative abundance, regardless of the reservoir's large hydrogeological differences. The bacterioplankton was characterized by high phylogenetic heterogeneity in the taxonomic structure, being distributed among 211 genera. The genera *Limnohabitans* and *Rhodospirillum rubrum* held the absolute predominance, implying their significance in the aquatic food webs. The obtained data can contribute to the better systematic understanding of the microbial diversity of freshwater environments.

7. Ivanova Z., Sablok G., Daskalova E., Zahmanova G., Apostolova E., Yahubyan G. and Baev V.: Chloroplast Genome Analysis of Resurrection Tertiary Relict *Haberlea rhodopensis* Highlights Genes Important for Desiccation Stress Response. *Front Plant Sci.* 2017 Feb 20; 8:204. Q1; SJR - 1,731; IF - 3,677  
Cited by: 44

**Abstract:** *Haberlea rhodopensis* is a paleolithic tertiary relict species, best known as a resurrection plant with remarkable tolerance to desiccation. When exposed to severe drought stress, *H. rhodopensis* shows an ability to maintain the structural integrity of its photosynthetic apparatus, which re-activates easily upon rehydration. We present here the results from the assembly and annotation of the chloroplast (cp) genome of *H. rhodopensis*, which was further subjected to comparative analysis with

the cp genomes of closely related species. *H. rhodopensis* showed a cp genome size of 153,099 bp, harboring a pair of inverted repeats (IR) of 25,415 bp separated by small and large copy regions (SSC and LSC) of 17,826 and 84,443 bp. The genome structure, gene order, GC content and codon usage are similar to those of the typical angiosperm cp genomes. The genome hosts 137 genes representing 70.66% of the plastome, which includes 86 protein-coding genes, 36 tRNAs, and 4 rRNAs. A comparative plastome analysis with other closely related *Lamiales* members revealed conserved gene order in the IR and LSC/SSC regions. A phylogenetic analysis based on protein-coding genes from 33 species defines this species as belonging to the *Gesneriaceae* family. From an evolutionary point of view, a site-specific selection analysis detected positively selected sites in 17 genes, most of which are involved in photosynthesis (e.g., *rbcL*, *ndhF*, *accD*, *atpE*, etc.). The observed codon substitutions may be interpreted as being a consequence of molecular adaptation to drought stress, which ensures an evolutionary advantage to *H. rhodopensis*.

8. **Apostolova, E.**, Todorov, K., Dimitrova-Dyulgerova, I., Stoyanov, P., Mladenov, R., Yahubyan, G., & Naimov, S. (2017). Analysis of the sequence of ITS1 and ITS2 regions of Three *Mentha* species. *Plant Genetic Resources*, 15(6), 563-565. **Q2; SJR - 0,353; IF - 0,712**

**Abstract:** *Mentha* (Nepetoideae, Lamiaceae) is a taxonomically complex genus consisting of 29 species and 11 different hybrids including a number of economically important species among them medical plants used for more than 250 years. Because of its high incidence of polyploidy, diverse morphology, high degree of chromosome number variations and interspecies hybridization, *Mentha* is considered to be a taxonomical challenge. In this paper, we describe cloning and sequencing of internal transcribed spacers (ITS) 1 and 2 of three mint species: *Mentha aquatic* L., *Mentha longifolia* L. (Huds) and *Mentha piperita* L., collected from natural habitats of Bulgaria. The applicability of ITS1 and ITS2 for molecular fingerprinting and further comparative studies, including the newly obtained ITS1 and ITS2 sequences, was tested in phylogenetic analysis of all eight *Mentha* species, found in Bulgarian flora.

9. **Apostolova E.**, Anachkov G., Todorov K., Dimitrova-Dyulgerova I., Mladenov R., Stoyanov P., Yahubyan G., Naimov S.: Genetic Variability of Chosen Bulgarian *Mentha* Species. *Comptes rendus de l'Academie bulgare des Sciences*, Vol 69, N 6, 2016, 725-730. **Q3; SJR - 0,209; IF - 0,251**

Cited by: 3

**Abstract:** The systematics of genus *Mentha* from the family *Lamiaceae* of the major group *Angiosperms* is well known as complicated. It was previously shown that using morphological characters alone is insufficient when differentiating the mint cultivars. It is important to identify and characterize mint species for medical and industrial applications. The present study investigated the efficiency of ISSR markers for assessing the genetic variability within this genus and to make an attempt to differentiate the genotypes within the group of species. Eighteen different ISSR primer sets were tested on five Bulgarian mint species. On the basis of molecular characterization, a clear allocation of the genotypes was found on the dendrogram.



10. Naydenov M., Baev V., **Apostolova E.**, Gospodinova N., Gozmanova M., Yahubyan G.: High-temperature effect on genes engaged in DNA methylation and affected by DNA methylation in *Arabidopsis*. *Plant Physiology and Biochemistry* 02/2015; 87. Q1; SJR - 1,185; IF - 2,928

Cited by: 39

**Abstract:** Along with its essential role in the maintenance of genome integrity, DNA methylation takes part in regulation of genes which are important for plant development and stress response. In plants, DNA methylation processes can be directed by small RNAs in a process known as RNA-directed DNA methylation (RdDM) involving two plant-specific RNA polymerases e PolIV and PolV. The aim of the present study was to investigate the effect of heat stress on the expression of genes encoding key players in DNA methylation - DNA methyltransferase (MET1, CMT3, and DRM2), the largest subunits of PolIV and PolV (NRPD1 and NRPE1 respectively) and the DNA demethylase ROS1. We also examined the high- temperature effect on two protein-coding genes e At3g50770 and At5g43260 whose promoters contain transposon insertions and are affected by DNA-methylation, as well as on the AtSN1, a SINE-like retrotransposon. To assess the involvement of PolIV and PolV in heat stress response, the promoter methylation status and transcript levels of these genes were compared between wild type and double mutant lacking NRPD1 and NRPE1. The results demonstrate coordinated up-regulation of the DRM2, NRPD1 and NRPE1 in response to high temperature and suggest that PolIV and/or PolV might be required for the induction of DRM2 expression under heat stress. The ROS1 expression was confirmed to be suppressed in the mutant lacking active PolIV and PolV that might be a consequence of abolished DNA methylation. The increased expression of At3g50770 in response to elevated temperature correlated with reduced promoter DNA methylation, while the stress response of At5g43260 did not show inverse correlation between promoter methylation and gene expression. Our results also imply that PolIV and/or PolV could regulate gene expression under stress conditions not only through RdDM but also by acting in other regulatory processes.

11. Baev V., Milev I., Naydenov M., Vachev T., **Apostolova E.**, Mehterov N., Gozmanva M., Minkov G., Sablok G., Yahubyan G.: Insight into small RNA abundance and expression in high- and low-temperature stress response using deep sequencing in *Arabidopsis*. *Plant Physiology and Biochemistry* 09/2014; 84. Q1; SJR - 1,061; IF - 2,756

Cited by: 20

**Abstract:** Small RNA profiling and assessing its dependence on changing environmental factors have expanded our understanding of the transcriptional and post-transcriptional regulation of plant stress responses. Insufficient data have been documented earlier to depict the profiling of small RNA classes in temperature-associated stress which has a wide implication for climate change biology. In the present study, we report a comparative assessment of the genome-wide profiling of small RNAs in *Arabidopsis thaliana* using two conditional responses, induced by high- and low-temperature. Genome-wide profiling of small RNAs revealed an abundance of 21 nt small RNAs at low temperature, while high temperature showed an abundance of 21 nt and 24 nt small RNAs. The two temperature treatments altered the expression of a specific subset of mature miRNAs and displayed differential expression of a number of miRNA isoforms (isomiRs). Comparative analysis demonstrated that a large number of protein-coding genes can give rise to differentially expressed small RNAs following temperature shifts. Low temperature caused accumulation of small RNAs, corresponding to the sense strand of a number

of cold-responsive genes. In contrast, high temperature stimulated the production of small RNAs of both polarities from genes encoding functionally diverse proteins.

12. Cabrita L., [Apostolova E.](#), Neves A., Marreiros A., Leitão J.: Genetic diversity assessment of the almond (*Prunus dulcis* (Mill.) D.A. Webb) traditional germplasm of Algarve, Portugal, using molecular markers. *Plant Genetic Resources* 07/2014; 12(S1):164–S167. **Q2; SJR - 0,338; IF - 0,580**

Cited by: 4

**Abstract:** In this study, 123 almond (*Prunus dulcis* (Mill.) D.A. Webb) trees identified among traditional orchards in the Algarve region and 53 trees of the local field collection managed by the regional office of the Portuguese Ministry of Agriculture (DRAALG) were assessed using isozyme, inter-single sequence repeat and simple sequence repeat or microsatellite techniques for the evaluation of genetic diversity and genetic relatedness and identification of new accessions for the field collection. The isozyme analysis allowed the distribution of the 176 plants into 13 different classes of enzyme similarity, while the use of DNA markers increased the distribution of the analyzed trees among 140 discriminating DNA patterns. Multiple cases of homonymy and synonymy were identified in the local germplasm. Some traditional varieties, such as Lourencinha, appeared to be relatively homogeneous, while other local denominations, e.g. Galamba, included diverse genotypes. Of the 13 commercial varieties analyzed in this study, 11 assembled in one major cluster clearly differentiated from the majority of the local genotypes. These results reinforced the perception that the Algarve traditional germplasm constitutes an important repository of genetic diversity, eventually carrying alleles of high agricultural interest such as the recently identified *Phomopsis* resistance in the traditional variety Barrinho Grado.

13. Valkova R., [Apostolova E.](#), Naimov S.: Plant molecular farming: Opportunities and challenges. *Journal of the Serbian Chemical Society* 01/2013; 78(3):407-415. **Q3; SJR - 0,269; IF - 0,889**

Cited by: 2

**Abstract:** Modern human life is impossible without products derived from classical, contemporary biotechnology. However, large-scale production of biotechnology wares opens a discussion about the economic impact, waste management, biosafety, and bioethical issues. Plant molecular farming offers a relatively inexpensive option for the yielding of many valuable products and demonstrates a number of advantages over classical technologies, but also raises the questions of further development perspectives, hazard identification and risk assessment. This review is focused on these two questions: opportunities offered and challenges faced by modern plant molecular farming systems.

14. [Apostolova E.](#), Rashkova M., Anachkov N., Denev I., Toneva V., Minkov I., Yahubyan G.: Molecular cloning and characterization of cDNAs of the superoxide dismutase gene family in the resurrection plant *Haberlea rhodopensis*. *Plant Physiology and Biochemistry* 04/2012; 55:85-92. **Q1; SJR - 1,144; IF - 2,775**

Cited by: 12



**Abstract:** Resurrection plants can tolerate almost complete water loss in their vegetative parts. The superoxide dismutases (SODs) are essential enzymes of defense against the oxidative damage caused by water stress. Here, we cloned and characterized cDNAs of the SOD gene family in the resurrection plant *Haberlea rhodopensis*. Seven full-length cDNAs, and their partial genomic clones, were obtained by combination of degenerate PCR, RT-PCR and RACE. The derived amino acid sequences exhibited a very high degree of similarity to cytosolic Cu,Zn-SODs (HrCSD2, HrCSD3), chloroplastic Cu,Zn-SODs (HrCSD5), other Cu,Zn-SODs (HrCSD4), Mn-SODs (HrMSD) and Fe-SODs (HrFSD). One cDNA turned out to be a pseudogene (HrCSD1). All identified SOD genes were found expressed at transcriptional level - the HrCSD2, HrCSD5, HrMSD and HrFSD were constitutively expressed in all organs, while the HrCSD3 and HrCSD4 were organ-specific. The transcripts of the housekeeping SOD genes were detected at significant levels even in air-dry leaves. The multigene SOD family of *H. rhodopensis* is the first studied SOD family amongst resurrection plant species. Our finding of well-expressed SOD transcripts in fully dehydrated leaves correlates with retention of SOD activity, and with the ability of *H. rhodopensis* to revive upon rehydration. Because of the endemic relict nature of that species, our findings may help to further elucidate the evolutionary relationships among different SOD isoforms from distinct plant species.

#### SCIENTIFIC PUBLICATIONS IN JOURNALS WITHOUT IMPACT FACTOR

15. Popova T., Mustafov A., Stankova G., **Apostolova E.** (2019) Mutagenic and Cytotoxic Effects of Pesticide Lambada 5EK (Lambda-Cyhalothrin) on Sweet and Hot Pepper (*Capsicum annuum* L.), Beetroot (*Beta vulgaris*) and Onion (*Allium cepa*) In Vivo. *Ecologia Balkanica*. 11(2): 79-86. Q4; SJR - 0,134

**Abstract:** The aim of the present work was to study the potential mutagenic and cytotoxic effects of synthetic pyrethroid insecticide Lambada 5EK in the root meristems of some vegetables. Two concentrations of the pesticide were investigated (0.03% - recommended in agricultural practices, and tenfold lower concentration - 0.003%), and three plant-species, widely used as food, were tested - sweet and hot pepper (*Capsicum annuum* L.), beetroot (*Beta vulgaris* L.) and onion (*Allium cepa* L.). The results obtained regarding the influence of pesticide on mitotic cell division and chromosome status showed that the species have different sensitivity towards the pesticide action. The hot pepper was the most sensitive of all the tested plant species towards the impact of the pesticide in mitosis, and lower mitotic index values were recorded in both tested concentrations compared to the control. The most common observed chromosomal aberrations were: anaphase/telophase bridges, lagging acentric chromosome and/or chromosome fragments, and cells with micronuclei; they were found in all tested plant species, except beetroot treated with 0.003% solution of the pesticide. These results suggested that the beetroot possesses some endurance to the mutagenic action of the insecticide and probably it is an appropriate culture in crop rotation in agricultural practice when lambda-cyhalothrin pesticides have been used.

16. **Apostolova E.**, Naimov S. (2019) Development of Copy DNA Library of *Haberlea rhodopensis* Friv. National scientific conference with foreign participation “Science and Society, 2019”; 2-3 October 2019, Kardzhali, Bulgaria, 396-401.

**Abstract:** The aim of the study is to develop a cDNA library of *Haberlea rhodopensis* Friv. and subsequent sequence analysis. From the cDNA library were obtained single clones for isolation of highly purified DNA, suitable for Sanger’s method sequencing. 384 DNA sequences from the cDNA library were analyzed, 374 sequences of them were annotated. 332 sequences of them were linked to the specific function. It was determined sequences of 40 regulatory proteins of which 18 will be used for the development of probes for Real Time PCR analysis. Full-length cDNA clones presented there were translated into a data format for publication in NCBI database.

17. **Апостолова Е.**, Н. Хаджиева, Д. Иванова, Н. Томлекова, Г. Яхубян, В. Баев, М. Гозманова. (2019) Характеризиране на малки РНК в PSTVd – индуцирания отговор на два български сорта пипер – ЮБИЛЕЙНА НАУЧНА СЕСИЯ „ДНИ НА НАУКАТА – 2019”, посветена на 75-годишнината от създаването на Съюза на учените в България; 31 октомври 2019 г. Дом на науката –Пловдив.

**Abstract:** Potato Spindle Tuber Viroid (PSTVd) is a pathogenic RNA of 359nt that invades many species of the *Solanaceae* family, including pepper cultivars developing mild symptoms in response to infection. PSTVd is an inducer and target of post-transcriptional gene silencing (PTGS) - an important antiviral defense mechanism for plants. Two major classes of small non-coding RNAs, namely micro RNAs (miRNA) and small interfering RNAs (siRNA) mediate a defense response in plant-pathogen interactions. In order to explore the role of siRNAs in PSTVd pathogenicity in pepper we performed a PSTVd infection on two Bulgarian pepper cultivars: Djulunska shipka (DS) and Kurtovska kapia (KK). Only DS infected plants (DSI) have developed a mild PSTVd specific phenotype. Total RNA was isolated from healthy and infected plants of both cultivars and the small RNA populations were analyzed at 28dpi. Small RNA profiling was performed based on small RNA sequencing data derived from Illumina platform.

18. Valkova R., Zahmanova G., **Apostolova E.**, Kostova M., Minkov I.: Ethyl methanesulfonate induced mutation phenotype in M1 generation of *Paulownia tomentosa*. *Journal of Mountain Agriculture on the Balkans*. 2017, 20 (5), 283-295.

**Abstract:** *Paulownia* is a deciduous fast-growing, hardwood and multipurpose tree species that is native to China. It exhibits a number of desirable characteristics, such as rot resistance, dimensional stability and a high ignition point. It is a beautiful ornamental tree, which is also suitable for the bioethanol industry and as a source of secondary metabolites. As an economically important species over the past several decades, research on *Paulownia* has been conducted to develop biotechnological approaches for plant propagation and genetic improvement.

Mutagenesis has been used to increase genetic variability in crop plants through chemical mutagens or irradiation. Mutagen such as ethyl methane sulphonate (EMS) has been widely used to induce a large number of functional variations.

A study has been conducted to enhance genetic variability in *Paulownia tomentosa* using ethyl methane sulphonate. Exposure to an EMS concentration of 0.6%, v/v for 12h was used to mutagenize

100 seeds for the first generation (M1). It was observed one phenotypic mutation with altered growth behaviors than in wild type (WT) plants. Produced plant had dwarflike phenotype with broken apical dominance; increased number of branches; smaller leaves and short internodes.

In response to gibberellins (GA3) treatment they grow as tall as normal tall varieties but with multiple branched stems and small leaves. Treatment with auxins Indole-3-acetic acid (IAA) and 1-Naphthaleneacetic acid (NAA) had no result on the dwarflike phenotype either. In vitro DWL plants grown on media supplemented with Epibrassinosteroids (EBR) had bigger leaves and unbranched stems but in vivo DWL plants treated with EBR didn't change their phenotypes. Dwarf phenotypes could be the result of reduced BR biosynthesis or through up- or down- regulation of other genes.

19. **Apostolova E.**, Krastev V, Yahubyan G, Svetleva D, Parvanova P, Mitrovska Zh, Chankova S. Molecular Analysis of Bulgarian Common Bean Genotypes and their Characterization after Growing under Rainfed and Irrigated Conditions. *International Journal of Agricultural Sciences and Natural Resources*. 1(4) 2014, 50-57.

**Abstract:** Experiments were conducted in the field of Agricultural University in Plovdiv, Bulgaria. A standard method for cultivation in 5 replicates was applied. Biometric evaluation of common beans (*Phaseolus vulgaris* L.) - 10 mutant lines and 10 varieties, grown under rainfed and irrigated conditions was conducted. Main traits, associated with productivity in common beans: plant height, mass of plants with pods, number of branches, height of budding on the first pod, number of fruit branches, number of pods per plant, weight of pods with seeds, number of seeds per plant, weight of seeds and average length per 10 pods, were characterized. Mutant line D2-0.0125 M EMS (6) has the best manifestation of the studied traits among other mutant lines and it may be included in breeding schemes for evaluation as a new cultivar. BAT 477 (20) differs significantly by its traits from other genotypes, irrespectively of the cultivation mode. RAPD and ISSR analyses were done to the studied genotypes. On the basis of molecular characterization clear allocation of genotypes was found on dendrograms built by NTSYS program. It was demonstrated that the studied Bulgarian varieties are promising germoplasma for their introduction in hybridisation breeding schemes, as well as in application of mutagenesis and biotechnological approaches.

20. Рашкова М., **Апостолова Е.**, Дончева С., Тонева В., Минков И. и Яхубян Г. (2011) Проследяване на експресията в условия на воден стрес на гени, кодиращи изоформи на супероксид дисмутаза при родопския силивряк *Haberlea rhodopensis*. *Trav. Sci. Univ. Plovdiv, Plantarum*, Том 41, Кн.6, 17-24.

**Abstract:** A group of vascular plants known as resurrection plants are extremely resistant to water stress. To these belongs the tertiary relic and endemic for Balkan peninsula species *Haberlea rhodopensis* Friv. Drought stress significantly increases the formation of highly reactive oxygen species and plants respond to it by strengthening the non-enzymatic and enzymatic antioxidative processes. In prokaryotes and eukaryotes, oxidative stress induces or increases the activity of Superoxide dismutase (SOD) isoforms - metalloenzymes that have the ability to catalyze the dismutation of two superoxide radicals to H<sub>2</sub>O, and O<sub>2</sub>. In this study we have analysed the expression of SOD genes in *Haberlea rhodopensis* under drought conditions by semiquantitative RT-PCR. For this purpose, in vitro plants were subjected to dehydration for 72 and 144 hours and then were rehydrated. The cDNA was amplified

with gene-specific primers for CSD1, CSD2, CSD2<sup>a</sup>, MSD and FSD. The transcript of the constant expressing EF-1a gene was used as a standard for comparing the levels of expression of the other transcripts. The expression of all SOD genes was increased at transcriptional level in the plants exposed to dehydration, and during the subsequent rehydration the level of expression was decreased.

21. Krasteva L., **E. Apostolova**, D. Dimova, D. Svetleva: Correlation dependences between twenty-one traits of some Bulgarian common bean genotypes. *Acta horticulturae* 06/2009. Q3; SJR - 0,223

Cited by: 2

**Abstract:** The main aim of this investigation was to estimate the interrelationships between common bean quantitative traits using correlation coefficients, as well to select the most perspective of the investigated genotypes, which possess the best traits interesting for further breeding works. Three-year investigations (2003–2006) were conducted into thirty-one Bulgarian common bean genotypes. Twenty-one important seed, pod and plant traits were evaluated. Strong positive correlations were determined between the following traits: weight of pods with seeds and length of pods; number of pods with weight of seeds per plant; weight of seeds per plant with weight of over grounded plant part and number of first pod-bearing nodes; number of pods per plant with number of pod-bearing nodes; weight of over grounded plant part with number of pod-bearing nodes, thickness of hypocotyls and number of pods per plant. Negative correlations were found between the following traits: height of the set on the lowest pod with number of pods (under 10 cm), weight of seeds per plant, number of branches, pod-bearing nodes, pods per plant and seeds per pod. Breeders can use all these correlations for further work in breeding programs.

22. **Apostolova E.**, D. Svetleva: ISSR Markers Linked to the Two Genes Determining Indeterminate Growth Habit (*Fin*) and Seeds' Color (*P*) in Common Bean. *Acta horticulturae* 06/2009. Q3; SJR - 0,223

**Abstract:** Two bulked DNA samples of segregating population obtained by single cross (A 195 x Kristal 137) were prepared for bulked segregant analysis (BSA). Each pool, or bulk, contained identical individuals of a particular trait or genomic region dispersed arbitrary at all unlinked regions. BSA was used to identify inter simple sequence repeats (ISSR) markers linked to the genes determining indeterminate growth habit (*Fin*) and seeds' color (*P*) in F<sub>2</sub> progeny. Cultivar A 195 has determinate growth habit and brown seeds, while Kristal 137 has indeterminate growth habit and white seeds. Two different bulked DNAs were prepared from equal volumes of standardized DNA of those plants. Among the 72 ISSR primers used to discriminate the two parental genotypes and between the two DNA bulks (determinate versus indeterminate), only 32 (44.44%) amplified polymorphic markers. Previously confirmed 10 primers amplified clear polymorphic bands. They were further tested among 100 F<sub>2</sub> plants. The analysis of the genetic linkage between analyzed molecular markers and the loci for indeterminate growth habit and seeds' color was performed using the program Join Map 3.0 (Van Ooijen and Voorrips, 2001). The logarithm of odds (LOD) of 3.0 was used as a linkage threshold for linkage grouping. Map distances (cM) between loci were calculated based on recombination coefficient values using Kosambi's mapping function (Kosambi, 1944). ISSR marker L11, at distance – 40.9 cM

from the *P* gene (LODlinkage 3.21) and 41.5 cM from the gene *Fin* (LODlinkage 3.48), was mapped. ISSR marker L8 was also mapped to the *Fin* gene at distance 44.1 cM (LODlinkage 3.10). The coupling-phase ISSR markers detected in that study could be used to select for some levels of quantitative traits associated with *Fin* and *P* genes in common bean. Investigations have to continue for discovering more closely related ISSR markers with *Fin* and *P* genes.

23. Panayotov N., **E. Apostolova**, D. Dimova, D. Svetleva: Inheritance of Some Common Bean (*Phaseolus vulgaris* L.) traits in the cross A 195 × Kristal 137. *Acta horticulturae* 06/2009; 830(830):221-228. **Q3; SJR - 0,223**

Cited by: 1

**Abstract:** Three-year (2003–2006) investigations were conducted to study the inheritance of some common bean (*Phaseolus vulgaris* L.) traits in the cross A 195 x Kristal 137. It was found out that most of the investigated traits: height of plants, weight of over grounded plant parts, number of branches, pod-bearing nodes and pods, height of placement of the first pod, length and width of pods, number of seed- buds and seeds, mass of seeds, mass of one seed and length of seeds were with higher degree of inheritance. This is an important indication for successful selection according to these traits. The coefficients of inheritance of the traits – length of pod peak (23,08%) and weight of pods per plant (25,74%) were lower but over 20%. The number of branches was strongly influenced by the environmental conditions. The characteristics: number of seeds in pods, weight of pods with seeds, size and number of pods, number of pod-bearing nodes and weight of over grounded plant parts were controlled by the genotype and environmental conditions in equal degrees. The influence of the genotype was determined for manifestation of traits – size of seeds and pods as well as the number of pods placed under 10 cm. The characteristics: number of seeds in pods, weight of pods with seeds and number of pods per plant are of great importance for the formation of common bean yields, but they showed higher variability in our investigations. It is known that the high level of inheritance, in a wide sense, is important for the first steps in selection – the choice of elite plants with desired traits. Coefficient of inheritance, in a narrow sense, is important in the second part of selection – creation of new breeding lines with better combinations of desired traits.

24. Apostolova E., J. Carlier, J. Leitão, D. Svetleva: Identification of RAPD markers linked to the *Fin* and *P* loci in *Phaseolus vulgaris* L. *Acta horticulturae* 06/2009; 830(830):101-106. **Q3; SJR - 0,223**

**Abstract:** Bulk segregant analysis was used to identify random amplified polymorphic DNA RAPD markers linked to the genes for indeterminate growth habit (*Fin*) and seed color (*P*) in common bean (*Phaseolus vulgaris* L.) using an F<sub>2</sub> population derived from the cross A 195 x Kristal 137. The variety A 195 is characterized by determinate growth habit and brown colored seeds, while Kristal 137 exhibits indeterminate growth habit and white colored seeds. Among the 140 RAPD primers used to discriminate between two parental genotypes and between two bulks of DNA, respectively, from determinate and indeterminate plants, only 64 (45.71%) amplified polymorphic markers. Nineteen primers amplified clear polymorphic bands and were further tested among 100 F<sub>2</sub> plants. The analysis of the genetic linkage between analyzed molecular markers and the loci for indeterminate growth habit and seed color was performed using the program Join Map 3.0. The logarithm of odds (LOD) of 8.0



and 10 were used as thresholds for linkage grouping, respectively of locus Fin and locus P. One RAPD marker, AA18\_600-P1, was found linked to the Fin locus that determines plant habit type and mapped at 17.6 cM apart from it (rec. freq. = 0.162). Five RAPD markers were found linked to the P locus, with distances to this locus varying from 4.1 cM (marker AB18\_1500-P2, rec. freq. = 0.034) to 42.6 cM (marker Q03\_700-P1).

## BOOK CHAPTERS

25. Galina Yahubyan, [Elena Apostolova](#), Ivan Minkov, Vesselin Baev: Small RNAs in Crop Response to Temperature Stress Non-coding RNAs in Plants. Handbook of Plant and Crop Physiology, Third Edition edited by Mohammad Pessarakli, 04/2014: chapter 35: pages 785-794; CRC Press, Taylor & Francis Group., ISBN: 9781466553286

**Abstract:** The evidence presented in this chapter disclosed important roles of a diverse set of small RNAs in temperature response of crop plants. By now, great efforts were made in characterizing the involvement of conserved and species-specific miRNAs in stress response. Future studies are likely to identify other types of small RNAs that take part in response to diverse stress factors. Our unpublished data from NGS analysis of temperature-treated *Arabidopsis* reveal that low and high temperatures bring about completely different genome-wide small RNA profiles and affect the sRNA production from particular sets of protein-coding genes. What the genome-wide small RNA response of crop species to temperature stress is remains to be answered.

26. Mariyana Gozmanova, Vesselin Baev, [Elena Apostolova](#), Gaurav Sablok, and Galina Yahubyan: Growing Diversity of Plant MicroRNAs and MIR-Derived Small RNAs. Plant Epigenetics, Part of the series RNA Technologies, 2017, pp 49-67. Editors: Nikolaus Rajewsky, Stefan Jurga, Jan Barciszewski, ISBN: 978-3-319-55519-5 (Print) 978-3-319-55520-1 (Online)

**Abstract:** Small, noncoding RNAs are essential regulatory molecules of the plant genome. Small RNAs (sRNAs) have been classified on the basis of their biogenesis and mode of action in two major types - microRNAs (miRNAs) and small interfering RNAs (siRNAs). Plant miRNAs are typically 21 nucleotides in length and derive from unique genetic loci (MIR genes). Next-generation sequencing approaches have increased significantly the number of known plant miRNAs and have revealed that MIR genes frequently produce sRNAs, known as miRNA variants, isoforms or isomiRs, which exhibit differences from their corresponding “reference” mature sequences. The main mechanism of action of canonical miRNAs is sequence-specific repression of gene expression on posttranscriptional level. Recent studies have revealed that noncanonical miRNAs and MIR-derived siRNAs (a particular subset of isomiRs) can act as well in sequence-specific transcriptional silencing thus influencing genome function through DNA methylation. Moreover, miRNAs can be regulated by epigenetic alteration such as DNA methylation and histone modifications of MIR genes. Having profound roles in genetic and epigenetic control, plant miRNAs, and MIR-derived siRNAs can potentially participate in most developmental processes, plant stress response, and adaptation. In this chapter, we discuss the biogenesis of miRNAs and MIR-derived sRNAs and their regulatory impact on plant gene expression. The algorithms for accurate annotation of novel miRNAs and isomiR sequences are still a challenging task, requiring integration of experimental and computational approaches. Here, we shortly present



some recent tools which have been developed to facilitate this task by providing a friendly user interface, without requiring computing skills for the purpose of the analysis.

27. „Нанонауки и нанотехнологии“; Сборник с обзорни статии; Самир Наимов и Елена Апостолова; ПУ Паисий Хилендар; 2017; ISBN: 978-619-202-282-2.

**Abstract:** The Nanoscience and Nanotechnology Handbook aims to briefly present to science teachers aspects of the relationship between nanotechnology and the modern sciences of physics, chemistry and biology. The idea is to provide an informational curriculum of a thematic course of the same name, designed to enrich the scientific knowledge of teachers, organized by the Faculty of Chemistry of Paisii Hilendarski University of Plovdiv. In particular, the chapter "DNA technologies" describes the main types of nanostructures obtained on the basis of deoxyribonucleic acid molecules and the methods of their assembly.

## TEXTBOOKS

28. Ръководство по биоинформатика; Веселин Баев, Елена Апостолова, Евелина Даскалова, Георги Минков. ПУ Паисий Хилендарски; 2013, v1.03; ISBN: 978-954-423-835-3.

This textbook aims to support the implementation of lectures and practical classes in Bioinformatics and Bioinformatics analysis, which are mandatory disciplines for the curricula of the specialties Molecular Biology, Pharmaceutical Biotechnology, Microbiology and Virology, Medical Biology and Bioinformatics in Biology Faculty, Plovdiv University “P. Hilendarski”. The textbook introduces students and prepares them to work with: databases for nucleotide sequences and protein sequences, specialized databases, pairwise alignment, multiple alignment, search for homologous sequences in databases, as well as and building phylogenetic trees.

29. „Практикум по генно инженерство“; Самир Наимов и Елена Апостолова; ПУ Паисий Хилендарски; 2020; ISBN: 978-619-202-598-4.

The textbook Genetic Engineering aims to support the implementation of practical classes in Genetic Engineering and Molecular Biotechnology, which are mandatory disciplines for the curricula of the specialties Molecular Biology, Pharmaceutical Biotechnology, Microbiology and Virology, and Bioinformatics, in the Faculty of Biology, Plovdiv University “P. Hilendarski”. The textbook introduces students and prepares them to work with methods and techniques used for bacterial transformation, to identify and demonstrate transformed with GFP (green fluorescent protein) bacterial branches - isolation, chromatographic purification and electrophoretic separation of GFP, as well as Polymerase Chain Reaction (PCR) to identify the recombinant plasmid pGLO carrying GFP.

The textbook contains 12 protocols, most of which are structured so that the student receives theoretical training on the topic of the exercise, to get acquainted with the planning process of the experiment (including laboratory equipment and consumables) and with the course of the work, as well as the ways of reporting the results, so that he can finally test his new knowledge through test tasks.

Students are assisted in deriving experimental work from multiple figures, illustrating the successive steps in the course of work.

## II Self-evaluation of scientific contributions

**The contributions of the submitted materials for participation in the competition for the academic position "Associate Professor" in the scientific specialty of Molecular Biology can be grouped in several areas:**

1. Contribution to the regulation of gene expression in plant development and stress response;
2. Contributions towards DNA markers and genotyping;
3. Contribution to the sequencing and annotation of plastomes;
4. Contribution to the molecular ecology;
5. Contributions towards the field of education.

### **1) Contributions to the regulation of gene expression;**

Small, noncoding RNAs are essential regulatory molecules of the plant genome. Small RNAs (sRNAs) have been classified on the basis of their biogenesis and mode of action in two major types - microRNAs (miRNAs) and small interfering RNAs (siRNAs). Plant miRNAs are typically 21 nucleotides in length and derive from unique genetic loci (MIR genes). Next-generation sequencing approaches have increased significantly the number of known plant miRNAs and have revealed that MIR genes frequently produce sRNAs, known as miRNA variants, isoforms or isomiRs, which exhibit differences from their corresponding “reference” mature sequences. The main mechanism of action of canonical miRNAs is sequence-specific repression of gene expression on post transcriptional level. Recent studies have revealed that noncanonical miRNAs and MIR-derived siRNAs (a particular subset of isomiRs) can act as well in sequence-specific transcriptional silencing thus influencing genome function through DNA methylation. Moreover, miRNAs can be regulated by epigenetic alteration such as DNA methylation and histone modifications of MIR genes. Having profound roles in genetic and epigenetic control, plant miRNAs, and MIR-derived siRNAs can potentially participate in most developmental processes, plant stress response, and adaptation. In this chapter, we discuss the biogenesis of miRNAs and MIR-derived sRNAs and their regulatory impact on plant gene expression. The algorithms for accurate annotation of novel miRNAs and isomiR sequences are still a challenging task, requiring integration of experimental and computational approaches. In **Publication 26** we shortly present some recent tools which have been developed to facilitate this task by providing a friendly user interface, without requiring computing skills for the purpose of the analysis.

Determining the profile of small RNAs and assessing its dependence on changing environmental factors deepens our understanding of the transcriptional and posttranscriptional regulation of plant stress responses. **Publication 25** reveals the importance of a diverse set of small RNAs in response to temperature stress in cultivated plants. In **Publication 11**, data from NGS analysis of temperature-treated *Arabidopsis thaliana* revealed that low and high temperatures bring about completely different genome-wide small RNA profiles and affect the sRNA production from particular sets of protein-coding genes. Genome-wide profiling of small RNAs revealed an abundance of 21 nt small RNAs at low temperature, while high temperature showed an abundance of 21 nt and 24 nt small RNAs. The

two temperature treatments altered the expression of a specific subset of mature miRNAs and displayed differential expression of a number of miRNA isoforms (isomiRs). Comparative analysis demonstrated that a large number of protein-coding genes can give rise to differentially expressed small RNAs following temperature shifts. Low temperature causes the accumulation of small RNAs, corresponding to the sense strand of a number of cold-responsive genes. In contrast, high temperature stimulates the production of small RNAs of both polarities from genes encoding functionally diverse proteins.

Small RNAs (sRNAs) are essential components of gene-regulatory, which guide plant development and tune it to environmental challenges. In **Publication 4**, high-throughput sequencing sRNAs indicated a higher complexity of the sRNA population, especially of a 24 nt sRNA category, in the desiccated vegetative tissue of *H. rhodopensis* compared to unstressed tissues. The cross-species discovery was performed to predict 77 mature microRNAs (miRNAs), most of which were assigned to 23 high-confidence conserved miRNA families in the leaf tissue. Several members of the miR156/157, miR166, and miR399 families were found to be desiccation-responsive. The miR156/157 family members were found up-regulated upon dehydration and down-regulated upon rehydration, while the miR166 and miR399 family members followed an opposite trend of expression. A probable miR156/157 target, orthologous to the SQUAMOSA promoter binding protein- like, was reconstructed in *H. rhodopensis* based on genomic data available for this species and the closely related *Boea hygrometrica*. Reverse transcription quantitative PCR analysis confirmed the expression profile of hrh-miR156a-5p and hrh-miR157-5p established by sRNA sequencing and revealed an inverse expression pattern between these miRNAs and their targets in the desiccated tissue. Our study suggests that the miR156/157 and miR399 families are essential for plant survival under severe drought due to their ability to control plant development and growth by modulating transcription factor expression.

Resurrection plants can tolerate almost complete water loss in their vegetative parts. The superoxide dismutases (SODs) are essential enzymes of defense against the oxidative damage caused by water stress. **Publication 14** and **Publication 20** provide data for the cloning and characterization of cDNA of the SOD gene family in the resurrection plant *Haberlea rhodopensis*. Seven full-length cDNAs and their partial genomic clones were obtained by a combination of degenerative PCR, RT-PCR and RACE. The derived amino acid sequences exhibited a very high degree of similarity with cytosolic Cu, Zn-SODs (HrCSD2, HrCSD3), chloroplastic Cu, Zn-SODs (HrCSD5), other Cu, Zn-SODs (HrCSD4), Mn-SODs (HrMSD) and Fe-SOD (HrFSD). One cDNA turned out to be a pseudogene (HrCSD1). All identified SOD genes were found to be expressed at the transcriptional level - HrCSD2, HrCSD5, HrMSD and HrFSD are constitutively expressed in all organs, while HrCSD3 and HrCSD4 are organ-specific. Transcripts of constitutive SOD genes have been found at significant levels even in air-dried leaves. The multigene SOD family of *H. rhodopensis* is the first studied SOD family amongst resurrection plant species. Our finding of well-expressed SOD transcripts in fully dehydrated leaves correlates with retention of SOD activity, and with the ability of *H. rhodopensis* to revive upon rehydration. Because of the endemic relict nature of that species, our findings may help to further elucidate the evolutionary relationships among different SOD isoforms from distinct plant species.

In **Publication 16** were analyzed 384 DNA sequences from a cDNA library of *Haberlea rhodopensis* Friv. 374 sequences were annotated, 332 of which were associated with a specific function and 42 were assigned to the group of sequences without homologues or orthologs. The sequences of 40 regulatory proteins were identified and 18 of them were selected as a basis for the development of probes for subsequent real-time PCR analysis. The full-length cDNA clones were translated into a form suitable for subsequent annotation in the NCBI database.

Viroids are infectious plant lncRNAs that carry the circular, single-stranded, non-coding RNAs.

Potato spindle tuber viroid (PSTVd) is one of the smallest pathogenic RNAs infecting plants. PSTVd pathogenicity is a complex phenomenon mediated by the sequence- and structure-specific characteristics of viroid strain, host species-specific genomic traits, and interactions between PSTVd and its host in a dynamic environment. Viroid-responsive miRNAs and their potential role as regulatory determinants in the pathogenicity of PSTVd have been studied in **Publication 1** and **Publication 17** in two Bulgarian pepper (*Capsicum annuum* L.) cultivars: Djulunska shipka (DS) and Kurtovska kapia (KK). In these cultivars, conserved miRNAs such as can-miR397, can-miR398, can-miR408, and can-miR482 display cultivar-specific expression upon PSTVd infection at 28 days post-inoculation (dpi), as shown by sRNA-seq and validated by RT-qPCR. Unlike in KK, the level of can-miR397a-5p was significantly reduced in PSTVd-infected DS, and the expression of its corresponding target gene, *Laccase-like 4*, was proportionately upregulated at 43 dpi, suggesting that this miRNA/mRNA circuit is implicated in the susceptibility of DS to PSTVd. These results advance our understanding of the cultivar-specific regulation of miRNAs associated with PSTVd response in pepper, and extend the functional role of miRNAs in regulatory networks that operate in the PSTVd–pepper pathosystem.

Genetic diversity caused by transposable element movement can play an important role in plant adaptation to local environments. Regarding genes, transposon-induced alleles were mostly related to gene bodies and a few of them to promoter regions. Transposable element-associated insertion/deletion (indel) polymorphisms were identified in 7 of the 12 promoter loci across accessions studied in **Publication 5**, which may be further developed as molecular markers. The transposable element absence in the promoter regions of orthologous genes in *A. lyrata* indicated that the insertion of these transposable elements in *A. thaliana* lineage had occurred after its divergence from *A. lyrata*. Sequence analysis of the promoter regions of CML41 (Calmodulin-like protein 41) and CHAP (chaperone protein dnaJ-related) confirmed the indel polymorphic sites in four accessions – Col-0, Wassilewskija, Shahdara, and Pirin. The observed indel polymorphism of the CHAP promoter region was associated with specific gene expression profiles in the different accessions grown at a normal and elevated temperature.

Along with its essential role in the maintenance of genome integrity, DNA methylation takes part in regulation of genes which are important for plant development and stress response. In plants, DNA methylation processes can be directed by small RNAs in a process known as RNA-directed DNA methylation (RdDM) involving two plant-specific RNA polymerases e PolIV and PolV. The results obtained in **Publication 10** demonstrate coordinated up-regulation of the DRM2, NRPD1 and NRPE1 in response to high temperature and suggest that PolIV and/or PolV might be required for the induction of DRM2 expression under heat stress. The ROS1 expression was confirmed to be suppressed in the mutant lacking active PolIV and PolV that might be a consequence of abolished DNA methylation. The increased expression of At3g50770 in response to elevated temperature correlated with reduced promoter DNA methylation, while the stress response of At5g43260 did not show inverse correlation between promoter methylation and gene expression. Our results also imply that PolIV and/or PolV could regulate gene expression under stress conditions not only through RdDM but also by acting in other regulatory processes.

In **Publication 27** - a collection of review articles "Nanosciences and nanotechnologies", briefly introduces science teachers to aspects of the relationship between nanotechnology and modern science. This material presents in detail the main types of nanostructures obtained on the basis of deoxyribonucleic acid molecules and the methods of their assembly.

## 2) Contributions towards DNA markers and genotyping;

The *Iridaceae* family is a well-defined family of about 1800 species and between 60 and 70 genera. The lack of modern research in terms of chorology, morphology, taxonomy and ecology of this group of higher flowering plants in our country is the basis for a study published in **Publication 3**. In addition, the species of the family Iridaceae attracts valuable economic qualities, which provokes in fact, it is of interest, because here you can take advantage of the most popular decorative species in the world, and therefore have a medical application. Molecular identification and DNA barcoding is a relatively new taxonomic method. It can be used to identify species, differentiate native taxa or hidden taxa. Nuclear ribosomal internal transcribed spacer (ITS) region was used to identify and determine the affiliation of members of the *Iridaceae* family. A phylogenetic tree was built using a Bayesian algorithm. Results have shown highest phylogenetic similarity with *C. adamioides* from Turkey. The closest relative *C. ranjeloviciorum* Kernd., Pasche, Harpke et Raca remains in proximity. Our morphological, anatomical and molecular analyses have revealed that the Bulgarian population shows a peculiar combination of characters specific to *C. adamioides*.

The systematics of genus *Mentha* from the family *Lamiaceae* of the major group *Angiosperms* is well known as complicated. Because of its high incidence of polyploidy, diverse morphology, high degree of chromosome number variations and interspecies hybridization, *Mentha* is considered to be a taxonomical challenge. The use of morphological characters alone is insufficient when differentiating the mint cultivars. The study in **Publication 9** examined the efficiency of ISSR molecular markers for assessing the genetic variability within this genus and to make an attempt to differentiate the genotypes within the group of species. Eighteen different ISSR primer sets were tested on five Bulgarian mint species. On the basis of molecular characterization, a clear allocation of the genotypes was found on the dendrogram. In addition, the internal transcribed spacer region (ITS) 1 and 2 was cloned and sequenced of three mint species: *Mentha aquatic* L., *Mentha longifolia* L. (Huds) and *Mentha piperita* L. - **Publication 8**. In the phylogenetic analysis of all eight species *Mentha* found in the Bulgarian flora tested the applicability of ITS1 and ITS2 for molecular fingerprints and as well as further comparative studies, including the newly obtained sequences of ITS1 and ITS2.

Genetic diversity and genetic relatedness in almond (*Prunus dulcis* (Mill.) DA Webb) were assessed using a number of molecular markers. It has been confirmed that traditional germplasm in the Algarve, Portugal, is an important repository of genetic diversity. The species has alleles of great agricultural interest in the area (**Publication 12**).

**Publications 19; 21; 22; 23 and 24** described the work with common beans (*Phaseolus vulgaris* L.). A number of molecular marker techniques and classical plant breeding programs have been applied to improve a number of Bulgarian common bean varieties. It has been proven that the studied Bulgarian varieties are promising germplasm and are suitable for their introduction into schemes for hybridization propagation, as well as for the application of mutagenesis and biotechnological approaches. Using RAPD and ISSR techniques, the markers we found near the *Fin* (habitus type) and *P* (seed color) genes can be used to select certain levels of quantitative traits related to genes in common beans.

The result of this long-term work is the recognition and inclusion in **List B** of the official cultivar list of the country under **Article 14, Paragraph 3, Volume 2 of LSPM** new cultivar of common bean (*Phaseolus vulgaris* L.) from a team with my participation - **Plovdiv 6M**.

## 3) Contribution to the sequencing and annotation of plastomes;

In **Publication 7** were presented the results of the assembly and annotation of the chloroplast

genome of *H. rhodopensis*, which was subsequently subjected to a comparative analysis with the chloroplast genomes of closely related species. The structure of the genome, the arrangement of the genes, the content of GC and the meaning of the codons are similar to the typical chloroplast genomes of angiosperms. The genome contains 137 genes, representing 70.66% of the plastomes, which includes 86 protein-coding genes, 36 mRNA and 4 rRNA. Comparative analysis of the plastomes with other closely related members of *Lamiales* revealed a preserved gene order in the IR and LSC/SSC regions. Phylogenetic analysis based on genes encoding proteins of 33 species identifies the species as a member of the *Gesneriaceae* family. From an evolutionary point of view, site-specific selection analysis detects positively selected sites in 17 genes, most of which are involved in photosynthesis (*rbcL*, *ndhF*, *accD*, *atpE*, etc.). The observed codon substitutions can be interpreted as a consequence of molecular adaptation to drought, which provides an evolutionary advantage of *H. rhodopensis*.

#### 4) Contribution to the molecular ecology;

The study in **Publication 2** aimed to determine whether inorganic and organic pollution lead to corresponding differences in the molecular, chemical and micromorphological markers of *Fontinalis antipyretica*. Suppressed large ribulose-1,5-bisphosphate carboxylase (*rbcL*) expression was assessed in moss-bags from two of the reservoirs, contaminated with heavy metals. The results show that *rbcL* gene expression is one of the promising tools for biomonitoring.

Microorganisms inhabiting the freshwater environment are an integral part of aquatic ecosystems. There is very little data available on the profiles of microbial communities in the reservoirs in Bulgaria, despite their key role in the biogeochemical processes. **Publication 6** provides the first comprehensive metagenomic analysis of the planktonic bacterial diversity of two large and economically important Bulgarian reservoirs - Batak and Tsankov Kamak, using next-generation sequencing of the 16S ribosomal RNA gene (16S rRNA). The obtained data can contribute to the better systematic understanding of the microbial diversity of freshwater environments.

The effect of the synthetic pyrethroid insecticide Lambada 5EK on mitotic cell division and chromosomal status shows that the species we tested: *Capsicum annuum* L., *Beta vulgaris* L. and *Allium cepa* L. have different sensitivity to pesticidal action. When treated with 0.003% pesticide solution, chromosomal aberrations were not detected in *Beta vulgaris* L. (**Publication 15**). These results suggested that the beetroot possesses some endurance to the mutagenic action of the insecticide and probably it is an appropriate culture in crop rotation in agricultural practice when lambda-cyhalothrin pesticides have been used.

Chemical and physical mutagenesis is used to increase the genetic diversity of cultivated plants, which has led to the creation of many new varieties. Among chemical mutagens, ethyl methanesulfonate (EMS) is one of the most potent. **Publication 18** describes the application of EMS to *Paulownia tomentosa* seeds. Subsequently, phenotypic changes in the resulting generation (M1) were followed. A plant with a dwarf-like phenotype (DWL) was identified - it has impaired apical dominance, multiple lateral branches, reduced leaf size and shortened internodes. Treated with gibberellin acids (GA3), the dwarf plants lengthened their stems, but remained with branched stems and smaller leaves. The dwarf phenotype did not change with the auxins Indole-3-acetic acid (IAA) and 1-Naphthaleneacetic acid (NAA). DWL cultured in vivo and sprayed with epibrasinosteroids (EBR) did not show a change in their phenotype, but DWL cultured in vitro on EBR medium had elongated internodes, leaves of normal size and remained without branches. The dwarf phenotype may be associated with brassinosteroid deficiency as a result of decreased biosynthesis or affecting the



regulation of other brassinosteroid-related genes.

Modern life is impossible without products obtained using the techniques used in classical, contemporary biotechnology. However, large-scale production of biotechnological products opens up a discussion of economic impact, waste management, biosafety and bioethical issues. Crop molecular agriculture offers a relatively inexpensive option for extracting many valuable products and demonstrates a number of advantages over classical technologies, but also raises questions about further prospects for development, hazard identification and risk assessment. **Publication 13** provides an overview of the opportunities offered and the challenges faced by modern plant-based molecular farming systems.

### **5) Contributions towards the field of education.**

Three textbooks (**Publications №28** and **№29**) for practical classes in Bioinformatics and Genetic Engineering, respectively, are presented, intended for students from different Bachelor's courses. They are in accordance with the curriculum of the students and the number of study hours, but can also be used by students in different biological fields and related specialties from other higher education institutions.

The Bioinformatics textbook examines theoretically and demonstrates in practice databases for nucleotide sequences and protein sequences, specialized databases, pairwise alignment, multiple alignment, search for homologous sequences in databases as well as the construction of phylogenetic trees.

In the textbook Practicum in Genetic Engineering introduces students and prepares them to work with methods and techniques used for bacterial transformation, to identify and demonstrate transformed with GFP (green fluorescent protein) bacterial branches - isolation, chromatographic purification and electrophoretic separation of GFP as well as Polymerase Chain Reaction (PCR) to identify the recombinant plasmid pGLO carrying GFP.



Plovdiv,  
January, 2021

Prepared by:  
/ch. assist. prof. Elena Apostolova-Kuzova PhD/