REVIEW

by Prof. Dr. Elena Ivanova Georgieva

regarding holding a competition for the academic position "Docent", announced for the needs of the "Genome dynamics and stability" laboratory at the Institute of Plant Physiology and Genetics (IFRG) - BAS; appointed as a member of the scientific jury by order of the director of IFRG No. RD 01-35/11.05.2023

1. General part

The competition for "Docent" in the specialty "Genetics", field of higher education 4. Natural sciences, mathematics and informatics; professional direction 4.3. Biological Sciences was announced in the Official Gazette, no. 24/17.03.2023 for the needs of the Institute of Plant Physiology and Genetics (IFRG) at the "Genome Dynamics and Stability" laboratory at the same Institute.

Documents for the announced competition were presented by only one candidate - Assistant Professor Georgi Nikolaev Bonchev, Ph.D., with a scientific research profile in genetics, performing research activities in molecular genetics, population genetics, stress and genomic dynamics in plants in the specified laboratory. The procedure for disclosure and announcement of the tender has been followed. The documents are very well prepared and fully comply with the requirements of the Law on the Development of the Academic Staff in the Republic of Belarus, its additions and the Regulations on the specific conditions and procedure for acquiring scientific degrees and for holding academic positions at IFRG-BAS. The submitted documents, as well as the copies of the scientific works, meet the requirements for the academic position "Docent" and certify that the procedure can be started.

2. Biographical data and career development of the candidate

In 1993, Dr. Bonchev graduated with a degree in Plant Physiology and Genetics from the State University, St. Petersburg, Russia, Faculty of Soil Biology, Department of Plant Genetics. Two years later, after defending a diploma thesis, at SU "St. Kliment Ohridski", Faculty of Biology, graduated as a master's degree in "Biotechnological processes and devices"; specialization in "Genetic and Cell Engineering". The professional realization of Dr. Bonchev began after his appointment as a specialist biologist in 1999 and he worked as such until 2002 in the Institute of Genetics at the BAS, "Molecular Genetics" section, which after the merger with the Institute of Plant Physiology became the Institute of Plant Physiology and genetics (IFRG). In 2011, after a successful defence at IFRG-BAS of a doctoral thesis: "Molecular-genetic characterization of sphaerococcum-type mutant forms in *Triticum aestivum* L. and *Triticale* using transposons", he acquired the National Academy of Sciences "Doctor", scientific specialty "Genetics" (code 01.06.06). From 2002 to 2014, he was appointed as an assistant at the Institute of Genetics, BAS, "Molecular Genetics" section, currently IFRG, performing research activities in the field of plant genetics. From March 2015 to the present, he holds the position of assistant professor, with a scientific specialty "Genetics" at IFRG-BAS, "Genome Dynamics and Stability" laboratory, and since 2017 he has been the head of the laboratory. Dr. Bonchev has been appointed to a basic, permanent employment contract at IFRG-BAS in the scientific department research direction "Molecular Biology and Genetics" and has a total work experience in the specialty of 22 years and 5 months. Direct scientific activity and scientific interests throughout his career have been focused on fundamental and scientific-applied research in the field of functional and molecular genetics in plants, structural dynamics of the plant genome in natural and stress-induced conditions, genome dynamics during evolution of barley, sphaerococcum mutant forms of common wheat and triticale,

cytoplasmic male sterility, pathogenic and medicinal plants, DNA molecular analyzes in plants - DNA marker methods: DNA barcoding, SSR, AFLP, transposon markers - SSAP, IRAP and REMAP; statistical and bioinformatic analyses. The scientific production of Dr. Bonchev is fully related to the topic of the competition, it is oriented towards strategically important directions for our country and deserves a high evaluation.

For his entire working experience, Dr. Bonchev has completed 5 short-term specializations under awarded scholarship programs, of which one scholarship of the Ministry of Education and Culture for training, specializations and scientific research abroad, two earned under programs of the Slovak government and carried out at Comenius University, Faculty of natural sciences, Bratislava, Slovakia, one post-doctoral fellowship under the Sciex program of the Swiss government at the University of Neuchatel, Switzerland; a six-month Marie Curie Fellowship held at the University of Sussex, Brighton, UK. The conducted specializations were won in a highly competitive environment, which is a reason to assume that Dr. Bonchev's scientometric indicators are high enough to meet the criteria of the announced competitions. It should also be noted that the acquired knowledge, experience and established scientific contacts, Dr. Bonchev managed to apply in his future scientific ideas at the national level and are the basis for the implementation of joint international and national scientific projects. In a rather difficult for experimental development research area, using and introducing the most modern methods for molecular genetic analyses, Dr. Bonchev has managed to solve and prove important scientific challenges related to the structural dynamics of the plant genome in natural and stress-induced conditions.

3. Scientific production and scientometric data of the candidate

It is clear from the submitted documents for the competition that the scientific production of Dr. Bonchev, including both his doctoral work and the publication activity after it, fully cover the minimum national requirements (MNI) of NACID, necessary for this academic position and fully correspond to the profile of the announced competition. For his entire scientific experience, Dr. Bonchev presents himself with a total of 23 publications, of which 2 were included for the acquisition of the ONS "Doctor". In the competition for "Docent" he participated with 16 scientific articles, of which 13 were experimental scientific articles and 3 scientific reviews. The publication of review articles is an indication of deep knowledge in the scientific field in which he works. The report on the implementation of the Ministry of Education and Culture by the Ministry of Education, Culture, Sports and Science for scientific field 4. Natural sciences, mathematics and informatics professional direction: 4.3 Biological sciences, in accordance with the additional regulations adopted to the law in BAS and IFRG, certifies that ch. assistant professor, Dr. Georgi Bonchev, exceeds the MNI and forms more than the required number of points for occupying the academic position "Docent". With a required minimum of 540 points for "Docent", Dr. Bonchev presented himself for the competition with 857 points. According to the individual indicators, the figures are as follows:

- Indicators from group A - dissertation work for ONS "doctor" - 50 points;
- Indicators from group B (indicators 3 or 4) - 100 points,
- Indicators from group D (sum of points in indicators from 5 to 10) - 234 points.
- Group D indicators (sum of points in indicator 11) - 132 points.
- Group E indicators (sum of points in indicators 14-18) – 341

The distribution of the candidate's scientific output by quartiles is remarkable: Q1 - 8; Q2 – 4; Q3 – 2; Q4 – 2, and is first author in 9 publications and corresponding author in 3 articles. The total JCR Impact Factor (IF) of all publications for the competition is 55.553, the total JCR IF is 47.248, and the JCR IF of those in which Dr. Bonchev is first or corresponding author is 33.242. All scientific works of the candidate included in the competition have been published in prestigious

indexed and referenced international journals on Scopus and WoS with a high IF, namely: Plants – IF = 9.3; Frontiers of Plant Science – IF = 6.627; Molecular Ecology Resources – IF = 5.6; New Phytologist - IF = 7.2; Journal of Fungi - IF = 5.7; Environmental Science and Pollution Research - IF = 5.2, etc. For the period 2010 – 2022, the noticed citations in WoS and Scopus, without auto-citations or cross-citations, of 13 of the candidate's publications are 66. The most cited article of Dr. Bonchev with 38 citations was published in Molecular Ecology Resources. Attached is a detailed reference to published information in plant depositories in the BOLD system for *Plantago atrata* [*rbcL:645*], and *Nepeta nuda* [*ITS: 593, matK:819, rbcL:630, trnH-psbA: 450*] (https://boldsystems.org/index.php/Public_BINSearch?searchtype=records), in NCBI GeneBank *Ludisia discolor* (orchid) - accessions ITS OP688578; *rbcL* OP719316; *matK* OP719315; *trnH-psbA* OP719317 and phytopathogenic fungi Colletotrichum Accessions: COLB001-22 - COLB018-22. The number of his participation in scientific forums, trainings and scientific visits is also remarkable. With posters and reports, the candidate has reflected his scientific activity in 23 international and national forums with international participation, in which he presents himself with posters, oral reports, with participation in training courses conducted by the International Atomic Energy Agency (IAEA) on PCR-based molecular markers in Katowice, Poland; in the city of Basel, Switzerland in Bioinformatics; on “Plant Epigenetics” in Gättersleben, Germany; in DNA barcoding at the Norwegian University of Science and Technology, Trondheim, Norway. Under the "European Scientific Networks" program, Dr. Bonchev has conducted a working scientific visit to Finland within the framework of the highly financially supported BULCode project, of which he is also the head. Within the framework of a project with the IAEA, Vienna BUL5016 had a business trip to Italy at the CREA Research Center for Genomics and Bioinformatics. The overall scientometrics of Dr. Bonchev is an indicator that in the field of genetics he is undoubtedly a successful scientist with high professional qualifications.

4. Participation in scientific projects and other activities

Dr. Bonchev participated as a member of the scientific team in 6 national projects funded by the Ministry of Education and Science and the National Institute of Scientific Research and 3 international projects, two of which were funded under the technical cooperation program of the International Atomic Energy Agency, Vienna. The BULCode project won by the candidate, financed under the National Program "European Scientific Networks", deserves special attention. Ministry of Education and Science, of which he is the head, and the beneficiary is IFRG. The funds raised for this project are impressive and amount to BGN 956,925, with which the applicant finances conferences, training programs and provides the Institute with some of the necessary materials and equipment for modern scientific activity.

Dr. Bonchev has very good organizational and managerial capacity accumulated over the years. In addition to his scientific commitments as head of the "Genome Dynamics and Stability" laboratory at IFRG, he is also involved in administrative activities. He is the head of a team for preparation and management of projects, organizational activity of scientific forums; a representative person is for IFRG as a partner in BIOSCAN Europe and is a member of the Scientific Committee of iBOL (International Barcode of Life) consortium IFRG - unit of the organization for Bulgaria.

As the coordinator of the BULCode project, this year, he organized and conducted a training course on DNA barcoding and working with databases in Bulgaria. For 2021, Dr. Bonchev has conducted two workshops on the topic "Introduction to DNA barcoding" and two training seminars - "Green School" and a coordination meeting of the BULCode project team. In 2018, as a participant in the "Student Practices" project, for practical training of students under the Operational Program "Development of Human Resources", he mentored one student from the

Faculty of Biology of SU "St. Kliment Ohridski" and one from New Bulgarian University. For the Master's program "Genetics" and "Genetic and cellular engineering" in the discipline "Functional genetics - genomics" at the Faculty of Biology of the University of Sofia, 165 hours of practical classes have been conducted.

5. Evaluation of scientific achievements in the candidate's research work

According to the presented reference and on the basis of the publication activity, Dr. Bonchev groups his achievements in two main areas:
1. Scientific research activity
2. Developing working networks and collaborations

The scientific research activity and interests of the candidate are mainly aimed at studying the structural dynamics of the plant genome in natural and stress-induced conditions and covers several directions on this topic, reflected in the publications presented for the competition. I accept this qualification and consider that, for the most part, the contributions received are pioneering and add new facts.

A dynamically researched field with significant achievements is focused on the development of DNA markers for genotyping and assessment of natural and mutant genetic diversity in plants. In this connection, the studies of mobile genetic elements, structural dynamics, development and application of transposon-based marker methods were carried out. Mobile genetic elements, or transposons, are the most widely represented fraction of the eukaryotic genome, and in plants they comprise up to 90% of the genome in cereals and maize, and 37% of the human genome. Transposons are discrete sequences in the genome that are mobile and can be transported to other locations within the genome. Various biotic and abiotic environmental stressors such as temperature stress, mutagens, tissue culture, pathogen attacks, etc. have a significant influence on the dynamics of transposons in the host genome. Activating and moving the transposons can lead to various genetic changes such as mutations changing the expression of the affected genes and chromosomal disorders such as translocations, inversions, deletions, etc. These events create structural and functional insertional polymorphism between individuals and define mobile genetic elements as a convenient and efficient system for molecular genetic analysis of the eukaryotic genome.

Research on transposons was initiated already during Dr. Bonchev's dissertation work with the study of the dynamics of Ac-like transposons (DNA transposons) in mutant lines of hexaploid wheat and triticale of the sphaerococcum type, obtained as a result of chemical mutagenesis with ethyl methane sulfonate (EMS) (article B4_5). Data are presented for variation in the profile of Ac-like elements in the wheat genome at the level of interphase nuclei and metaphase chromosomes by FISH analysis, as well as weak changes in the methylation profile, as a result of which it is assumed that EMS is able to activate the dynamics of the transposons. The candidate's personal involvement in this research is in genomic DNA isolation, PCR amplification of As-like element in wheat, conducting Southern blot analyses, in situ hybridizations and data analysis.

During a 6-month internship at the University of Sussex, Great Britain in the group of Prof. Stephen Pearce, Dr. Georgi Bonchev was able to get to know and train important transposon-based marker methods that enable studies of the impact of stress on individuals to study of genetic diversity, as well as in plant selection for genotyping selection material, namely: REMAP (Retrotransposon Microsatellite Amplification Polymorphism), IRAP (Inter Retrotransposon Amplification Polymorphism), SSAP (Sequence-specific amplification polymorphisms), TID (Transposon insertion display), iPBS (inter-primary binding site). For the first time, the iPBS transposon-based marker method was applied to study genetic divergence and evolution in the genus Hordeum, including cultivated and wild barley species (*H. agriocrithon, H. spontaneum* and

H. vulgare). The genetic structure of the species *Hordeum agriocrithon* has been characterized. It was found that the species is characterized by a highly heterogeneous structure, reflecting its hybrid character (B4_3). The obtained data contribute to the clarification of phylogenetic relationships in the genus Hordeum and show that the iPBS method is effective for studying molecular evolution and phylogeny in barley. The BARE-1 retrotransposon-based REMAP marker system has also been shown to be effective in assessing the phylogenetic relatedness of different barley species *H. spontaneum, H. vulgare, H. bulbosum* and *H. agriocrithon*, as well as elucidating profiles of genetic divergence in the evolution and domestication history of barley (B4_4). The Transposon Display marker system has also been applied to study the dynamics of different classes of retrotransposons in *Arabidopsis lyrata* populations from North America and shown how these dynamics are influenced by the mode of propagation of the species and transposon specificity (G7_6). Experiments were conducted on genotyping and establishing a relationship between the genetic proximity of inbred lines and genomic dynamics in hybrids of corn (G7_7) and representatives of the Asteraceae family from the collection of IFRG-BAS (species of *Helianthus, Echinaceae, Tagetes* and *Verbesina*, B4_2). Applied retrotransposon markers are also effective for assessing the impact of abiotic stressors such as neonicotinoid pesticides (G7_5), where iPBS markers were used to assess genomic integrity in treated and untreated sunflower plants.

An object of intensive analysis is also focused on the characterization of mobile genetic elements as structure and function, their role in microevolution, and as a DNA marker tool to assess genomic dynamics. The data from these studies are summarized in three review articles G7_8, 10, 11 and compared with those published so far.

A contribution with great potential for practical application is the data from the conducted taxonomic studies, through the application of DNA barcoding - a modern marker methodology, which has proven to be an effective approach for genetic identification of species. This method is applicable in taxonomic studies of both individual individuals and mixed samples such as biological composition, as well as for the study of species diversity at the population and ecological level. Dr. Bonchev optimized this method and proved its application for taxonomic identification of plants, fungi, algae through chloroplast DNA barcode regions such as *rbcL, trnH-psbA, matK* and ribosomal regions (ITS). The object of taxonomic analysis are species and genera of plants, which are of interest to Bulgaria from a medical point of view, endemic species whose genetic diversity has not been studied so far. This group includes the genera Crocus (crocus), Galanthus (snowdrop), Thymus (thyme), studied in a wider population ecological aspect. The most important achievement is the discovery in Bulgaria of a plant species new to science from the genus *Sideritis* - *Siderirtis elica* or *Rhodope elica* (B4_1), which is a local Bulgarian endemic. The discovery and description of this new species is of utmost importance both for the more complete characterization of the national biodiversity and for the conservation of rare and valuable species with limited distribution. On October 31, 2022, in the "Curious" column, this achievement was widely reported and noted in the national press as a new discovery. In the rubric Dr. Georgi Bonchev is cited as its discoverer, which is an achievement for BAS and IFRG.
(https://www.vesti.bg/lyubopitno/bylgarski-ucheni-otkriha-nov-vid-rastenie-snimki-6154218.)

DNA barcoding has been applied to investigate inter- and intra-species genetic diversity in the genus *Thymus*, thyme (G7_4), as well as to identify single plants such as catnip (*Nepeta nuda*) used as material in studies concerning the presence in extracts of *Nepeta nuda* of bioactive ingredients with antioxidant and anti-herpes virus activity (G7_2).

Other studies with contributing opportunities are the results obtained from the taxonomic identification of phytopathogenic fungi in crops from the Solanaceae family, through the application of the innovative method of IFRG, BAS and RBulgaria DNA barcoding for taxonomic identification of phytopathogenic fungi in crop plants. In a similar study (G7_1), a detailed species

differentiation and phylogenetic analysis of a group of 26 fungal isolates from the genus *Colletotrichum*, a fungal pathogen attacking important for Bulgaria vegetable crops from the family *Solanaceae* (mainly pepper, tomatoes and potatoes) was made. For this purpose, the universal ITS barcode was applied, as well as the secondary barcodes multiplying the protein-coding genes *ACT* (actin), *EF-1a* (elongation factor 1a) and *TUB2* (beta-tubulin). It has been found that successful intraspecies and interspecies differentiation in the studied group of fungal isolates is possible only by applying multilocus barcode analysis.

Discovery and identification of new loci for resistance to powdery mildew *Blumeria graminis* is another indisputable contribution of Dr. Bonchev to agricultural practice. The tetraploid wheat *Triticum turgidum* subsp. dicoccum (race GZ1) is characterized by resistance to a wide range of isolates of the powdery mildew *Blumeria graminis*. As a result of genotyping two GZ1-based mapping populations and subsequent QTL analysis, two new multigenic loci on chromosome A (QPm.GZ1-2A and QPm.GZ1-7A) were identified, determining resistance to the pathogen (G7_3). A dense genetic map was created and, as a result, additional saturation of the new effective recessive locus QPm.GZ1-2A with molecular markers was carried out, and this is the basis for its more detailed study and perspective for its effective use in breeding programs.

An excellent impression is made by the assessment that Dr. Bonchev makes regarding his personal participation in the conduct of all conducted and published results.

The second direction of the candidate's scientific achievements concerns the establishment of a network of collaborations and contacts at the national and international level in the field of DNA barcoding technologies for the study and preservation of biodiversity. Formulated in this way, this direction can be characterized as undertaken administrative commitments to the funded BULCode project, of which Dr. Bonchev is the head. It is my opinion that I accept the creation of a network of scientific contacts and a network of collaborations at the national and international level as an important scientific contribution, showing efforts supported by significant scientific results obtained by an established researcher. The high financial support for this winning project shows that the leader, Dr. Bonchev, not only has high scientific achievements, but is also an internationally recognized scientist. In 2023, Dr. Bonchev started holding coordination meetings between scientific organizations from Bulgaria for the creation of a National DNA Barcoding Network. This National DNA Barcoding Network (working name BulBOL) will include scientific organizations working in the field of biodiversity and interested in applying the technology to accelerate the speed and efficiency of taxonomic research. The scientific achievements and leadership functions of Dr. Bonchev are a prerequisite for raising the level of genetic taxonomic research by applying DNA barcoding technology in Bulgaria, attracting young scientists, exchanging experience, as well as for the visibility and capacity of the Institute as a partner in project initiatives at national and international level. In 2021, IFRG becomes the representative unit for Bulgaria of the International Barcode of Life Consortium (iBOL) - an organization coordinating the study of biodiversity at the global level with over 45 member countries, by applying the marker technologies DNA barcoding and metabarcoding. In 2022, IFRG-BAN became a partner of the European unit of iBOL - BIOSCAN EUROPE. This European network supports cooperation between the member countries of Europe in the field of studying biodiversity, participation in project initiatives, training activities, etc. These scientific events will improve the knowledge and expertise of the unit in this field of research through the exchange of experience from other competent working groups in Bulgaria and abroad, and will optimize technologies in various biological objects - plants, animals, fungi, algae. There is currently a collaboration between the Genomic Dynamics and Stability Laboratory and the Experimental Algology and Plant Growth and Development Regulators laboratories on the application of DNA markers, including DNA barcoding for the taxonomic study of microalgae, medicinal plants, rare and endangered species

and complementation of molecular, physiological and biochemical data for storage of the gene pool of biological resources, study of productivity, stimulation of biosynthesis of valuable secondary metabolites. The promotion of DNA barcoding as a method has the potential for application in practice and business for food composition analysis, composition of mixed samples, providing expertise for government bodies in the field of environmental studies and others.

CONCLUSION

I know Dr. Georgi Bonchev from our many years of joint work in the "Molecular Genetics" section at the Institute of Genetics - BAS, currently the Institute of Plant Physiology and Genetics, as an extremely hardworking, conscientious and motivated specialist. I can confidently say that all experiments and analyzes of the results obtained by him were carried out with high precision, and he achieved and published new, pioneering results for genetics using state-of-the-art methodologies. The overall analysis of Dr. Bonchev's scientific achievements shows that he is a highly qualified and erudite specialist in the field of classical and molecular genetics of plants. The candidate's high scientific achievements are proven by a large number of articles published in highly indexed and referenced international journals on Scopus and WoS. Dr. Bonchev possesses undeniable qualities as an organizer and leader of a network of collaborations and contacts at the national and international level in the field of DNA barcoding technologies. He is the discoverer in Bulgaria of a plant species new to science, the Rhodope heather, a local Bulgarian endemic plant. The scientific achievements of Dr. Georgi Bonchev are priority and strategically important for science and our country. An indicator of a leading role in the candidate's scientific developments is that in more than 50% of the publications he occupies first or corresponding place.

The assessment according to the minimum criteria of NACID for "associate professor" shows that assistant professor Dr. Georgi Nikolaev Bonchev fully satisfies and by some indicators almost twice exceeds the requirements of the Law on Academic Development in the Republic of Bulgaria, the Regulations for its Implementation and the Regulations for the Development of the Academic Staff at IFRG - BAS for occupying the academic position "Associate Professor". This gives me reason to confidently recommend to the respected Scientific Jury and the IFRG Scientific Council to award assistant professor, Dr. Georgi Nikolaev Bonchev, the academic position "Associate Professor" in the Higher Education Department 4. "Natural Sciences, Mathematics and Informatics", professional direction 4.3 Biological Sciences, scientific specialty "Genetics".

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