

# РЕЗЮМЕТА

на научните трудове на **доц. д-р Божин Максимов Божинов** от **катедра “Физиология на растенията, биохимия и генетика”** при **Аграрния университет – Пловдив**

които не повтарят представените за придобиване на ОНС „Доктор“, и използваните за верификация на АД „Доцент“

1. Lacape, J. M., Nguyen, T. B., Thibivilliers, S., Bojinov, B., Courtois, B., Cantrell, R. G., Burr, B., & Hau, B. (2003). A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. *Genome*, 46(4), 612-626.

**Abstract:** An interspecific *Gossypium hirsutum* × *Gossypium barbadense* backcross population of 75 BC1 plants was evaluated for 1014 markers. The map consists of 888 loci, including 465 AFLPs, 229 SSRs, 192 RFLPs, and 2 morphological markers, ordered in 37 linkage groups that represent most if not all of the 26 chromosomes, altogether spanning 4400 cM. Loci were not evenly distributed over linkage groups, and 18 of the 26 long groups had a single dense region. This paper proposes a partially revised list of the 13 pairs of homoeologous A/D chromosomes of the  $2n = 4x = 52$  tetraploid cotton genome. The major revisions, which involve the c3–c17, c4–c22, c5–D08, and c10–c20 homoeologous pairs, are based on the mapping of 68 SSR and RFLP loci with a known chromosome assignment, as well as on comparative alignments with previously published *G. hirsutum* × *G. barbadense* maps. The overall congruency in the locus orders and distances of common SSR and RFLP loci in these maps allows for an estimation of the consensus length that reaches a minimum of 5500 cM, and is encouraging for future efforts aimed at developing an integrated map of cultivated cotton. The present map also provides a firm framework for precision mapping of Mendelian components of quantitative traits in cotton.

2. Schroeder, P., Herzig, R., Bojinov, B., Ruttens, A., Nehnevajova, E., Stamatiadis, S., Memon, A., Vassilev, A., Caviezel, M., & Vangronsveld, J. (2008). Bioenergy to save the world - Producing novel energy plants for growth on abandoned land. *Environmental Science and Pollution Research*, 15(3), 196-204.

## **Abstract**

**Background and Aim.** Following to the 2006 climate summit, the European Union formally set the goal of limiting global warming to 2 degrees Celsius. But even today, climate change is already affecting people and ecosystems. Examples are melting glaciers and polar ice, reports about thawing permafrost areas, dying coral reefs, rising sea levels, changing ecosystems and fatal heat periods. Within the last 150 years, CO<sub>2</sub> levels rose from 280 ppm to currently over 400 ppm. If we continue on our present course, CO<sub>2</sub> equivalent levels could approach 600 ppm by 2035. However, if CO<sub>2</sub> levels are not stabilized at the 450-550 ppm level, the consequences could be quite severe. Hence, if we do not act now, the opportunity to stabilise at even 550 ppm is likely to slip away. Long-term stabilisation will require that CO<sub>2</sub> emissions ultimately be reduced to more than 80% below current levels. This will require major changes in how we operate.

**Results.** Reducing greenhouse gases from burning fossil fuels seems to be the most promising approach to counterbalance the dramatic climate changes we would face in the near future. It is clear since the Kyoto protocol that the availability of fossil carbon resources will not match our future requirements. Furthermore, the distribution of fossil carbon sources around the globe makes them an even less reliable source in the future. We propose to screen crop and non-crop species for high biomass production and good survival on marginal soils as well as to produce mutants from the same species by chemical mutagenesis or related methods. These plants, when grown in adequate crop rotation, will provide local farming communities with biomass for the fermentation in decentralized biogas reactors, and the resulting nitrogen rich manure can be distributed on the fields to improve the soil.

**Discussion.** Such an approach will open new economic perspectives to small farmers, and provide a clever way to self sufficient and sustainable rural development. Together with the present economic reality, where energy and raw material prices have drastically increased over the last decade, they necessitate the development and the establishment of alternative concepts.

**Conclusions.** Biotechnology is available to apply fast breeding to promising energy plant species. It is important that our valuable arable land is preserved for agriculture. The opportunity to switch from low-income agriculture to biogas production may convince small farmers to adhere to their business and by that preserve the identity of rural communities.

**Perspectives.** Overall, biogas is a promising alternative for the future, because its resource base is widely available, and single farms or small local cooperatives might start biogas plant operation

- Ivanova, B., Yancheva, S., & Bojinov, B. (2012). Molecular differentiation of Paulownia species and hybrids. *Journal of Central European Agriculture*, 13(1), 73 - 84.  
<https://doi.org/DOI:/10.5513/JCEA01/13.1.1016> Q3 - SJR - 0,23

#### ABSTRACT

*Paulownia* is the only genus in *Paulowniaceae* family and encompasses several species with similar characteristics. In the last years the interest for industrial use of the genus is rising in Bulgaria in relation to the possibilities for using it as bioenergy source and a raw material for wood industry. Knowledge of the genus however is very limited and poses difficulties even when species and hybrids that are marketed in the country are to be differentiated. Therefore a system for adequate identification of different genotypes is not just of scientific, but of practical interest as well. Due to the lack of adequately described methods for molecular differentiation of the species of *Paulownia* sp. the present study aims at assessing the efficiency of using ISSR markers within this genus and to make an attempt to differentiate the genotypes within the group of species and hybrids, that are available *in vitro* at the Laboratory of Plant biotechnology of the Agricultural University of Plovdiv.

- Bojinov, B. M., & Danailov, Z. P. (2009). Applicability of ISSRs for Genotype Identification in a Tomato Breeding Collection. *Acta Horticulturae*, 830(1), 63-70.  
<https://doi.org/https://doi.org/10.17660/ActaHortic.2009.830.6>

#### Abstract

The ISSR (Inter-Simple Sequence Repeats) technique was applied to a set of wild species, modern varieties and F1 tomato hybrids. Genotype identification is of great importance for seed production and variety propagation where the UPOV system still relies on phenotypic markers. The inherited instability of these markers often leads to disputes between seed producers, traders and farmers. The problem was aggravated by the introduction of molecular markers in plant breeding that was witnessed in the recent years. Marker-assisted selection (MAS) is becoming a tool that brings major advantages for the seed companies and they are readily adopting it in their breeding programs. Modern genetic technology allows for identification of genotypes based on the unique sequences in their genome. This opens the possibility for effective development of a system for variety identification. The aim of the present report is to present the results from testing ISSRs as candidate technique for identification of tomato genotypes with the assistance of highly polymorphic molecular markers. A specific profile was obtained for every accession from the group of wild species while the differences between the

domesticated forms are considerably less. The prospects of adoption of ISSRs by breeders and producers as well as their potential and shortcomings if proposed for inclusion in the standard DUS testing are discussed.

5. Todorovska, E., Ivanova, A., Ganeva, D., Pevicharova, G., Molle, E., Bojinov, B., Radkova, M., & Danailov, Z. (2014). Assessment of genetic variation in Bulgarian tomato (*Solanum lycopersicum* L.) genotypes, using fluorescent SSR genotyping platform. *Biotechnology & Biotechnological Equipment*, 28(1), 68-76.  
<https://doi.org/10.1080/13102818.2014.901683>

#### ABSTRACT:

Genetic variability in modern crops is limited due to domestication and selection processes. Genetic variation in eight Bulgarian tomato varieties and breeding lines (variety Plovdivska karotina, variety IZK Alya, L21b, L53b, L1140, L1116, L975, L984) differing in their morphological and biochemical composition was assessed using a highly efficient and lowcost fluorescent simple sequence repeat (SSR) genotyping platform. Genotyping was conducted with 165 publicly available microsatellite markers developed from different research groups under a number of projects in tomato (SOL Genomics SSRs, Kazusa TGS and TES, SLM, TMS and LEMDDNa) among which only five (3.03%) failed to amplify the expected PCR fragments. Of the remaining markers, 81 (50.62%) were polymorphic in the whole collection of eight genotypes. Among the marker groups used, SLM markers were most polymorphic, followed by TMS and SOL Genomics SSR markers. The total number of amplified alleles was 299, with a mean of 1.869; and the average polymorphic information content (PIC) was 0.196. The genetic diversity within the collection was relatively low (0.2222). Nei's genetic distance varied from 0.0953 to 0.3992. Cluster analysis using the un-weighted pair group method with arithmetic mean (UPGMA) method indicated that the studied tomato genotypes are grouped in four main clusters, which is to some extent consistent with the morpho- and hemo-types of the studied tomatoes. Variety IZK Alya (cherry type) and two of the breeding lines (L1140, L1116) formed three separate and more distant clusters. The fourth cluster includes the other five genotypes. The observed grouping of these genotypes in two sub-clusters reflects their similar morphological and biochemical composition. The genetic distance information from this study might be useful for further implementation of breeding strategies and crosses among these inbred lines.

6. Ricroch, A., Harwood, W., Svobodová, Z., Sági, L., Hundleby, P., Badea, E. M., Rosca, I., Cruz, G., Salema Fevereiro, M. P., Marfà Riera, V., Jansson, S., Morandini, P., **Bojinov, B.**,

Cetiner, S., Custers, R., Schrader, U., Jacobsen, H.-J., Martin-Laffon, J., Boisron, A., & Kuntz, M. (2016). Challenges facing European agriculture and possible biotechnological solutions. *Critical Reviews in Biotechnology*, 36(5), 875-883

## Abstract

Agriculture faces many challenges to maximize yields while it is required to operate in an environmentally sustainable manner. In the present study, we analyze the major agricultural challenges identified by European farmers (primarily related to biotic stresses) in 13 countries, namely Belgium, Bulgaria, the Czech Republic, France, Germany, Hungary, Italy, Portugal, Romania, Spain, Sweden, UK and Turkey, for nine major crops (barley, beet, grapevine, maize, oilseed rape, olive, potato, sunflower and wheat). Most biotic stresses (BSs) are related to fungi or insects, but viral diseases, bacterial diseases and even parasitic plants have an important impact on yield and harvest quality. We examine how these challenges have been addressed by public and private research sectors, using either conventional breeding, marker-assisted selection, transgenesis, cisgenesis, RNAi technology or mutagenesis. Both national surveys and scientific literature analysis followed by text mining were employed to evaluate genetic engineering (GE) and non-GE approaches. This is the first report of text mining of the scientific literature on plant breeding and agricultural biotechnology research. For the nine major crops in Europe, 128 BS challenges were identified with 40% of these addressed neither in the scientific literature nor in recent European public research programs. We found evidence that the private sector was addressing only a few of these “neglected” challenges. Consequently, there are considerable gaps between farmer’s needs and current breeding and biotechnology research. We also provide evidence that the current political situation in certain European countries is an impediment to GE research in order to address these agricultural challenges in the future. This study should also contribute to the decision-making process on future pertinent international consortia to fill the identified research gaps

7. Angelov, M., Ivanova, B., Pavlov, A., Ganeva, D., Danailov, Z., & Bojinov, B. (2017). Development of ISSR markers for a Bulgarian tomato breeding collection aiming to improve antioxidant compounds in fruits. *Bulgarian Journal of Agricultural Science*, 23 (3), 405–410

**Abstract:** The Inter-Simple Sequence Repeat marker technique was applied to a set of tomato genotypes that were found to diverge in antioxidant compounds in fruits. As marker-assisted selection is becoming a tool that brings major advantages for breeding programs need is rising to use it to quickly respond to consumer demand. Being part of a system for efficient plant

breeding in many crops the aim of the present study was to test Inter-Simple Sequence Repeats as candidate tool for incorporating in a breeding program aiming to improve antioxidant properties of tomato fruits. Application of Inter-Simple Sequence Repeat markers resulted in obtaining adequate representation of every accession from the selected group. This opens the opportunity for using the system in confirming the hybrid nature of the F1 progenies and further accelerating the breeding of genotypes for use as healthy food.

8. Kostova, M., & **Bojinov, B.** (2018). Application of ISSR markers for detection of genetic variation in two Bulgarian autochthonous goat breeds . Bulgarian Journal of Agricultural Science, 24(6), 1109-1113.

**ABSTRACT:** The conservation of genetic resources in livestock is of rising interest in recent years. The introduction of highly intensive selected breeds results in reducing the size of local ones, leading to narrowing of their genetic diversity. Therefore, estimating the remaining genetic variation is becoming of key importance for prioritizing conservation efforts and developing future breeding programs. In this respect, two local goat breeds with a distinctive exterior, stably transmitted in the progeny, were selected for the present study and their within-breed genetic variation assessed with ISSR markers. Our results demonstrate that ISSR markers are capable of revealing substantial genetic diversity in the autochthonous Bulgarian goat breeds. Furthermore, unique genetic profiles were obtained for each of the studied animals, thus providing usable tool for paternity testing in future breeding programs.

9. Bojinov, B. (2019). Quantitative trait loci detection in Bulgarian cotton mutant segregating population. Genetika, 51(1), 323 -334.

**ABSTRACT:** Identification of quantitative trait loci (QTL) linked to fiber quality is of primary interest in cotton breeding. As Bulgarian cotton varieties belong to a specific group (proles *Bulgaricum*) they are even more difficult for cross-breeding into them such quality characteristics. Therefore a segregating mutant population has been developed from the standard Bulgarian variety 'Chirpan 603' by applying seed gamma irradiation. M4 and M5 generations were used for identification of QTLs related to fiber quality characteristics. SSR markers developed in interspecific crosses and further confirmed in intraspecific crosses, together with in-house developed ISSR markers were used for association mapping of QTLs for fiber quality. Fiber strength, length, uniformity, micronaire and elongation were the main studied characteristics. QTLs with major effects on these traits identified in M4 and M5 were

confirmed in M6 generation. Further ones were identified and used for map saturation and linkage group confirmation

10. Sirakov, I., Strateva, T., Popova, R., Bojinov, B., & Mitov, I. (2019). Ecological Sources of Wild-type Shiga-toxin and Intimin-producing *Escherichia coli* isolates in Bulgaria: Antimicrobial Susceptibility and Multilocus Sequence Typing. *Journal of Environmental Protection and Ecology*, 20(2), 823–831.

**Abstract.** Ruminant animals bred freely in Bulgarian national and natural reserves in the recent years serve as the main reservoir of Shiga-toxin and intimin-producing *Escherichia coli* strains, which pose risks for the members of these ecosystems, including humans. The ability of *E. coli* to maintain viability in the environment for months provides it with opportunities to circulate throughout the biotope and the various members of the respective biocenosis. The process is often facilitated by the run-off water resulting from heavy rains. The possibility of horizontal transfer between different strains of genes coding for pathogenicity factors increases the risk of their dispersal in the environment. The epidemiological typing of the pathogenic *E. coli* strains impacting biosafety, human and animal health is of paramount importance to control their spread. The present work aims to study Bulgarian Shiga-toxin and intimin-positive *E. coli* isolates from different sources, including their antimicrobial susceptibility and the intimin toxin type, as well as to perform Multilocus Sequence Typing analysis. The results showed that the isolates had wild-type susceptibility to the antibiotics tested, intimin (type beta 1, theta and omicron) and belonged to various sequence types and clonal complexes. The information obtained will complement the profiles of circulating pathogenic strains of *E. coli* which will facilitate risk assessment and adoption of adequate measures for limiting contamination of biocenoses with pathogenic strains-infected faeces from agricultural animals

11. Trayanov, K., Samaliev, H., Kostova, M., Bojinov, B., & Besheva, A. (2020). Morphological and molecular identification of potato cyst nematode *Globodera rostochiensis* and *Globodera pallida* in Bulgaria. *Bulgarian Journal of Agricultural Science*, 26(2), 416-422.

**ABSTRACT:** The potato cyst nematodes (PCNs), *Globodera rostochiensis* and *Globodera pallida* are economically important parasites of the potato and are recognized as quarantine pests. In Bulgaria *G. rostochiensis* was identified for the first time in 1978, while *G. pallida* in 1992. Identifications of PCNs populations have been based only on morphological characters and *G.*

rostochiensis is prevailing species in potato fields. The present work was aimed to introduce a faster and more reliable method for identifying and differentiating the PCN species in three potato-producing regions in Bulgaria. For the morphological identification of both PCN species, 30 cysts and 30 second-stage juveniles (J2S) were studied. For a more accurate and rapid identification of *G. rostochiensis* and *G. pallida*, polymerase chain reaction (PCR) based method was applied. Multiplex PCR reaction was performed with primers designed for a small region between internal transcribed spacer 1 and the 5.8 S ribosomal RNA gene regions in order to identify the *Globodera* species. The mean morphometric values of the 30 experimental cysts and J2S were common within the expected range for *G. pallida* and *G. rostochiensis* with some variations observed. The present results demonstrate the occurrence of both species of PCNs, as *G. pallida* was identified in 24 from the soil samples and *G. rostochiensis* in 6 of them. The correct and rapid identification of PCN species in Bulgarian potato fields is essential for adopting more effective measures for its control.

12. Bojinov, B., Vasileva, S., Ganeva, D., Georgiev, V., & Pavlov, A. (2024). An integrative approach to developing new tomato varieties with elevated fruit antioxidant content . *Bulgarian Journal of Agricultural Science*, 30(2), 363-374.

**ABSTRACT:** Carotenes have been identified as the most essential carotenoids in the human body, together with lycopene, lutein, and  $\beta$ -cryptoxanthin due to their beneficial antioxidant activities. Because of these properties, we have designed and conducted in 2018–2021 an experiment where various tomato accessions were metabolically profiled for their antioxidant content and other fruit parameters for further usage in crop improvement programs. Some genotypes were phenotyped and profiled with DNA markers to characterize their relative genetic distance. Aggregated data from metabolic and genetic profiling were complemented by the phenotypic data to select for the most promising cross (Pl. karotina  $\times$  L1116) that was performed and developed into F1 and F2 generations. After phenotyping and genetic profiling of the 104 F2 individuals obtained from that cross, 24 individuals were selected for metabolic profiling for antioxidant contents. In relation to carotenoids accumulated in fully matured fruits the highest lycopene concentration detected in our study was 587.03  $\mu\text{g/g}$  DW, while  $\beta$ -carotene reached 440.29  $\mu\text{g/g}$  DW. Our results support the use of such integrative approach to accelerate tomato breeding when improved metabolic content of the fruits is aimed.