ABSRTACTS

of scientific publications of Prof. Bojin Bojinov, PhD

in connection with the dissertation for obtaining the scientific degree

“Doctor of sciences”

Higher education area ***4.0. Natural sciences, mathematics and computer science;***

Professional field: ***4.3. Biological Sciences***;

Scientific specialty: “***Genetics"***

1. Angelov, M., and **Bojinov, B.** (2015). Determining genetic variability in traditional and newly created genotypes with the help of DNA markers. Agraren Universitet Plovdiv-Nauchni Trudove/Scientific Works of the Agrarian University-Plovdiv, 59, 29-34.

Abstract:

Tomato is one of the main vegetable crops, therefore the selection of new varieties that meet the increasing demands of consumers and producers is of great importance. The aim of this study was to test the applicability of the ISSR marker system for characterizing genetic diversity in breeding samples selected for the needs of a breeding program aimed at increasing the amount of antioxidants in tomato fruit. Eight genotypes distinguished by the antioxidant content of the fruits were included in the study. The results obtained from the analysis showed that the ISSR marker system is successfully applicable to tomatoes and could be used for the needs of selection, seed production and certification of existing and newly created varieties.

2. Angelov, M., Ivanova, B., Pavlov, A., Ganeva, D., Danailov, Z., and **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, 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.

3. **Bojinov, B.** (2014). Mutation breeding as a tool for developing new Bulgarian cotton varieties, adapted for mechanical harvesting. In "Turkey 5-th Seed Congress with International Participation" (S. BAŞBAĞ, B. T. BİÇER and V. PİRİNÇ, eds.), pp. 100-106, Dyarbakir.

Abstract:

Gamma irradiation of seeds from Chirpan 603 variety was used in 2004 to produce a mutant segregating population. As population reached M4 generation, breeding was started at the Experimental station of the Agricultural University of Plovdiv for plant architecture that would make it easier for mechanical harvesters to pick most of the seedcotton produced in the field in a single pass. Plant height and the height and length of the first fruiting branch were the main targets of the breeding program while yield and fiber quality were followed as additional parameters, essential for efficient crop production under Bulgarian conditions. As a result mutant lines were obtained that are suitable for intensive cotton production. Under high plant density that is typical for Bulgarian cotton farming the best selected lines demonstrated natural cut-out in vegetative growth and uniform maturation of semi-compact plants with ripening bolls positioned adequately for easier mechanical harvesting.

4. **Bojinov, B.** (2019). Quantitative trait loci detection in Bulgarian cotton mutant segregating population. Genetika, 51, 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.

5. **Bojinov, B.** and J.-M. Lacape (2003). Molecular markers for DNA-fingerprinting in cotton. Proceedings World Cotton Research Conference-3, 9-13 March, Cape Town, Republic of South Africa, pp. 343-349.

Abstract:

Molecular markers are long proposed as suitable for fingerprinting of crop species. With the development of the techniques the attention has shifted from protein to DNA-based markers as age-, tissue- and status-independent and, therefore, more reliable and reproducible. Several such markers (RFLP, RAPD, AFLP, SSR) were used in cotton for developing a high-density genetic map for genomic studies and with the prospect for marker-assisted selection. The availability of these markers, together with the existing possibility for genetic transformation of the crop, make the development of DNA "fingerprints" for the varieties an issue of rising interest among breeders and seed companies. To the best of our knowledge no concentrated effort for developing such fingerprints has been made up to now in spite of the several reports proposing the potential usefulness of microsatellites and AFLPs in this respect. Here we present our work on comparing applicability of three types of molecular markers (microsatellites, AFLPs and SAMPLs) towards establishing a reliable, reproducible and relatively inexpensive protocol for DNA-fingerprinting for cotton. Furthermore, the intended transferability to the widest possible range of laboratories and, therefore, bringing it the closest to the breeder's fields, was attempted through the replacement of the standard radioactive DNA labeling procedure with the less hazardous

"silver staining" protocol. The outcomes and the possible extensions of the present work are discussed.

6. **Bojinov, B. M.,** and Danailov, Z. P. (2009). Applicability of ISSRs for Genotype Identification in a Tomato Breeding Collection. Acta Horticulturae, 830, 63-70.

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 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.

7. **Bojinov, B**., and Bozhinova, R. (2020). Intra- and inter-cultivar genetic variability in Bulgarian tobacco. Trakia Journal of Sciences, 18, 279-284.

Abstract:

The genus *Nicotiana* is a well-defined group of species of which tobacco (*Nicotiana tabacum* L.) is the most important crop plant and plays a significant role in the economies of many countries. Recent advances in molecular genetics of the crop allowed the identification of a number of important genes and their location on chromosomes. However, the genetic analysis of this inbreeding, highly homozygous plant that serves as a model in many other studies is still very much work in progress.

A large proportion of the tobacco genome is represented by highly repeated DNA sequences, which makes molecular markers, based on them, an obvious first choice in diversity studies. Here we present an assessment with Inter-Simple Sequence Repeat (ISSR) markers of the intra- and inter-cultivar variability in a set of Bulgarian tobacco varieties. The screening of the 4 primers revealed variable number of bands generated by different primers. The total number of bands varied between 4 and 13. No correlation between the total number of bands and the number of polymorphic bands was observed. The present study demonstrates the power of ISSR technique as a tool for diversity identification in tobacco.

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

Abstract:

Carotenes have been identified as the most essential carotenoids in the human body, together with lycopene, lutein, and β-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. Same 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 × 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 μg/g DW, while β-carotene reached 440.29 μg/g DW. Our results support the use of such integrative approach to accelerate tomato breeding when improved metabolic content of the fruits is aimed.

9. Grozeva, S., Ganeva, D., Pevicharova, G., **Bojinov, B.**, and Danailov, Z. (2014). Characterization of quality and productivity traits of new tomato genotypes with high biological value. Genetics, Plant Breeding and Seed Production, 1, 239-243.

Abstract:

The aim of this work was morphological and phenotypic evaluation of new tomato genotypes. Eight tomato genotypes were characterized by plant habit, fruit colour, shape, weight, firmness and dry matter, number of fruits per plant, plant weight, biomass, harvest index and yield. Sensory analysis was conducted also. For most traits, a wide range of genetic variability was found. The established contrasting differences between studied genotypes by main plant and fruit traits are a good base for breeding program aimed to determine the nature of inheritance and identify of donors of valuable characters.

10. Ivanova, B., and **Bojinov, B.** (2009). Identification of QTLs for fiber quality in a Bulgarian cotton breeding collection. Genetics and Breeding, 38, 55-60.

Abstract:

A segregating mutant population has been developed from the standard Bulgarian variety “Chirpan 603” for identification of QTLs related to fiber quality characteristics. SSR markers developed in interspecific crosses and confirmed in intraspecific crosses in other studies were used for association mapping to the fiber strength, length, uniformity, micronaire and elongation. QTLs were identified with major effect on all traits with the ones related to fiber strength, uniformity and micronaire co-localizing on the same region of one linkage group (LG3).

11. Ivanova, B., and **Bojinov, B.** (2012). Identification of QTLs for cotton fiber quality in a M5 mutant segregating population. In "A Joint Workshop and Meeting of All Working Groups, of the Inter-Regional Cooperative Research Network on Cotton for the Mediterranean and Middle East Regions", Antalya, Turkey.

Abstract:

A segregating mutant population has been developed from the standard Bulgarian variety “Chirpan 603” 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 fiber strength, length, uniformity, micronaire and elongation. QTLs were identified with major effect on all traits with the ones related to fiber strength, uniformity and micronaire co-localizing on the same region of one linkage group.

12. Ivanova, B., Angelov, M., Pavlov, A., Ganeva, D., Danailov, Z., and **Bojinov, B.** (2014). Applicability of inter-simple sequence repeat markers in developing tomato breeding population. Agricultural Sciences / Agrarni Nauki, 6, 33-38.

Abstract:

The Inter-Simple Sequence Repeat marker technique was applied to parental tomato genotypes and their F 1 hybrids differing in the antioxidant compounds in the fruits. As the marker-assisted selection is becoming a tool that brings major advantages for breeding programs, the need to use it to quickly respond to the consumer demand has greatly increased. Modern genetic technology opens the possibility for effective application of a system for efficient plant breeding in many crops, including tomatoes. The aim of the present study was to test the Inter-Simple Sequence Repeats as a candidate tool to use in Marker-Assisted Selection in tomatoes. The application of the Inter-Simple Sequence Repeat markers allowed for efficient verification of the hybrid origin of the progeny from parental genotypes that were crossed for improving the antioxidant content in tomato fruits.

13. Ivanova, B., Yancheva, S., and **Bojinov, B.** (2012). Molecular differentiation of *Paulownia* species and hybrids. Journal of Central European Agriculture, 13, 73 - 84.

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 at the Laboratory of Plant biotechnology of the Agricultural University of Plovdiv.

14. Kostova, M., and **Bojinov, B**. (2018). Application of ISSR markers for detection of genetic variation in two Bulgarian autochthonous goat breeds. Bulgarian Journal of Agricultural Science, 24, 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.

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

Abstract:

An interspecific *Gossypium hirsutum x 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 x 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.

16. Lacape, J.-M., Nguyen, T.-B., Courtois, B., **Bojinov, B.,** and Hau, B. (2003b). Genome mapping of tetraploid cotton: towards a saturated and unified map. In "World Cotton Research Conference 3". ARC-IIC, FAO. Rustenburg.

Abstract:

DNA-based genetic maps have been produced in nearly all major crop species, thus facilitating the analysis of genome structure and evolution, and improving efficiency and accuracy of breeding. We have developed at CIRAD/France a combined and saturated RFLP-AFLP-SSR genetic map of tetraploid cotton from the analysis of the 1st and 2nd backcross generations of an interspecific *Gossypium hirsutum* (cv 'Guazuncho 2') x *G. barbadense* (cv 'VH8') cross. The BC1 and BC2 maps were independently constructed from the analysis of 75 and 200 individual plants respectively. As a recent development, a microsatellite-enriched library had been developed and 418 new microsatellite primers defined. One hundred and sixty one microsatellites showing at /east one polymorphism between Guazuncho 2 and VH8 had been screened on the BC1 population, and 185 new loci were added on the BC1 map. Having 360 loci in common between the BC1 (1107 loci in total) and the BC2 (513 loci in total) map proved helpful to confirm loci orders along linkage groups and allowed small linkage groups to be joined to larger ones. A total of 138 additional loci, mainly AFLP, of the BC2 map were added to the skeleton 60 map. Finally, after merging the SC 7 and 6C2 mapping data, the Guazuncho 2 x VH8 map now comprises 1260 loci divided mainly between AFLPs (40%), SSRs (34%) and RFLPs (15%). The 26 linkage groups span altogether over 5400 cM. Details of the Guazuncho 2 x VH8 map are commented. An important number of microsatellite and RFLP markers used in the present study are common with other published genetic maps also derived from interspecific *G. hirsutum x G. barbadense* populations. As a preliminary step towards an integration of the different maps, we used a "neighbor approach" applied to 4 of the 26 chromosomes by aligning these maps using these bridge loci. An overall agreement in locus order and distances has been observed. Integration of genetic maps into a denser consensus unified map of tetraploid cotton and future feasibility of genetic and physical maps integration are discussed.

17. Todorovska, E., Ivanova, A., Ganeva, D., Pevicharova, G., Molle, E., **Bojinov, B.,** Radkova, M., and Danailov, Z. (2014). Assessment of genetic variation in Bulgarian tomato (Solanum lycopersicum L.) genotypes, using fluorescent SSR genotyping platform. Biotechnology & Biotechnological Equipment 28, 68-76.

Abstract:

Genetic variability in the 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, L21β, L53β, L1140, L1116, L975, L984) differing in their morphological and biochemical composition was assessed using highly efficient and low-cost fluorescent 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 eighty-one (50.62%) are polymorphic in the whole collection of 8 genotypes. Among the markers groups used, SLM- markers were the most polymorphic followed by TMS- and Sol genomics SSR- markers. The total number of amplified alleles was 299 with a mean 1.869, and the average polymorphic information content (PIC) - 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 UPGMA method indicated that tomato genotypes are grouped in 4 main clusters, that is consistent to some extent with morpho- and hemo-types of the studied tomatoes. Variety IZK Alya (cherry type) and two of the breeding lines (L1140, L1116) formed 3 separate and more distant clusters. The fourth cluster includes the remaining 5 genotypes. The observed grouping of these genotypes in 2 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.

18. Vuchkov, A., Kostova, M., and **Bojinov, B.** (2017a). Genetic Variation in Two Bulgarian Domestic Goat Breeds as Detected With ISSR Markers. In "XXII SAVETOVANJE O BIOTEHNOLOGIJI" (P. M. Gordana Šekularac, Milun Petrović, Gorica Paunović, Milomirka Madić, Srđan Bošković, ed.), Vol. 1, pp. 387-394. Univerzitetu Kragujevcu, Agronomski fakultet u Čačku, Čačak, Serbia.

Abstract:

The issue of conservation of genetic resources in livestock is of growing interest in recent decades. Local breeds of farm animals are usually with relatively lower productivity but well adapted to the specific conditions of the environment.

Due to the advent of a number of highly intensive selected breeds the existence of local genetic resources is threatened in our country. The risk comes from reducing the size of local populations of purebred animals, leading to narrowing of genetic diversity. Two local goat breeds with distinctive exterior, stably transmitted in the progeny were selected for the present study and their within-breed genetic variation assessed with ISSR markers.

19. Vuchkov, A., Kostova, M., and **Bojinov, B.** (2017b). Variations of the color of coat in two autochthonous goat breeds in Southwest Bulgaria. Journal of Mountain Agriculture on the Balkans, 20, 25-36.

Abstract:

The present study shows a summary analysis of variations in the color of coat in two Bulgarian autohtonous goat breeds – Kalofer longhaired and Bulgarian screw-horned longhaired goat breeds, reared in Southwest Bulgaria. The specimens included in the investigation, form a representative sample of breeds – 120 typical animals (60 of each breed). For the purposes of the study were selected purebred animals, without common grandparents from 10 herds, representing the full diversity of color of the coat, typical for the populations of both breeds. On the base of this investigation conducted on the hair coverings of specimens from the populations of the Kalofer longhaired and Bulgarian screw-horned longhaired goat grown in Southwestern Bulgaria, the results show that 5 basic colorations of the coat can be differentiated – Black, Red-brown, Silver-gray, Paecock "barza", Black and tan. Two of them (black and redbrown) have been determined by the Blocus, defining the Eumelanic pigmentation, and three (silver-gray, "barza", black and tan) have been determined by Agouti-locus.