Vol. 43 - No. 1 Special Edition 2021

Biologia Serbica

Department of Biology and Ecology,
Faculty of Sciences, University of Novi Sad, Serbia

BELBI 2021

Book of Abstracts
Belgrade BioInformatics Conference 2021
21-25 June 2021, Vinča, Serbia

http://ojs.pmf.uns.ac.rs/index.php/dbe_serbica/index

Biologia Serbica

JUNE 2021
Vol. 43 - No. 1 Special Edition

Biologia Serbica is formerly Review of Research, Faculty of Sciences, Biology Series (1971-2004), published by Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia

Aim and Scope

Biologia Serbica (BS) is an international scientific, peer-reviewed, open access journal that publishes original research articles, review articles, and short communications in all areas of biology.

Publisher

Department of Biology and Ecology
Faculty of Sciences
University of Novi Sad
Trg Dositeja Obradovića 2
21000 Novi Sad, Serbia
: +381 21 485 26 59
: +381 21 450 620
www.dbe.uns.ac.rs

Biologia Serbica is published biannually.

Printed by

"Sajnos"
Momčila Tapavice 2,
21000 Novi Sad
+381/21/499-461, +381/21/499-088
sajnosns@gmail.com

Date od publication: June 2018.

Subscription rates

Instructions available at
http://ojs.pmf.uns.ac.rs/index.php/dbe_serbica/index

ISSN 2334-6590

Biologia Serbica

Book of Abstracts
Belgrade BioInformatics Conference 2021

Volume 43 - No. 1
(Special Edition)
2021

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Bioinformatics pipeline for genotyping and genotype - phenotype association study in maize (*Zea mays* L.)

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Abstract
Multidisciplinary research is today commonly used in plant breeding for improving important agronomic traits. High throughput genotyping technologies and genotype – phenotype association studies as widely used for improving breeding programs, depend on bioinformatics analysis for extracting information from the gathered data. In this research, among plethora of widely used bioinformatics approaches, the custom made one was chosen, based on the current recommendations in the field.
The material includes a set of 46 maize inbred lines commonly used in maize breeding programs. Phenotyping was done for thirteen important quantitative agronomic traits in 8 environments during two years (2018 and 2019). For the purpose of genotyping, plants of all inbred lines were grown under optimal conditions and sampled after completing the V4 growth stage. Total RNA was isolated from the third leaf of three plants per inbred line and used for cDNA preparation by Illumina TruSeq Stranded RNA LT kit. Pair-end RNA-Seq based on Next Generation Sequencing methodology was performed on MiSeq Illumina sequencer using MiSeq Reagent kit, v2 (2 x 150bp). Raw sequencing data of maize leaves' transcriptionally active genome regions at the moment of sampling were used for identification of single nucleotide polymorphisms (SNPs) in each of 46 inbred lines.
Bioinformatics pipeline for data manipulation and analysis was custom made and included FastQC (for quality control (QC) of raw data), Trimmomatic tool v0.32 (for adapter and contaminants removal, as well as for the removal of regions with QC below 30), TopHat (insert size 130, standard deviation 50, maximum intron size 100.000 – for mapping filtered reads onto the B73 maize reference genome v3.0), Cufflinks v2.2.1 (for reads assembly), Cuffmerge (for the final transcriptome assembly) and an intersection output of two independent SNPs calling tools FreeBayes and BCFtools (to minimize false positive results). With the aim to find SNP markers which show strongly statistically supported relationship with favorable values of investigated quantitative traits, genotype - phenotype association analysis was conducted. It was performed using two approaches – one relying on the TASSEL software, widely used in agronomics and the other based on machine learning software like WEKA, rarely used in agronomics. The results of two approaches were compared and discussed.

Keywords:
maize, bioinformatics, genotyping, RNA-Seq, genotype-phenotype association

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CIP – Каталогизација у публикацији
Библиотека Матице српске, Нови Сад 57
BIOLOGIA Serbica / editor-in chief Milica Matavulj. –
Vol. 34, no. ½ (dec. 2012) - . – Novi Sad : Faculty of Sciences, Department of Biology and Ecology, 2012-. –
Ilustr. ; 28 cm
Dva puta godišnje. – Je nastavak: Зборник радова Природно-математичког факултета. Серија за биологију = ISSN 0352 – 1788
ISSN 2334 – 6590 = Biologia Serbica
COBISS.SR – ID 275725831

ISSN 2334-6590

2021
Vol. 43 - No. 1 Special Edition

Biologia Serbica

ISSN 2334-6590