IDENTIFIKACIJA GENOTIPOVA GRAHA OTPORNIH NA SUŠU FENOTIPIZACIJOM VISOKE PROPUSNOSTI

Sažetak

Grah (Phaseolus vulgaris L.) jedna je od najznačajnijih mahunarki na globalnoj razini zbog svoje važne uloge u ljudskoj prehrani. Njegova se proizvodnja, poput drugih poljoprivrednih vrsta, sve češće suočava s izazovima klimatskih promjena, među kojima suša predstavlja jedan od najizraženijih stresnih čimbenika. Suša negativno utječe na biljku na svim razinama, od osnovnih fizioloških procesa i razvoja do konačnog prinosa. S obzirom na široku genetsku varijabilnost unutar vrste, identifikacija tolerantnih genotipova i razumijevanje mehanizama njihove prilagodbe ključni su za osiguranje dugoročno održive proizvodnje. U istraživanju je korišteno 200 tradicionalnih genotipova graha uzgojnih morfotipova: trešnjevac, zelenčec, biser, puter, bijeli, sitni crni, dan i noć, crveni meksički, crni, tetovac i kukuruzar. Genotipovi su podvrgnuti analizama fenotipizacije visoke propusnosti (HTP), te genotipizaciji. Od svakog genotipa šest biljaka je bilo u kontrolnoj skupini sa dovoljnom količinom vode i šest biljaka u skupini koja je predstavljala tretman suše. Kroz period od devet dana biljke su bile izložene postupnom utjecaju suše te su u tom vremenskom periodu provedene multispektralne analize, analize klorofilne fluorescencije, 3D skeniranje i mjerenja provodljivosti puči. Bayesovskom analizom određena je pripadnost pojedinih genotipova različitim genetskim skupinama. Generirani fenotipski podaci povezani su s genotipskim informacijama te je provedena cjelogenomska studija pridruživanja (GWAS). Na temelju rezultata GWAS analize, identificirani su lokusi kvantitativnih svojstava (QTL) i geni od značaja za fiziološke i biokemijske procese ključne za preživljavanje biljaka u uvjetima suše. Njihova biološka relevantnost dodatno je ispitana analizom povezanosti fenotipske varijabilnosti i alelne distribucije značajnih biljegaa te validacijom pripadnih genetskih regija. Suša je imala značajan utjecaj na fiziološke reakcije biljaka graha, pri čemu su se kao najosjetljivija i najresponzivnija svojstva izdvojila svojstva klorofilne fluorescencije; nefotokemijsko gašenje (NPQ), fluorescencija u stabilnom stanju (Fs') i efektivna efikasnost fotosustava PSII (Fq'/Fm'), svojstvo multispektralnih analiza; Indeks zelenila listova (GLI) i morfološko svojstvo ukupna lisna površina (TLA). Na temelju navedenih svojstava provedena je selekcija genotipova tolerantnih na sušu i GWAS analiza. Među analiziranim genotipovima, genotip 405 (morfotip biser) izdvojio se kao najtolerantniji, svrstavajući se u 5% najboljih genotipova u četiri od pet ključnih svojstava (Fq'/Fm', Fs', GLI i TLA). Bayesovskom analizom strukture populacije, utvrđeno je da 20 genotipova pripada genetskoj skupini srednjeameričkog podrijetla, 149 genetskoj skupini andskog podrijetla, dok je 7 genotipova klasificirano kao mješovitog podrijetla. Pomoću GWAS analize identificirane su četiri značajne genetske regije povezane sa svojstvima klorofilne fluorescencije; Fs' i Fq'/Fm'. Za svojstvo Fq'/Fm' identificirana su tri signifikantna biljega na kromosomima Pv1, Pv3 i Pv7. Sukladno detektiranim biljezima anotirani su potencijalni kandidat geni sa regulacijskom ulogom u uvjetima suše; elongation factor 1-beta (EEF1B), ZINC FINGER CCCH DOMAIN-CONTAINING PROTEIN 32 i ALPHA/BETA-HYDROLASES SUPERFAMILY PROTEIN. U slučaju svojstva Fs' detektiran je jedan biljeg na kromosomu Pv5 anotiran kao Pectinesterase/Pectin methylesterase. Analizom alelne distribucije značajnih SNP biljega utvrđen je jasan alelni obrazac za svojstvo Fs', pri čemu su genotipovi koji nose alel GG pokazali više fenotipske vrijednosti u odnosu na genotipove s alelom TT. Time je potvrđen alelno-ovisan učinak biljega na varijabilnost svojstva Fs'. Za svojstvo Fq'/Fm' nije utvrđen jasan alelni obrazac, stoga je procijenjena stabilnost pronađenih QTL na temelju efekata i jačine GWAS signala. Dobiveni rezultati potvrđuju da tradicionalni genotipovi graha predstavljaju vrijedan genetski resurs za razvoj tolerantnih genotipova. Integracija HTP-a i GWAS-a pokazala se učinkovitom strategijom za identifikaciju ključnih svojstava, genotipova i genetskih regija relevantnih za prilagodbu graha uvjetima suše.

Ključne riječi: multispektralne analize, klorofilna fluorescencija, 3D skeniranje, struktura populacije, cjelogenomska studija pridruživanja, lokus kvantitativnih svojstva

IDENTIFICATION OF DROUGHT-TOLERANT COMMON BEAN GENOTYPES USING HIGH-THROUGHPUT PHENOTYPING

Common bean (Phaseolus vulgaris L.) is one of the most important legume crops worldwide due to its significant role in human nutrition. Like other agricultural crops, its production is increasingly challenged by climate change, with drought emerging as one of the most severe stress factors. Drought negatively affects the plant at all levels, from basic physiological processes and development to final yield. Given the wide genetic variability within the species, the identification of drought-tolerant genotypes and an improved understanding of the mechanisms underlying their adaptation are essential for ensuring long-term sustainable production.

In this study, 200 traditional common bean genotypes which could be classified into agronomic morphotypes – trešnjevac, zelenčec, biser, puter, bijeli, sitni crni, dan i noć, crveni meksički, crni, tetovac i kukuruzar– were analysed. The genotypes underwent high-throughput phenotyping (HTP) and genotyping. For each genotype, six plants were grown under control conditions with adequate water supply, and six plants under drought stress. Over a nine-day period, plants were subjected to gradually increasing drought stress, during which multispectral analyses, chlorophyll fluorescence measurements, 3D scanning, and stomatal conductance measurements were performed. Population structure was determined using Bayesian analysis. The phenotypic data were integrated with genotypic information, and a genome-wide association study (GWAS) was conducted. Based on the GWAS results, quantitative trait loci (QTL) and genes important for physiological and biochemical processes critical to plant survival under drought conditions were identified. Their biological relevance was further examined through analyses of the association between phenotypic variability and allelic distribution of significant markers, as well as through validation of the corresponding genetic regions.

Drought had a significant impact on the physiological responses of common bean plants, with chlorophyll fluorescence traits – non-photochemical quenching (NPQ), steady-state fluorescence (Fs′), and effective quantum yield of photosystem II (Fq′/Fm′)—as well as the multispectral trait green leaf index (GLI) and the morphological trait total leaf area (TLA) identified as the most sensitive and responsive. Based on these traits, drought-tolerant genotypes were selected and GWAS analysis was conducted. Among the genotypes analysed, genotype 405 (biser morphotype) was identified as the most tolerant, ranking within the top 5% of genotypes for four out of five key traits (Fq′/Fm′, Fs′, GLI, and TLA). Bayesian population structure analysis revealed that 20 genotypes belonged to the Mesoamerican genetic group, 149 to the Andean genetic group, and 7 genotypes were classified as admixed. GWAS identified four significant genomic regions associated with chlorophyll fluorescence traits; Fs′ and Fq′/Fm′. For Fq′/Fm′, three significant markers were detected on chromosomes Pv1, Pv3, and Pv7. Based on these markers, potential candidate genes with regulatory roles under drought conditions were annotated, including elongation factor 1-beta (EEF1B), ZINC FINGER CCCH DOMAIN-CONTAINING PROTEIN 32, and ALPHA/BETA-HYDROLASES SUPERFAMILY PROTEIN. For Fs′, one significant marker was detected on chromosome Pv5 and annotated as Pectinesterase/Pectin methylesterase. Analysis of allelic distribution of significant SNP markers revealed a clear allelic pattern for Fs′, with genotypes carrying the GG allele exhibiting higher phenotypic values than those carrying the TT allele, confirming an allele-dependent effect of the marker on Fs′ variability. For Fq′/Fm′, no clear allelic pattern was observed; therefore, the stability of the identified QTL regions was assessed based on the generated effect and the strength of the GWAS signal.

The results confirm that traditional common bean genotypes represent a valuable genetic resource for the development of drought-tolerant genotypes. The integration of HTP and GWAS proved to be an effective strategy for identifying key traits, genotypes, and genomic regions relevant to the adaptation of common bean to drought conditions.

Keywords: Multispectral imaging, chlorophyll fluorescence, 3D scanning, population structure, genome-wide association studies, quantitative trait locus