

Common bean (*Phaseolus vulgaris* L.) is a species of great interest worldwide while in diet it provides macro- and micronutrients. In Croatia the production is based on landraces which display high levels of diversity and are important reservoirs of natural genetic variations (Carović-Stanko et al., 2017). In the last ten years genome-wide association studies (GWAS) were successfully carried out in common bean mainly focusing on disease resistance (Shi et al., 2011; Persegui et al., 2016; Zuiderveen et al., 2016; Tock et al., 2017; Fritsche-Neto et al., 2019) and agronomic traits (Nemli et al., 2014; Kamfwa et al., 2015; Moghaddam et al. 2016; Ates et al., 2018; Nascimento et al. 2018).



Fig. 1: Morphological diversity of Croatian common bean landraces

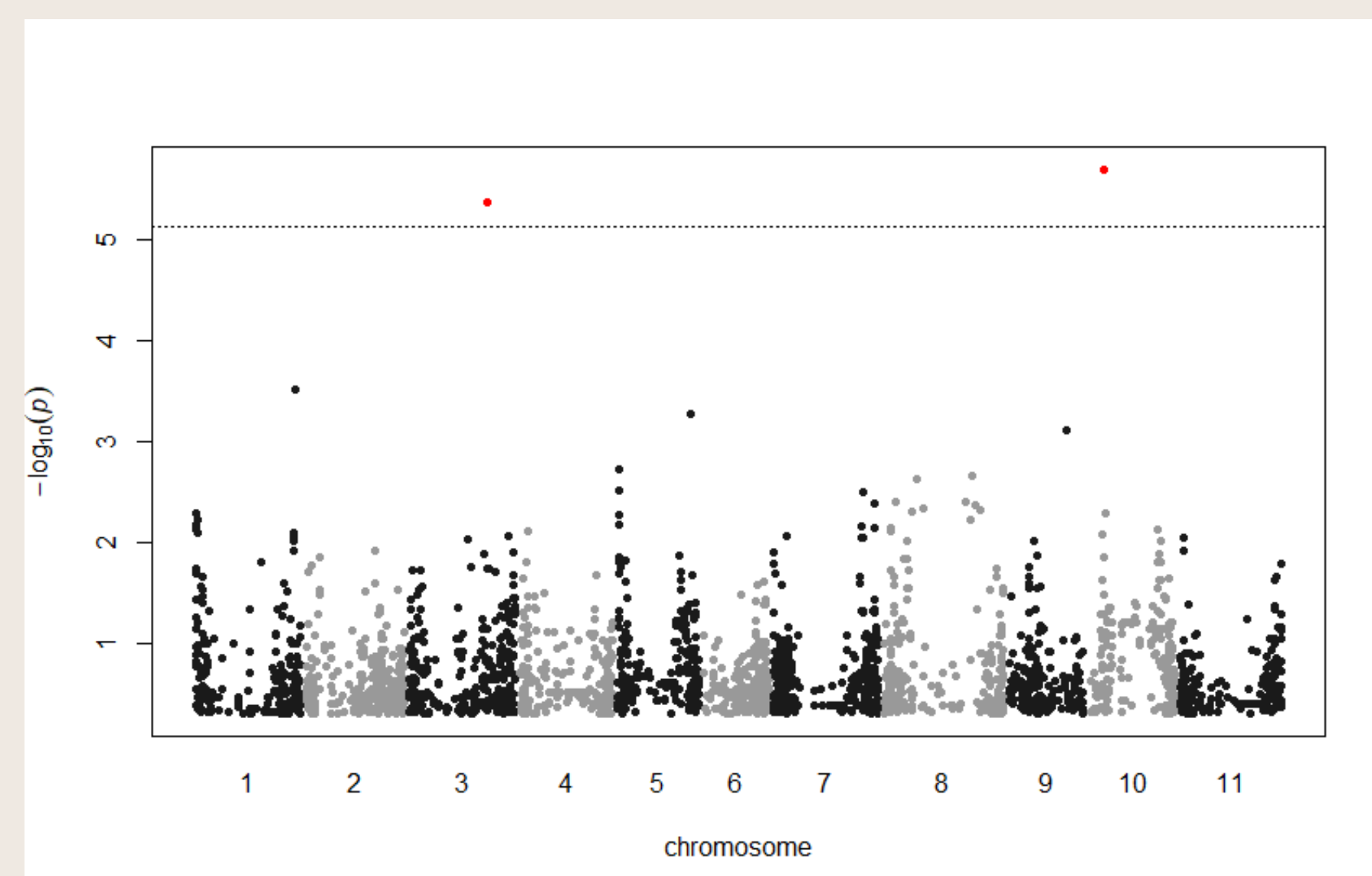


Fig. 2: Manhattan plot of MLMM fit for nitrogen content

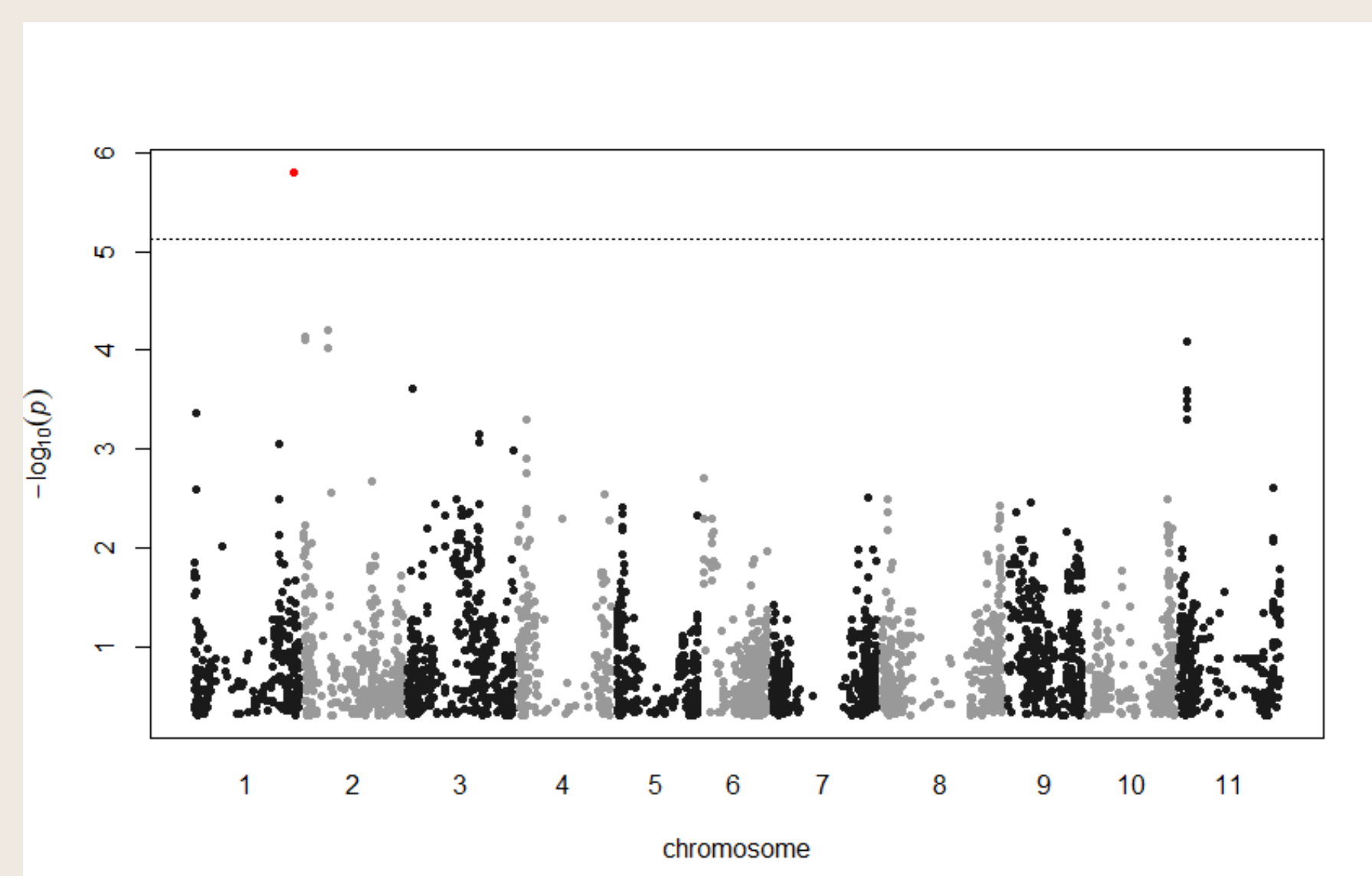


Fig. 3: Manhattan plot of MLMM fit for potassium content

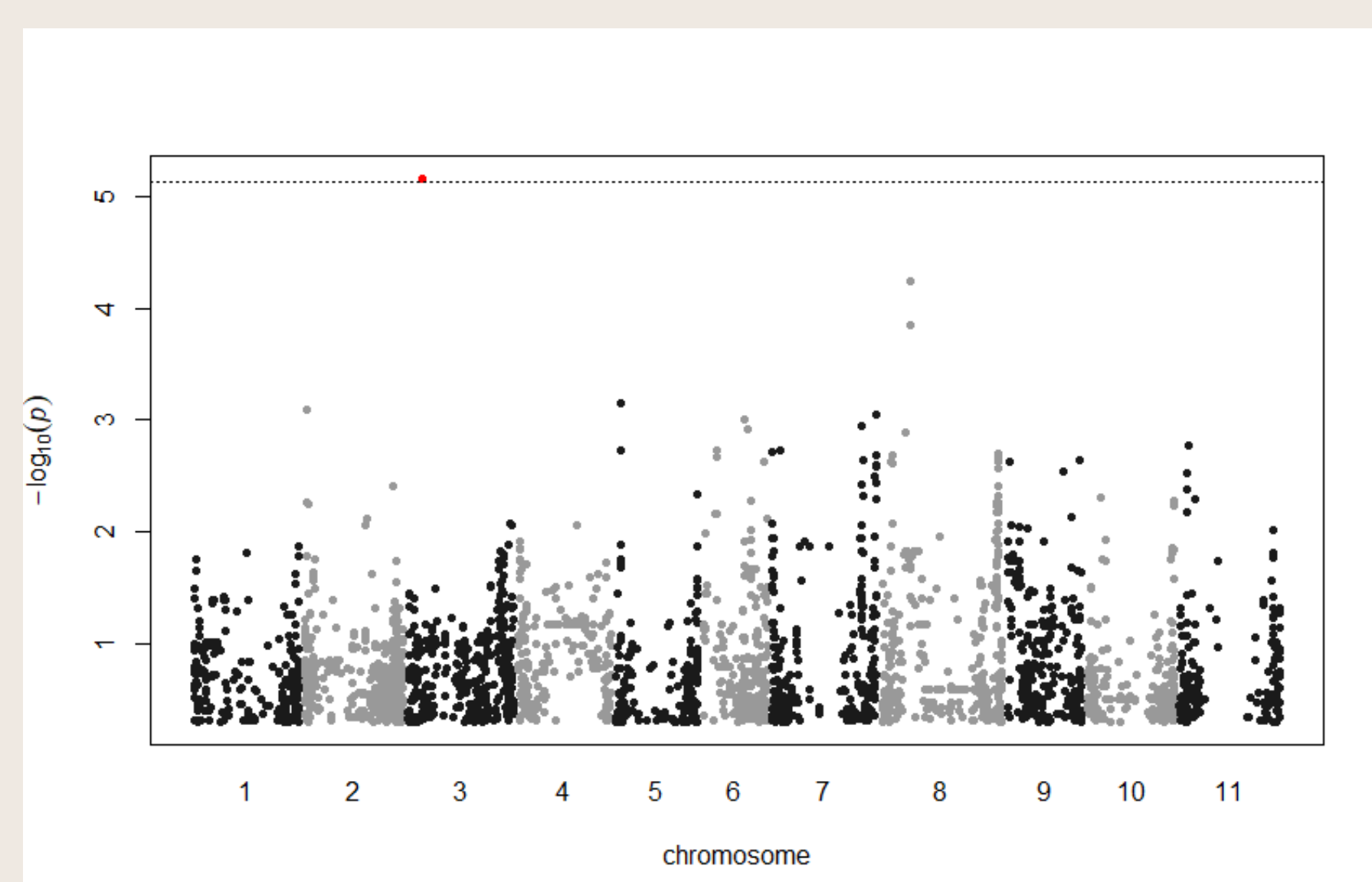


Fig. 4: Manhattan plot of MLMM fit for iron content

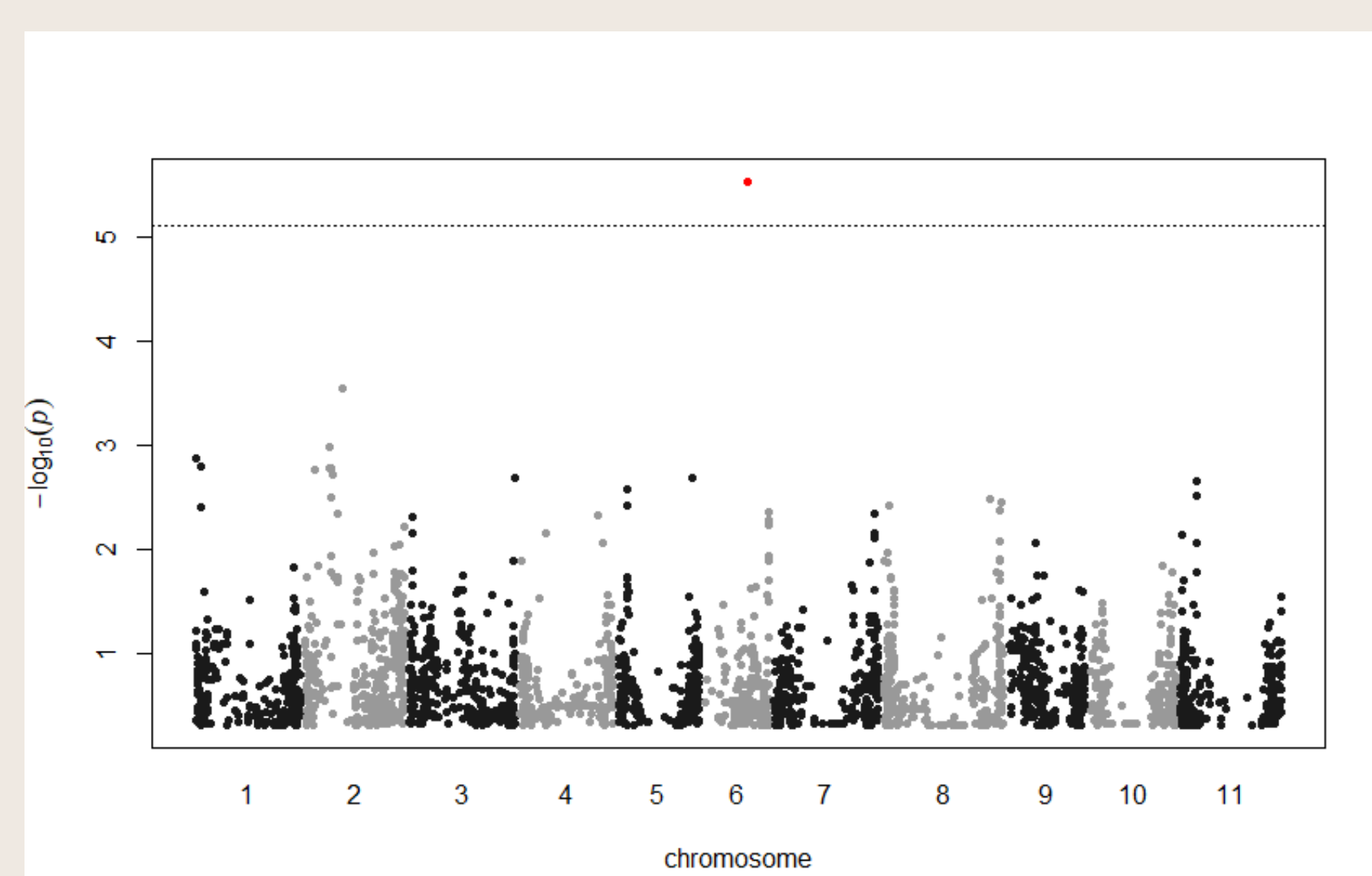


Fig. 5: Manhattan plot of MLMM fit for zinc content

Materials and methods

The study was performed using 174 accessions representing most commonly used Croatian landraces of common bean (Carović-Stanko et al., 2017)

The seed content of eight nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc and manganese) was assessed using standard methods. Seed samples (dried at 70 °C) were divided into three subsamples and analyzed. Nitrogen was determined using the Kjeldahl method.

After digestion with concentrated HNO₃ (MILESTONE 1200 Mega Microwave Digester), phosphorus content was determined using a spectrophotometer, potassium by a flame photometer, while calcium, magnesium, iron, zinc and manganese were analyzed by an atomic absorption spectrophotometer (AAS) (AOAC, 2015).

Genotyping was carried out using 26 microsatellites and 6,599 high-quality DArTseq-derived SNP markers (<https://www.diversityarrays.com/>).

Genome-wide association study (GWAS) was carried out using two methods for detection of marker-trait associations. Single-locus approach was based on unified mixed-model (Yu et al., 2006) implemented in TASSEL 5.2.52 (Bradbury et al., 2007), while multi-locus approach was based on stepwise mixed-model regression (MLMM) implemented in R package 'mlmm' (Segura et al., 2012). Missing genotypes were imputed using Random Forest method, as implemented in R package 'missForest' (Stekhoven and Buehlmann, 2012). Effects controlling the population structure and individual relatedness were incorporated in both models. Population membership estimates were derived from microsatellite data using STRUCTURE (Pritchard et al., 2000). Kinship matrix was calculated using Dominance Normalized IBS method (Zhu et al., 2015) implemented in TASSEL. Missing phenotypes were imputed using multiple-phenotype imputation method implemented in R package 'phenix', incorporating previously calculated kinship matrix (Dahl et al., 2016). Bonferroni corrected threshold for $\alpha=0.05$ was used to detect significant associations.

Results and discussion

Using either single or multi-locus method, two markers associated with seed nitrogen content were located on chromosomes 3 (M1967) and 10 (M5749) (Fig. 2). Both methods detected one loci associated with seed potassium content, located on chromosome 2 (M0510) (Fig. 3). Single-locus method did not detect any loci associated with seed iron content, but multi-locus model detected one marker on chromosome 3 (M1641) (Fig. 4). Moreover, both methods detected one loci associated with seed zinc content, located on chromosome 6 (M3410) (Fig. 5).

No significant associations were detected for all other nutrients. Correlations between traits were found to be quite weak, and consistently all significant markers were located on different chromosomes. No exception is made by markers associated with N and Fe, which are located on the opposite sides of chromosome 3.

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