SCREENING COMMON BEAN (*P. vulgaris* L.) GERMPLASM FOR Fe AND Zn BIOFORTICATION

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The aims of our work were: (i) to explore the diversity of a group of near-homozygous genotypes for iron, zinc and phytate content, (ii) to identify donor accessions for biofortification traits and (iii) to identify genome regions involved in determining iron, zinc and phytate seed content by means of Genome Wide Association Study (GWAS).

1. INTRODUCTION

Among Plant Genetic Resources for Agriculture, **landraces** are excellent genetic materials for developing new varieties. Due to their genetic diversity, these populations have maintained potentially useful alleles for increasing valuable traits in crops.

A diet mostly relying on pulses as source of proteins could not guarantee a proper intake of mineral such as **iron** (Fe) and **zinc** (Zn). Indeed **Fe and Zn deficiency** can lead to serious medical conditions, especially in developing countries. In this context, **biofortification** is regarded as a sustainable and an effective approach to cope with such a form of malnutrition. **Common bean**, the most widely used pulse in the world, shows wide diversity regarding Fe and Zn seed content and of anti-nutritional compounds such as **phytate** (phytic acid, InsP6, and its derivatives, InsP5 and InsP4); the latter reduces absorption of trace-elements including the two above-mentioned minerals. Exploring the natural variability of these compounds in purposely developed collections is the first step towards biofortification of common bean.

2. MATERIALS & METHODS

Plant Materials. **A near-homozygous genotypes** (i.e. pure lines) collection was developed starting from 179 common bean landraces and 13 cultivars using a **Single-Seed Decent (SSD)** approach for five generations. In 2017 the collection was multiplied in a nursery using a **partially replicated randomised experimental design** based on the replication of 7 different genotypes.

Phenotyping. Seed samples were collected, hoven-dried and milled using Teflon capsules and zirconium oxide beads for subsequent seed minerals and total phytate analyses. **X-ray fluorescence spectrometry** was used to quantify Fe and Zn using an X-Supreme 8000 (Oxford Instrument). Total phytate were extracted and purified using **anion-exchange chromatography** and then quantified in duplicates by means of **spectrophotometry**, according to Latta and Eskin[1]. For each trait, Best Linear Unbiased Predictors were estimated.

Genotyping. Genomic DNA was isolated from young leaf tissues. Genotyping was performed at IGATech (Udine, Italy) using a double digest Restriction-site Associated DNA sequencing approach (**ddRAD-seq).** Produced sequences were mapped against the common bean reference genome. Several quality control (QC) steps were used to filter the identified SNPs. SNPs characterized by missingness > 0.1, minor allele frequency (MAF) < 0.05 and heterozygosity ≥ 0.02 were discarded.

GWAS. Association analyses were performed for Fe, Zn and total phytates using a **mixed linear model,** including corrections for population structure and relatedness, as implemented in TASSEL (v. 5.2).

1.Latta, M. & Eskin, M. A Simple and Rapid Colorimetric Method for Phytate Determination. J. Agric. Food Chem. 28, 1313–1315 (1980).

3. RESULTS

Phenotyping. The collection showed high level of diversity for the considered traits (Fig. 1, left). The concentration of Fe ranged between **38.4 and 93.7 ppm** while Zn between **18.9 and 53.4 ppm.** Phytate concentrations ranged from **4.8 to 19.9 mg/g**, with an average of 12.0 mg/g. The average coefficient of variation (CV) between duplicates was 2.7% showing high level of method reproducibility.

Genotyping. **After QC control**, the genotyping produced a dataset of **49,518 SNPs** used to perform association analyses. Results of structure and cryptic relatedness analyses, carried out on a reduced dataset of 2,518 independent SNPs, showed the presence of two major groups consisting of genotypes characterized by different level of relatedness.

GWAS. A significant and meaningful association was found for zinc on chromosome 1 where five significant SNPs were identified spanning in an area of 42 Kbp.
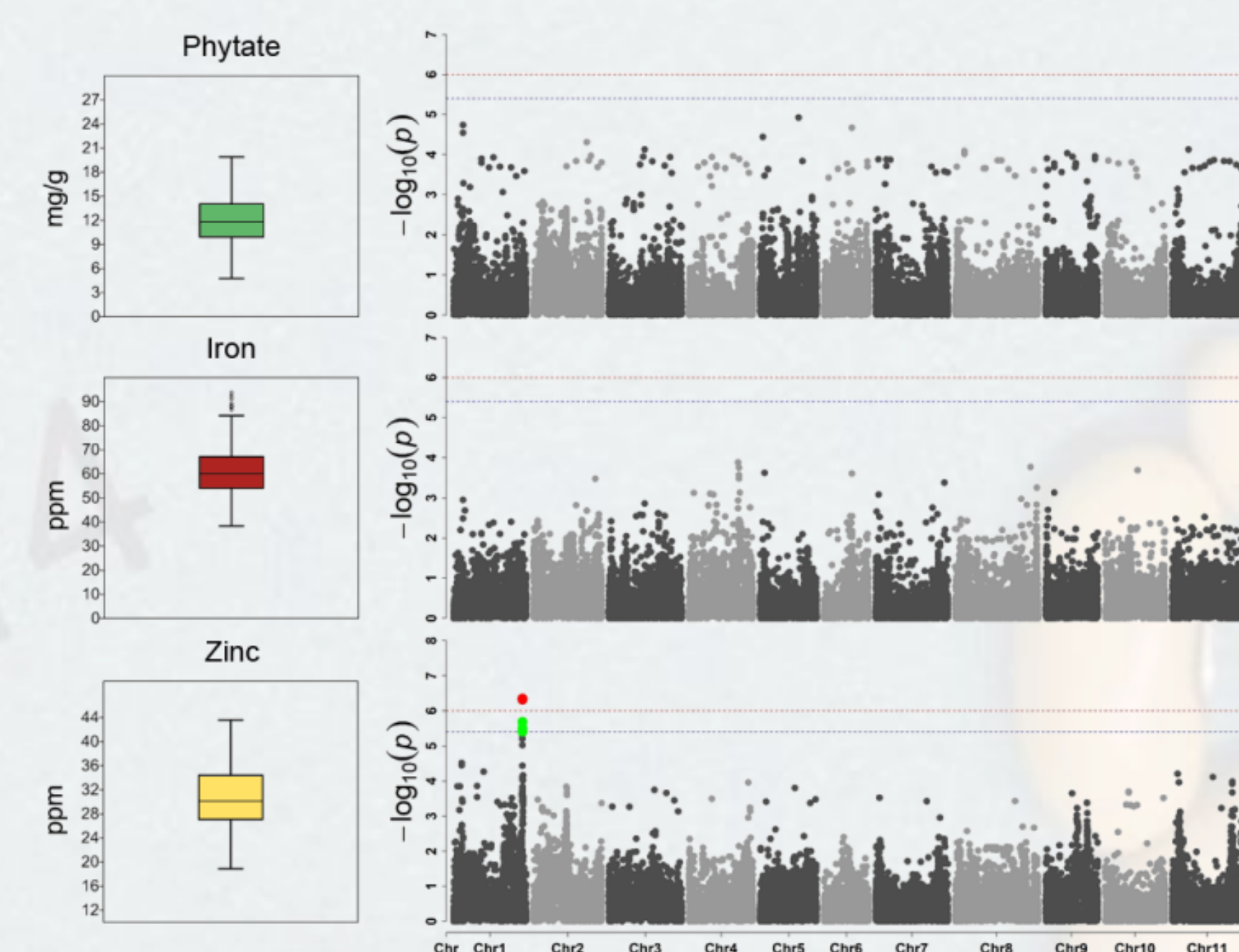
Figure1.
Box plots (left) and **Manhattan plots** (right) of the three biofortication-related traits investigated: phytate, iron and zinc. In the Manhattan plots, the horizontal lines indicate the genome-wide significance thresholds: 5.4 (blue, Bonferroni correction based on the number of recombination blocks) and 6 (red, Bonferroni correction based on total number of SNP markers). SNPs with a *p* value above the selected thresholds are highlighted in green and red, respectively.

4. DISCUSSION

The **collection of near-homozygous genotypes** developed in this study showed a **high level of diversity** for the seed nutrient related traits. The application of the **ddRAD** approach resulted in large and robust SNPs dataset with markers distributed over the 11 *P. vulgaris* chromosomes. Results of the GWAS analysis allowed the identification of a region significantly associated with seed zinc content (chromosome Pv01). Further investigation are needed to **detect candidate genes for the observed association**.