Genome-wide SNP identification and association mapping for seed mineral nutrients in Mung bean (*Vigna radiata* L.)

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INTRODUCTION

Mung bean (*Vigna radiata*) is an important pulse crop mainly cultivated in South, East and Southeast Asia. Pulses are an important source of essential minerals for human and biofortification, the application of breeding with the goal of increased ability of grain to acquire mineral elements, is an immediate strategy not only to increase mineral concentrations in edible crops but to solve nutritional deficiencies in human beings which can lead to stunted growth and development in children, lower resistance to disease, and increased mortality rates (White et al., 2009). Nutritional deficiencies are especially prevalent in developing countries where people do not have the full access to diverse vegetables, fruits and animal products.

OBJECTIVE

The goals of this research were to develop molecular marker tools for mung bean and to conduct nutrient analysis of a core collection for the species *V. radiata*. Specific objectives were to 1) identify genome-wide single nucleotide polymorphisms (SNPs) using genotyping by sequencing (GBS) and 2) perform genome-wide association studies (GWAS) for levels of seed calcium, iron, potassium, manganese, phosphorous, sulfur, and zinc across two years.

MATERIALS AND METHODS
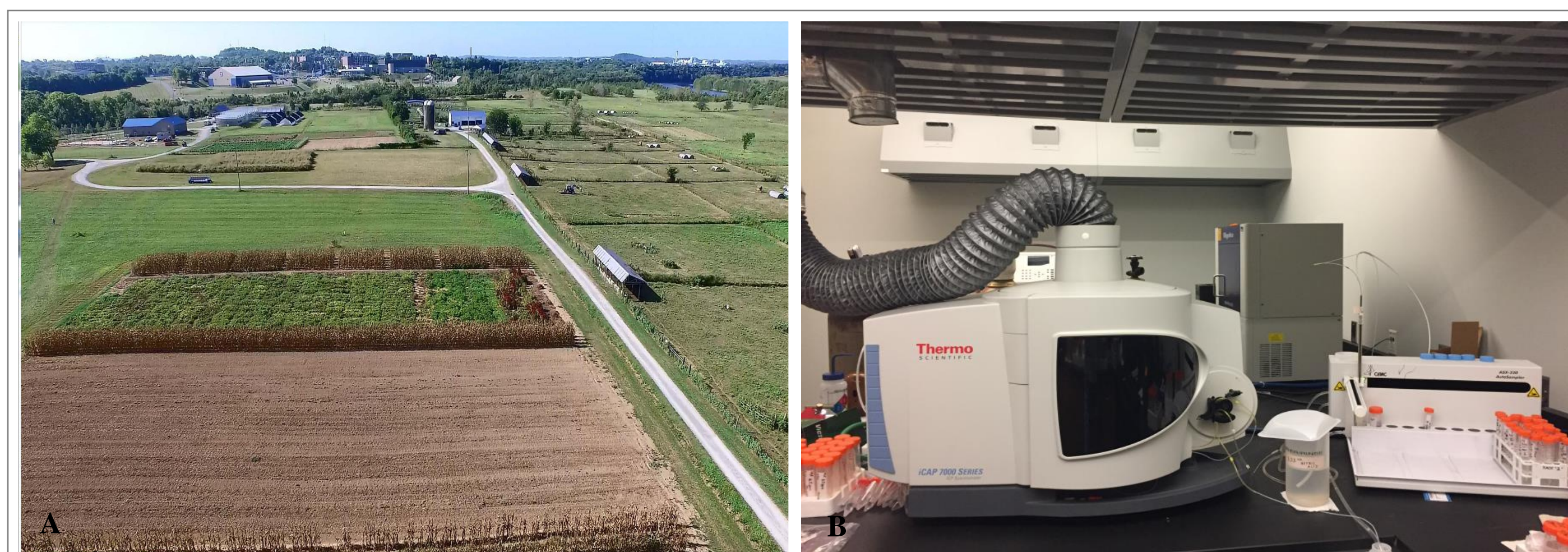
PHENOTYPING

Figure 1. Mung bean field in 2015, the first of two years' experiments for biofortification testing (A) and Inductively Coupled Plasma Optical Emission spectrum (ICPOES) equipment for seed mineral detection (B).

GENOTYPING

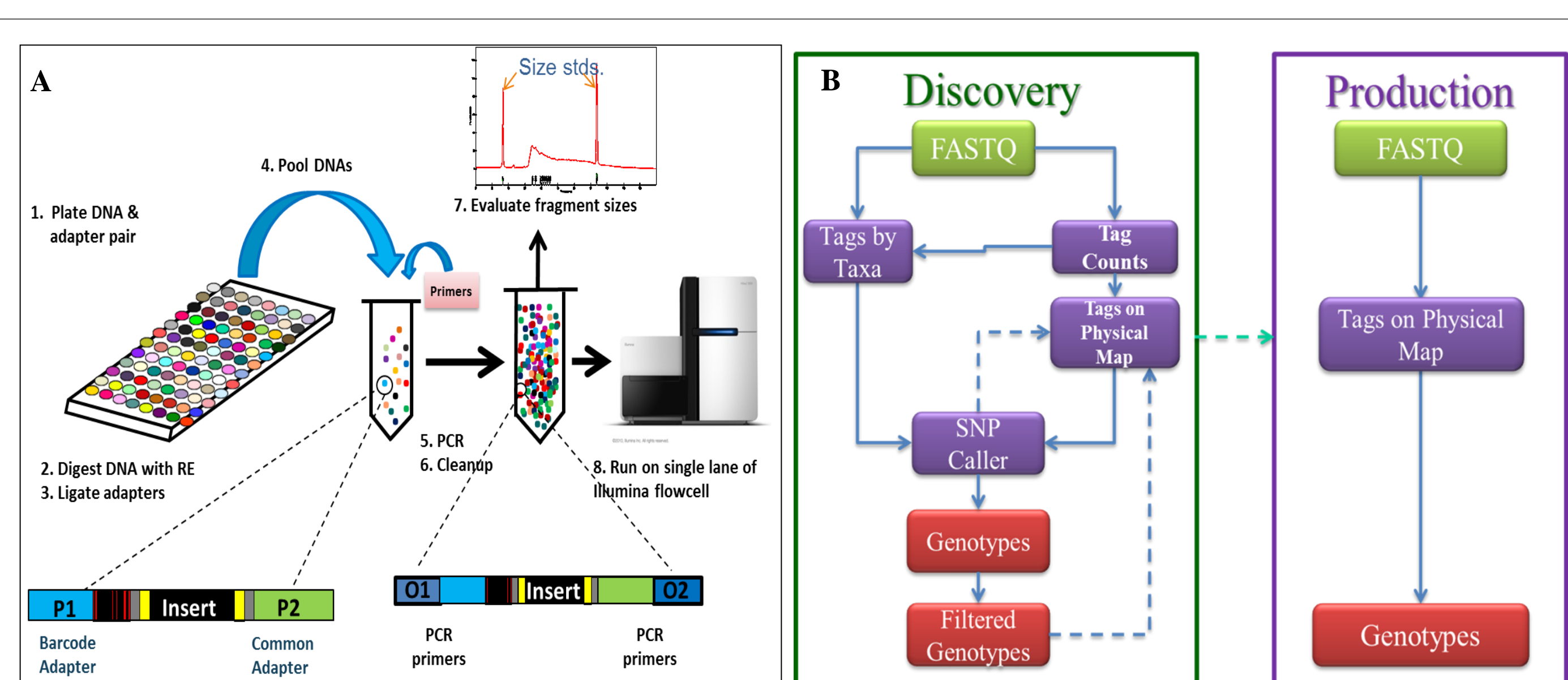**Figure 2.** Genotyping by sequencing procedure (Elshire et al., 2011) (A) and bioinformatics pipeline for SNP calling (Bradbury et al., 2006) (B).
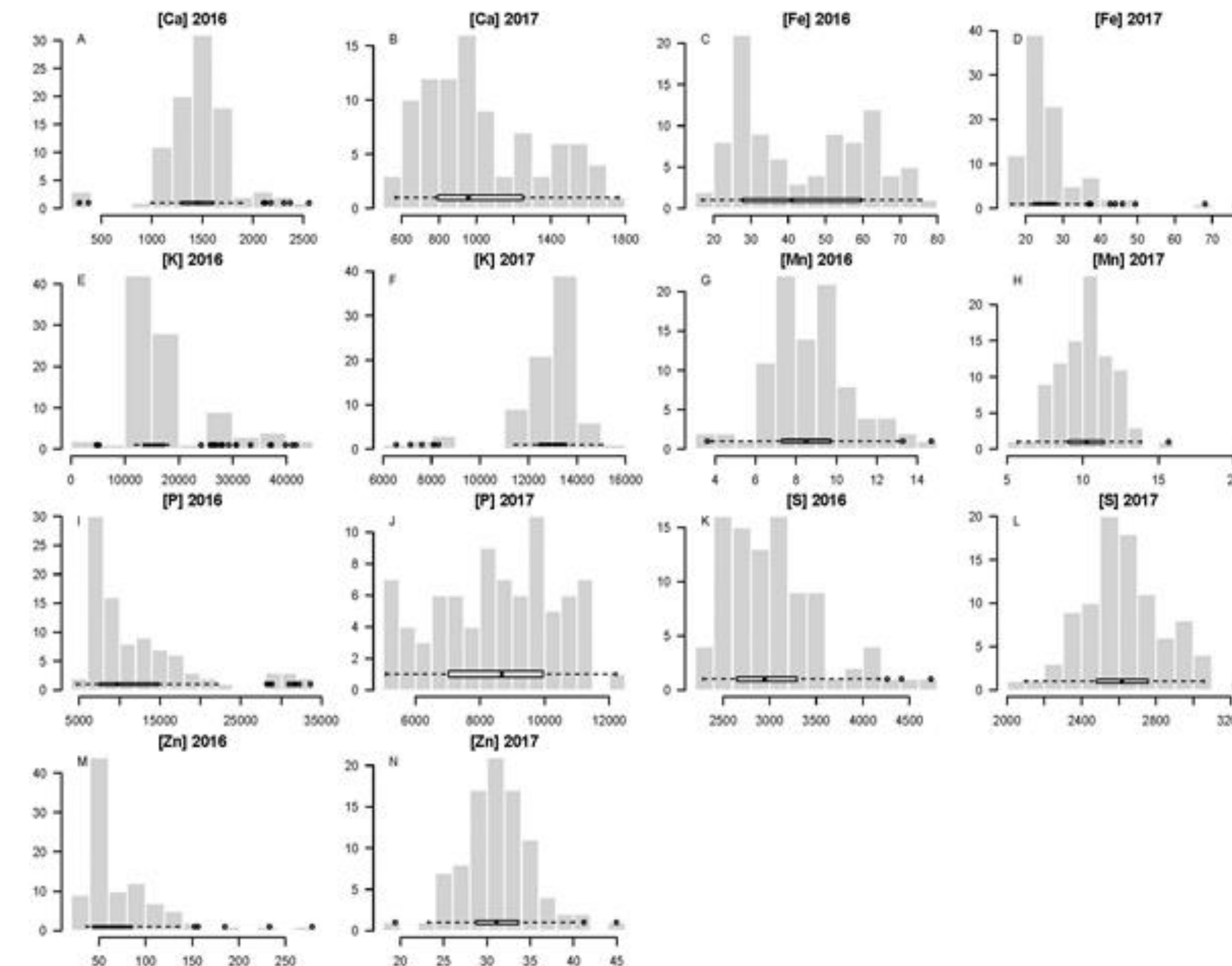
RESULTS

Figure 3. Histograms and horizontal box plots for the seven minerals measured in two years (2016 and 2017) in 92 mung bean accessions.
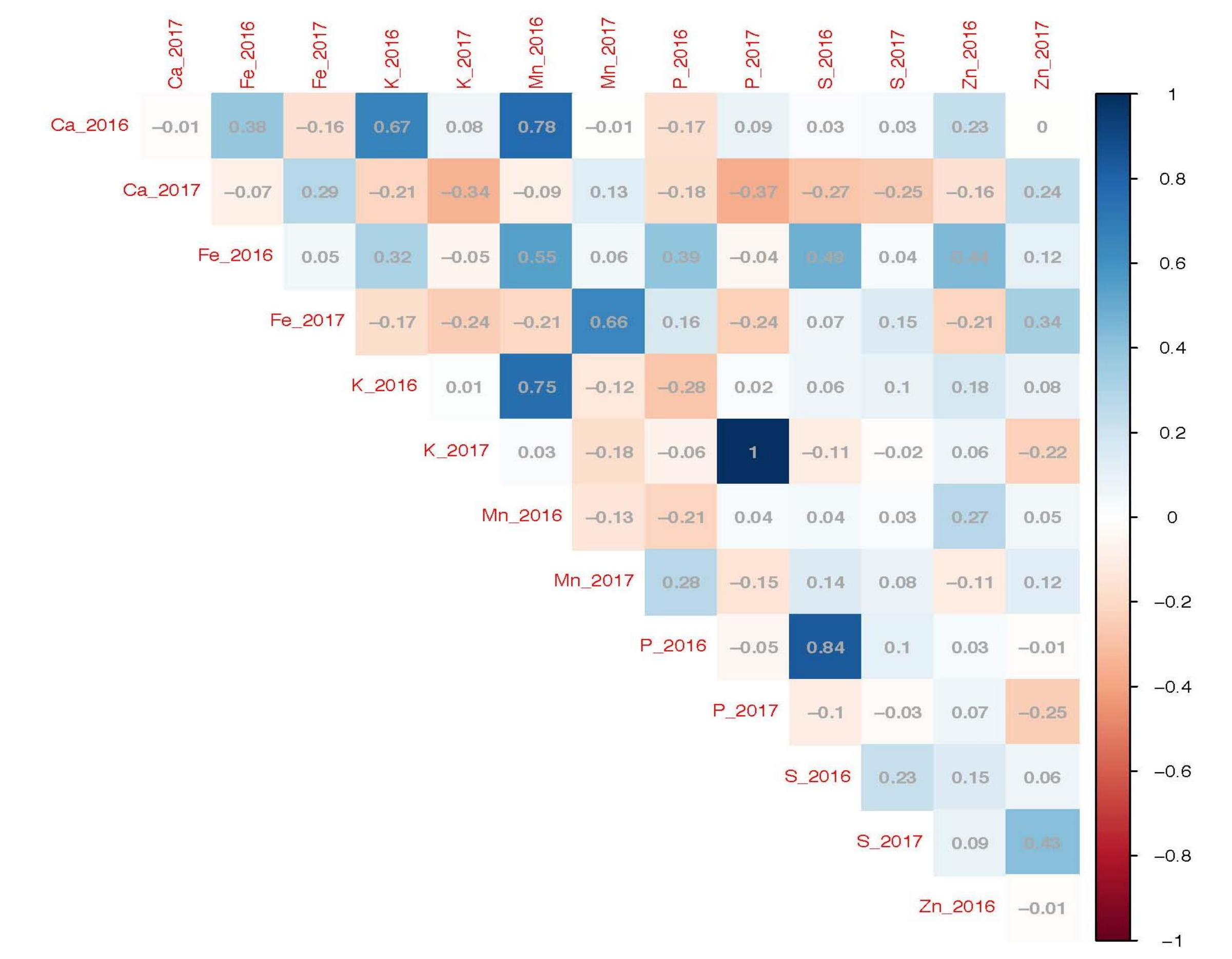

Figure 4. Pairwise correlation coefficients (R^2) for the seven minerals measured in two years (2016 and 2017) in mung bean accessions.
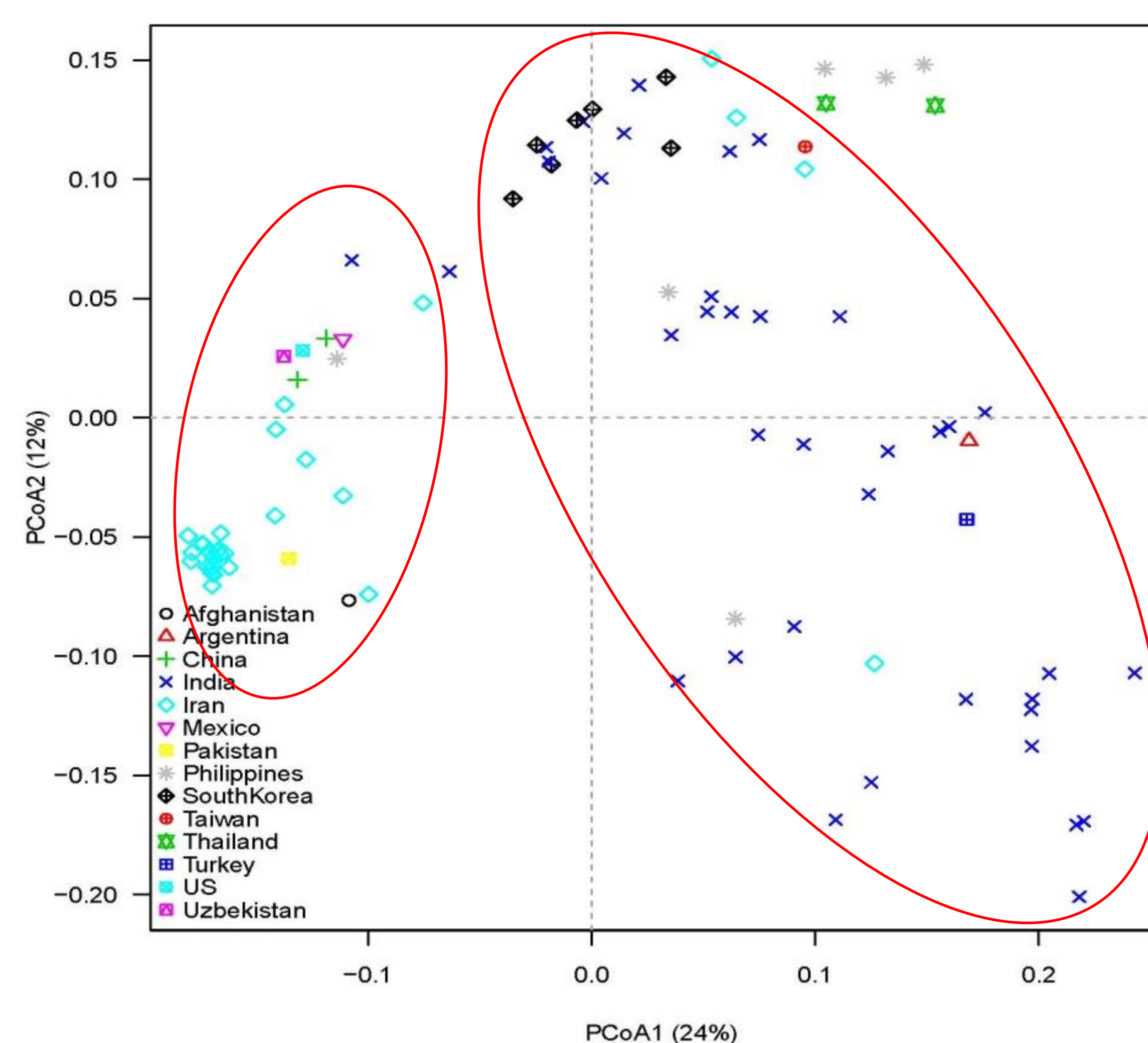

Figure 5. Population structure revealed by principal coordinates analysis (PCoA) based on 6,486 SNP markers in mung bean.
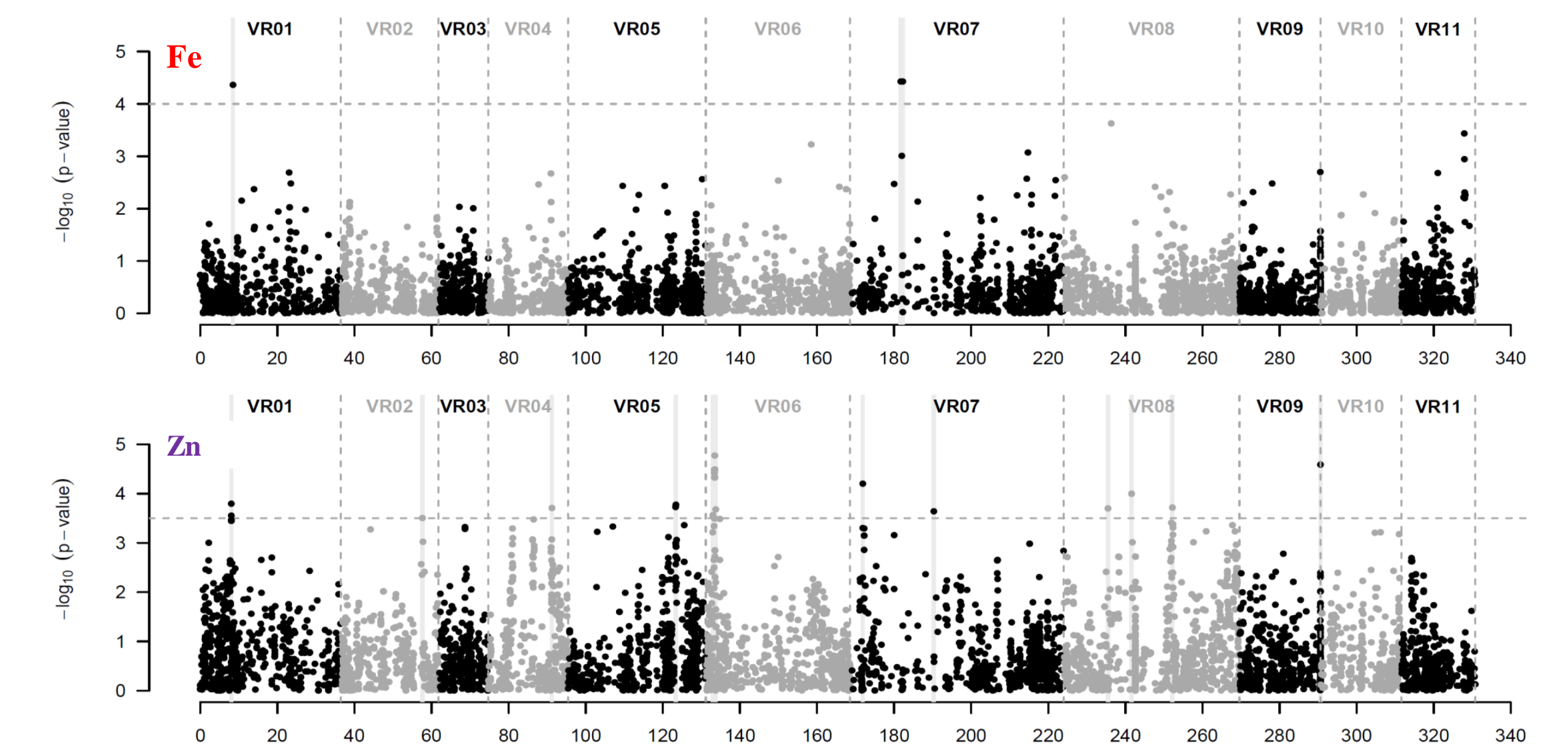

Figure 6. Manhattan plot of genome-wide association analyses for minerals measured in two different years in 92 mung bean accessions based on 6,486 SNP markers. The Manhattan plots show per-marker $-\log_{10}$(P-value) for Iron (Fe) and Zinc (Zn).

Figure 7. SNP validation pipeline by using KASP genotyping.

SUMMARY

- 6,486 high quality SNPs were discovered;
- 43 associated SNPs explained average 22 % of the overall variation in seed mineral contents;
- 38 genes were found by blasting with 1000 bp flanking regions of the significant SNPs;
- 20 SNPs were validated by using KASP pipeline.

References

Elshire, Robert J., et al. "A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species." *PloS One* 6.5 (2011): e19379.
Bradbury, Peter J., et al. "TASSEL: software for association mapping of complex traits in diverse samples." *Bioinformatics* 23.19 (2007): 2633-2635.
Kang, Yang Jae, et al. "Genome sequence of mung bean and insights into evolution within *Vigna* species." *Nature Communications* 5 (2014): 5443.
White, Philip J., and Martin R. Broadley. "Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine." *New Phytologist* 182.1 (2009): 49-84.

Acknowledgement