

## Exploration of genetic architecture of major agronomical traits in *Vicia sativa* collection via Genome-Wide Association Studies (GWAS)

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**Motivation and aim:** Legumes are valuable crops for human and livestock nutrition, offering health benefits. *Vicia sativa* (common vetch) enhances soil fertility through nitrogen fixation and supports intercropping, boosting productivity of cereals and climate resilience. However, its cultivation has declined since the late 20th century due to excessive use of nitrogen fertilizers and reduced profitability, thus, limiting genetic research. Renewed focus on sustainable agriculture makes intercropping practices more vital. This study aims i) to explore the genetic diversity in the unique vetch germplasm; ii) and to identify new Single Nucleotide Polymorphisms (SNPs) associated with key agronomic traits in *V. sativa* to aid breeders in developing improved cultivars.

**Materials and methods:** 289 accessions of *V. sativa* were genotyped using GBS (Genotype-by-Sequencing) approach. Phenotypic data for the same collection was obtained in a multi-environment trial according to alpha-lattice block design, in three different countries (Spain, Morocco, and Lebanon) over three campaigns from 2021 to 2023. In total, 17 major agronomical traits were recorded (including grain yield, plant biomass, day to flowering, maturation, and etc.), with three traits common to at least two locations. Due to the high environmental effect on *V. sativa* trait performances, Best Linear Unbiased Estimators (BLUEs, genetic effects in this case) were estimated using mixed linear models fitted i) per year×location; ii) per location over the years; iii) and for all the locations and years. Population structure was inferred based on the LD pruned SNP (Single Nucleotide Polymorphisms) data set (18k markers) with both ADMIXTURE and Discriminant Analysis of Principal Components (DAPC) methods. 320k SNP data set was employed for GWAS analyses, where five GWAS statistical models (GLM, MLM, SUPER, FarmCPU, and BLINK) were implemented and compared.

**Results:** SNP-based population genomics identified ten populations within the collection, pinpointing some discrepancy with the botanical description of some samples and thus leveraging the usage of whole-genome high-throughput genotyping for the refining of botanical classification. GWAS revealed 41 associated SNP, including 37 SNPs specific to a particular location and year for different traits, and 4 SNP, associated with a plant height trait, one of the few traits common to several environments. Such strong Genotype×Environment effect highlights that most detected SNPs might be employed in marker-assisted selection, depending on the region for growing this crop. After the quality control on the false positive discovery, we developed KASP (Kompetitive allele specific PCR) markers for the most promising genomic variants.

**Conclusions:** This study provides insights into the genetic diversity and major agronomical traits genomic architecture of *V. sativa*, a crop with ecological and agricultural benefits. Owing to comprehensive genotyping and multi-environment phenotypic evaluations, Genome-wide association analyses revealed SNPs associated with key agronomic traits, though their significance varied across environments, underscoring the strong genotype-by-environment (G×E) interactions in *V. sativa*. These findings suggest that breeding strategies should account for regional environmental conditions to optimize trait performance. Future studies should further validate these SNPs in diverse environments and explore their functional roles to enhance breeding precision and crop productivity.

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