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*Proceeding Paper*

# Genome-Wide Association Analysis of Yield-Related Traits of Soybean Using Haplotype-Based Framework †

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**Abstract:** Haplotype-based breeding involving multi-marker association analysis is a promising approach to developing custom-designed, high-yielding crop varieties. Here, we reported multi-marker association analysis for the number of pods per plant (PNP), the number of seeds per plant (SNP), 100-seed weight (HSW), and seed yield per plant (SYP) using 211 cultivated soybean accessions. The field experiment was conducted across six environments following a randomized complete block design with three replications. A genome-wide association study (GWAS) explored 12,617 single-nucleotide polymorphism (SNP) markers from NJAU 355K SoySNP array to identify significant marker associations for the studied traits across the six environments. Six markers that were consistently associated with the yield traits in two or more environments were considered stable and selected as the reference markers for building haplotype block/loci. The multi-marker association analysis within the haplotype-based framework revealed various allelic combinations regulating the phenotypic variations for the studied yield-related traits in soybean. These haplotype alleles may serve as genomic resources in breeding programs aimed at improving the yield potential of soybean.

**Keywords:** GWAS; haplotype analysis; SNP; soybean; yield-related traits

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## 1. Introduction

Yield characters are complex quantitative traits that posed some difficulties to breeding efforts. Analyses of family linkage maps and linkage disequilibrium among unrelated individuals have been widely explored for the understanding of the genetic basis of complex quantitative traits, such as the yield characters in several plant species, including soybean [1,2]. These procedures represent the genome-wide studies of these characters for the identification of marker-trait association using single-marker analysis. Recently, haplotype-based breeding has emerged as a promising approach to developing custom-designed crop varieties. It involves the identification and exploration of superior alleles from a combination of many markers within a locus associated with the traits of interest.

Haplotype analysis has great potential in crop improvement programs. It allows plant breeders to maximize the genetic variation underlying complex gene actions in a

given locus. In soybean, Patil et al. [3] conducted haplotype analysis for candidate gene regulating salinity tolerance (GmCHX1). They identified various haplotypes for GmCHX1, including SV-2 which provide maximum salinity tolerance in soybean. Moreover, Wang et al. [4] identified superior haplotypes for grain quality, such as cooking traits and eating quality traits, in rice. Abbai et al. [5] performed haplotype analysis in rice's 3K panel for 120 genes and identified desirable haplotypes for agronomically important traits. Similarly, the five candidate genes regulating the phenotypic performance of the direct-seeded rice were subjected to haplotype analysis [6] (Chen et al. 2019). Sinha et al. [7] performed a haplotype analysis of five genes controlling drought tolerance in pigeonpea.

Furthermore, using haplotypes for QTL mapping could compensate for several limitations of single SNPs, including their biallelic nature, and substantially improve the efficiency of QTL mapping [8]. Moreover, haplotype-traits association analyses are helpful for the precise mapping of important genomic regions and the location of favored alleles or haplotypes for breeding [9].

The present work is aimed at identifying superior combinations of alleles within the haplotype-based framework for yield-related traits of soybean in different environments.

## 2. Materials and Methods

### *2.1. Plant Materials and Field Experiment*

A panel of 211 diverse genotypes were selected from widely cultivated soybean germplasm across wide geographic areas, including the Peoples' Republic of China and the United States of America [10]. The selected genotypes were phenotyped for two years at three locations (six environments), including the experimental field of Nanjing Agricultural University in Nanjing (E1 and E2), the experimental field of Jiangsu Yanjiang Institute of Agricultural Sciences in Nantong (E3 and E4) and the experimental farm of the Agricultural College of Yangzhou University in Yangzhou (E5 and E6). In each of the environments, the genotypes were planted in a randomized complete block design (RBD) with three replications. Each genotype was planted in three rows per plot, each row 200 cm long and with a 50 cm row spacing. Normal agronomic cultural practices were followed for the cultivation of the soybean germplasm at each location, as previously described by Zhang et al. [11]. Phenotypic data were recorded for yield-related traits, including the number of pods per plant (PNP), the number of seeds per plant (SNP), 100-seed weight in grams (HSW), and the seed yield per plant in grams (SYP).

### *2.2. Genome-Wide Haplotype Association Analysis*

The genome-wide association study (GWAS) explored 12,617 single-nucleotide polymorphism (SNP) markers from NJAU 355K SoySNP array to identify significant marker associations for the studied traits across the six environments. GWAS was conducted using five different statistical models, including the general linear model (GLM) with PCA [12] (Price et al. 2006), the compressed mixed linear model (CMLM) [13] (Zhang et al. 2010), the multiple-locus mixed linear model (MLMM) [14] (Segura et al. 2012), the fixed and random model circulating probability unification (FarmCPU) [15] (Liu et al. 2016) and the Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) [16] (Huang et al. 2019). The population structure was corrected with principal component analysis (PCA) using the Bayesian-information criterion (BIC) to estimate the optimal numbers of PCA [12,17] (Schwarz, 1978; Price et al. 2006).

Haplotype analysis was conducted using PLINK, v1.07 [18]. The stable markers were considered as reference markers for building haplotype block/loci. All markers that are in proxy association with the reference markers within the LD decay distance ±670 Kbp made up a haplotype block/locus. The contribution of each haplotype to the observed phenotypic variance across the environment was estimated using the "–hap-assoc" command.

## 3. Results and Discussion

In practical breeding, understanding the genetics underlying traits of interest is the ultimate objective. In this study, a genome-wide association study identified a total of 57 significant markers underlying the studied traits across six individual environments plus the combined environment (Figures 1 and 2). These were distributed across 18 of the 20 soybean chromosomes, indicating a complex genetic control of these traits, as similarly reported by Li et al. [19] and Hu et al. [20]. The highest number of significant markers/QTLs were detected on Chr.15 (10) followed by Chr.20 (8) and Chr.11 (5), respectively. Four were found each on Chr.04, Chr.06 and Chr.13 while three each were located on Chr.08 and Chr.12.

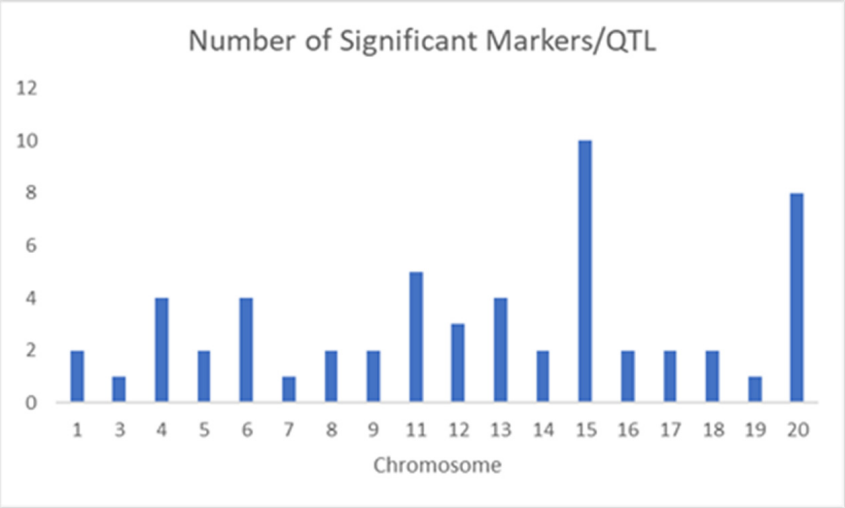



**Figure 1.** Distribution of significant markers/QTL across the soybean chromosomes.

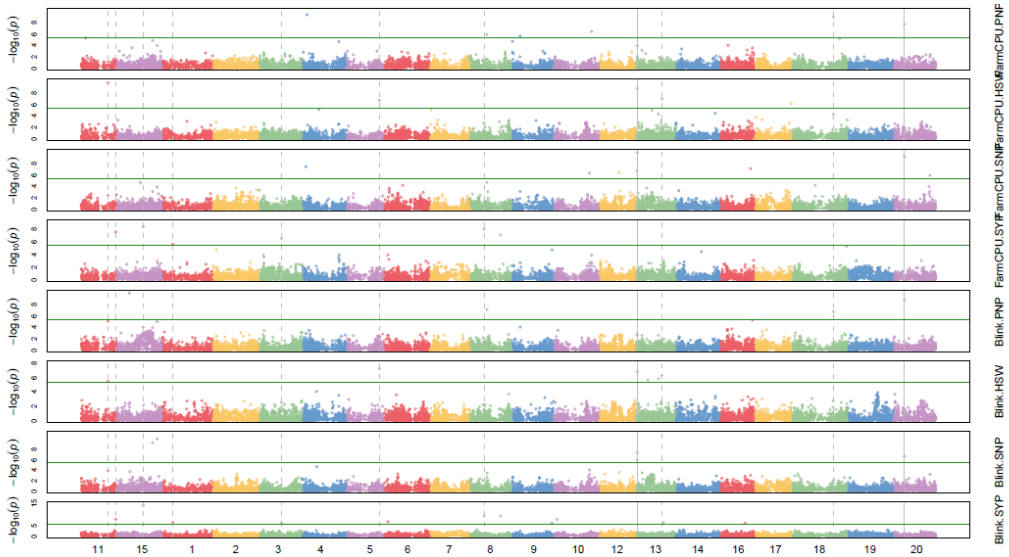



**Figure 2.** Manhattan plot showing the significant association of markers with yield-related traits in the combined environment based on the five GWAS models: BLINK, CMLMM, FarmCPU, GLM and MLMM.

Furthermore, in many studies, stable genomic regions or quantitative trait loci (QTL) are defined by markers consistently associated with a given trait across multiple environments or genetic backgrounds [21,22]. In the present study, stable genomic regions were found for three of the studied traits, including HSW, SNP and PNP on chromosomes 4, 5,

11, 13, 18 and 20 (Table 1). The stable QTL on chromosomes 11 and 13 was associated with both HSW and SNP, while those on chromosomes 4 and 20 were associated with PNP and SNP. The stable QTL on chromosome 5 is associated with HSW and the one on chromosome 18 is associated with HSW and PNP. The stable QTLs for 100-seed weight on chromosomes 5 and 11 have been respectively reported by Han et al. [23] and Du et al. [24], and Han et al. [23].

**Table 1.** Stable QTLs/genomic regions were identified for the yield-related traits consistently across the environments.

| QTL/Marker | Chromosome | Physical Position (bp) | Trait (Environment) | Related QTL |
|---|---|---|---|---|
| AX-93703924 | 4 | 4,291,705 | SNP (COM and E6); PNP (E3) | No related QTL |
| AX-93922099 | 5 | 36,599,702 | HSW (COM, E1 and E5) | *Seed weight 34–9* [17]; *Seed yield 22–10* [18] |
| AX-93793210 | 11 | 29,587,057 | HSW (COM, E1, E3 and E4); SNP (E2, E3 and E5) | *Seed weight 35–9* [17] |
| AX-93807406 | 13 | 1,843,185 | HSW (COM, E1, E2, E4 and E5); SNP (COM, E1 and E6) | No related QTL |
| AX-94176727 | 18 | 46,137,043 | PNP (COM and E1); HSW (E2) | No related QTL |
| AX-94199992 | 20 | 12,095,298 | PNP (COM and E3); SNP (COM and E1) | No related QTL |

Based on the haplotype-based framework, we conducted multi-marker association analyses using the stable markers as reference loci for the identification of superior allele combinations underlying the studied traits. Superior haplotype alleles for agronomically important traits have been reported in several crop species [5–7,25–27]. Our study revealed various allelic combinations regulating the phenotypic variations for the studied yield-related traits in soybean. Figures 3–5 highlight the haplotype alleles and the proportion of phenotypic variance contributed by these haplotypes to the associated traits across the six environments.

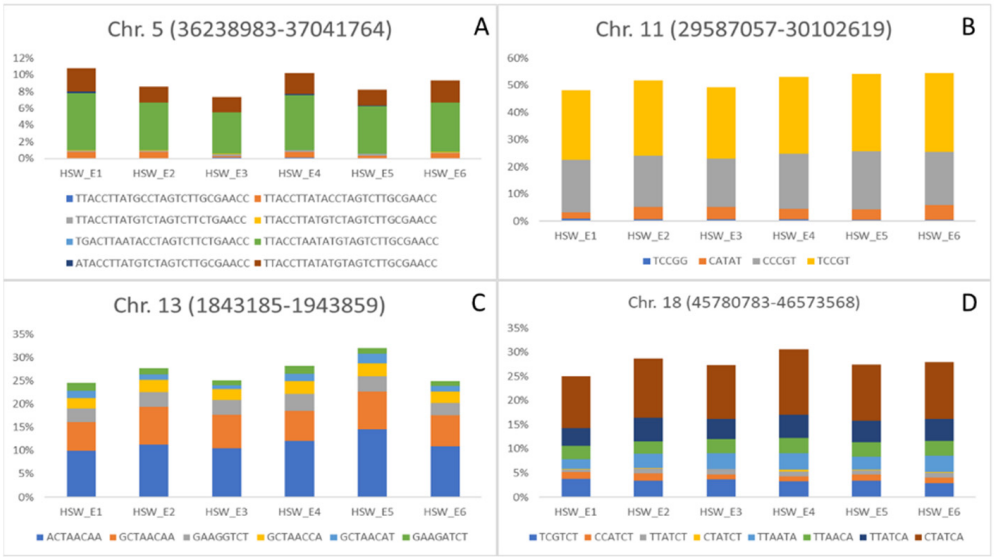



**Figure 3.** Haplotype alleles within the loci on chromosomes 5 (**A**), 11 (**B**), 13 (**C**) and 18 (**D**), and their contribution to the phenotypic variation of 100-seed weight across the environments.

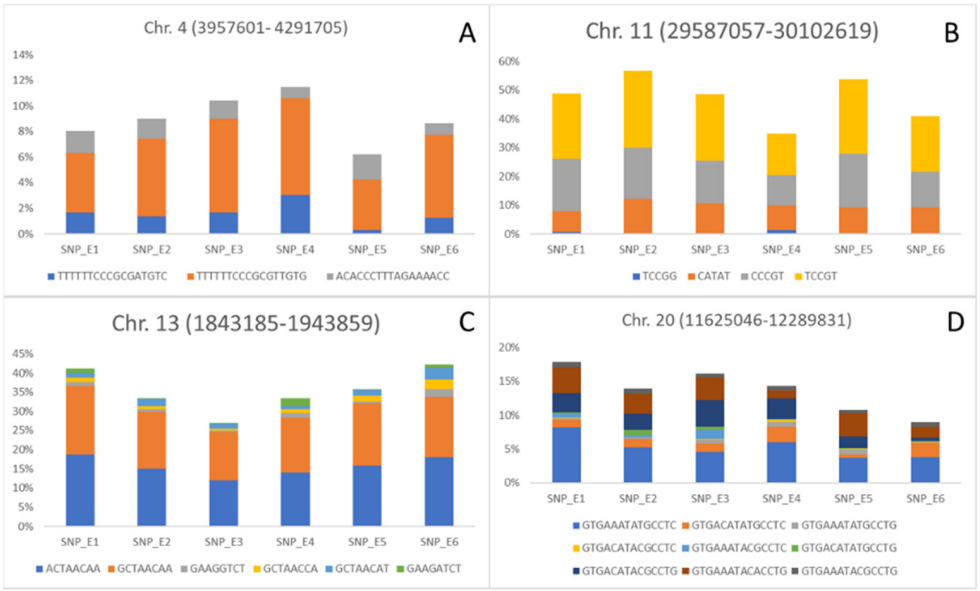



**Figure 4.** Haplotype alleles within the loci on chromosomes 4 (**A**), 11 (**B**), 13 (**C**) and 20 (**D**), and their contribution to the phenotypic variation of seed number per plant across the environments.

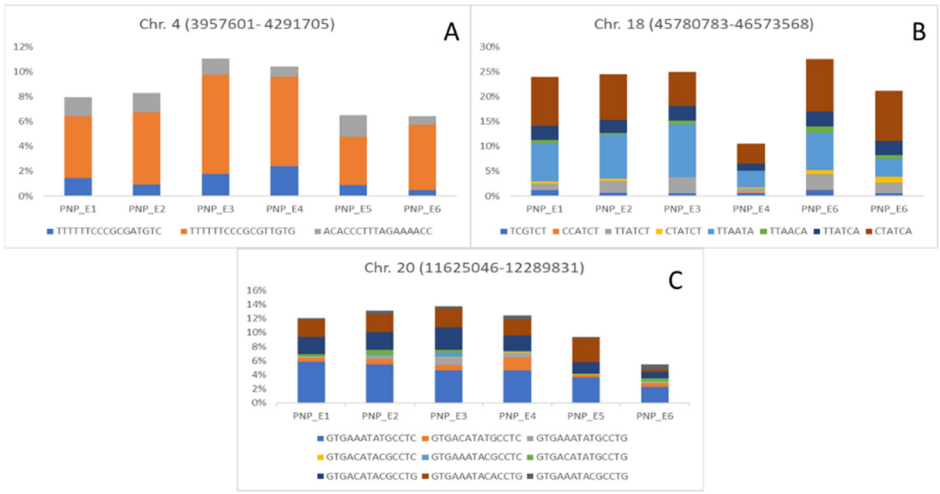



**Figure 5.** Haplotype alleles within the loci on chromosomes 4 (**A**), 18 (**B**) and 20 (**C**), and their contribution to the phenotypic variation of panicle number per plant across the environments.

## 4. Conclusions

The six stable QTL/Markers and the haplotype alleles identified in the present study may serve as genomic resources in breeding programs aimed at improving the yield potential of soybean.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/IECPS2021-12036/s1.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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