

**Fig. S1** Correlations between protein and amino acids by scaled BLUP values across all environments among the 621 plant introductions (*P* < 0.0001 for Met, Thr and Lys).



**(b**)



**Fig. S2** Results of *Delta K* analysis that determined the most like number of sub-populations from the STRUCTURE output



**Fig. S3** Linkage disequilibrium ( $r^2$ ) as a function of physical distance (kb) between marker pairs in heterochromatic and euchromatic chromosome regions.



**Fig. S4** QQ-plots for GWAS of 621 soybean accessions for seed protein content using CMLM. 34,014 SNPs were plotted for each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S5** QQ-plots for GWAS of 621 soybean accessions for seed oil content using CMLM. 34,014 SNPs were plotted for each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S6** QQ-plots for GWAS of 621 soybean accessions for seed protein content using MLMM. 34,014 SNPs were plotted for each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S7** QQ-plots (right) for GWAS of 621 soybean accessions for seed oil content using MLMM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S8** QQ-plots for GWAS of 621 soybean accessions for seed protein and oil content using MTMM-opposite. 34,014 SNPs were plotted for each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.





**Fig. S9** Manhattan plots for protein and oil content by each environment for a 5-Mb region surrounding significantly associated SNPs on chromosome 15. Negative  $\log_{10} P$ -values of MLMM are plotted against physical position (Glyma.W82.a2.v1). Red and blue horizontal lines represent genome-wide significant threshold (5%) and suggestive thresholds (25%), respectively.



**Fig. S10** Manhattan plots for protein and oil content by each environment for a 10-Mb region surrounding significantly associated markers on chromosome 20. Negative  $\log_{10} P$ -values of MLMM are plotted against physical position (Glyma.W82.a2.v1). Red and blue horizontal lines represent genome-wide significant threshold (5%) and suggestive thresholds (25%), respectively.

## 🔵 : Protein, 🛑 : Oil

Maturity\_OHW14



**Fig. S11** Manhattan plots (left) and QQ-plots (right) for GWAS of 621 soybean accessions for maturity using CMLM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue horizontal lines in the Manhattan plots and markers in the QQ-plots represent the genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively, and the SNPs significantly associated at those levels. Shaded region of the QQ-plots represent a 95% confidence interval.

Maturity\_OHW14



**Fig. S12** Manhattan plots (left) and QQ-plots (right) for GWAS of 621 soybean accessions for maturity using MLMM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue horizontal lines in the Manhattan plots and markers in the QQ-plots represent the genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively, and the SNPs significantly associated at those levels. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S13** Marker associations in the 43-48 Mb region surrounding significantly associated markers on Chr 10. Negative log<sub>10</sub> *P*-values of MTMM for trait-specific protein and oil effects for OHW14 and MLMM for maturity in OHW14 and NC15 are plotted against physical position (Glyma.W82.a2.v1). Red and blue horizontal lines represent genome-wide (5%) and suggestive significance thresholds (25%), respectively.



**Fig. S14** QQ-plots (right) for GWAS of 621 soybean accessions for Met using CMLM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S15** QQ-plots for GWAS of 621 soybean accessions for Cys using CMLM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S16** QQ-plots for GWAS of 621 soybean accessions for Lys using CMLM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S17** QQ-plots for GWAS of 621 soybean accessions for Thr using CMLM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S18** QQ-plots for GWAS of 621 soybean accessions for Met using MLMM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S19** QQ-plots for GWAS of 621 soybean accessions for Cys using MLMM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S20** QQ-plots for GWAS of 621 soybean accessions for Lys using MLMM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S21** QQ-plots for GWAS of 621 soybean accessions for Thr using MLMM. 34,014 SNPs were plotted by each environment and all environments combined. Red and blue dots represent the SNPs significantly associated at genome-wide significant threshold (5%) and suggestive significance thresholds (25%), respectively. Shaded region of the QQ-plots represent a 95% confidence interval.



**Fig. S22** The LD blocks from Chr 20 which were significantly associated with seed protein and oil contents (current study) compared with the genomic regions from six previous studies (Bandillo et al. 2015; Bolon et al. 2010; Hwang et al. 2014; Kim et al. 2016; Van and McHale 2017; Vaughn et al. 2014). LD blocks were defined by four-gamete method. Significant SNPs of the current study were indicated by red box. Asterisks represent the putative positional candidate genes mentioned in the main text.