Brief Communication

Mining novel kernel size-related genes by pQTL mapping and multi-omics integrative analysis in developing maize kernels

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As a sink organ for starch, protein, oil and essential nutrients, the maize (Zea mays) kernel is not only the main target for yield and quality improvement but also a model system for genetic and molecular biology studies. We identified many candidate genes for maize kernel quality and size quantitative trait loci (QTLs) at the genomic, transcriptomic, metabolomic and phenomic levels by genome-wide association studies (GWAS) and joint-linkage mapping (Fu et al., 2013; Liu et al., 2017b; Liu et al., 2017a; Wen et al., 2014; Yang et al., 2014) using a widely adopted Chinese association panel (Yang et al., 2011) and five recombinant inbred line (RIL) populations (Liu et al., 2017b). However, maize kernel proteome studies at the population scale have lagged behind.

Protein QTL (pQTL) analysis has proven to be useful in the diagnosis of various human diseases and has provided genome–proteome networks for clinical applications (Suhre et al., 2017). It is also necessary for elucidating the functional context of gene expression variation during modern maize breeding (Jiang et al., 2019). However, how pQTLs control maize kernel traits remain to be investigated. Here, we identified 468 clear and consistent protein spots in developing kernels of 210 inbred lines by combining 2-D gel electrophoresis with LC-MS/MS. These protein spots were translated from 283 unigenes, 84 of which encode moderate or low correlations between the transcript level and the protein abundance of the same gene; examples included several genes previously reported genes for kernel development (Dai et al., 2021), GRMZM2G068506 (Bt2), GRMZM2G429899 (Sh2), GRMZM2G089713 (Sh1), GRMZM2G141539 (Md4h), GRMZM2G306345 (Pdkt1) and GRMZM2G097457 (Pdkt2), among which only Bt2 exhibited a strong correlation between the transcript and protein levels (r = 0.78, P < 0.01). These genes clustered into different subnetworks at the transcript level (Figure 1b) but into the same subnetwork at the protein level (Figure 1c). The results reveal that the transcript level alone does not always reliably predict protein abundance at the population scale, and protein abundance variation may play an important role in orchestrating the biological functions of genes involved in the same biological pathways. pQTL analysis is therefore necessary to fully elucidate the molecular basis of kernel-related phenotypes.

Using GWAS based on 1.25 million SNPs, we identified 421 independent significant SNPs for the abundance of 40 protein spots encoded by 38 unigenes using the recommended P-value (1/N, P ≤ 2.04 × 10⁻⁶). Forty-six non-redundant pQTLs were defined within ±50 kb flanking regions of their lead SNPs based on the linkage disequilibrium of 50 kb (r² ≥ 0.1) in genome-wide average of 210 inbred lines. These included 13 local pQTLs and 33 distant pQTLs that distributed unevenly across the ten maize chromosomes (Figure 1d). Chromosome 7 and 2 had the lowest and highest density of pQTLs, respectively.
Five protein spots, P3206, P4506, P3005, P5202, and P2111, were underlain by two or more pQTLs, and the remaining protein spots by only one pQTL. Two pQTLs were found to regulate proteins that function in post-transcriptional/translational modifications (PS315 and P6207, P6508 and P7502).

We performed population-scale multi-omics integration to achieve a comprehensive understanding of the mechanistic basis of maize kernel development (Figure 1f). Eighty-seven previously reported eQTLs (Liu et al., 2017a) were found to regulate the transcript levels of genes that encode 30 protein spots, whereas co-localization for eQTL and pQTL was only observed for protein...
spots P1107, P1208, P6508, P8302 and P5211. We then compared the identified pQTLs with previously reported quality QTLs and normal QTLs for kernel (Liu et al., 2017b; Wen et al., 2014; Yang et al., 2014). Seventeen pQTLs coincided with ten QTLs from the association panel and ten QTLs from the five RIL populations (Figure 1d). In only four cases did a phenotypic QTL co-segregate with its eQTL and pQTL. For example, the abundance of P6508 and P7502 proteins significantly correlated with their transcript levels, and their pQTL co-localized with a kernel width (KW) QTL (LOD = 7.03, $R^2 = 9.37\%$) identified in the ZHENG58 × SK RIL population. The pQTL for P8302 coincided with its local eQTL, a QTL for (Glutamine and Glutamic acid)/total amino acid levels, and with an mQTL for metabolites n0565 and n0579 from the association panel, as well as with a kernel length (KL) QTL (LOD = 3.19, $R^2 = 4.81\%$) from the ZHENG58 × SK RIL population. The P1107’s pQTL coincided with a local eQTL and a kernel thickness (KT) QTL (LOD = 3.16, $R^2 = 5.11\%$) from the BY815 × KU13 RIL population (Figure 1g). The abundance of P5211 significantly correlated with its local eQTL, which co-localized with a previously reported KW QTL (LOD = 4.28, $R^2 = 7.22\%$) from the K22 × CI7 RIL population. Taken together, these results demonstrate the power of multi-omics integration to uncover novel relationships between genetic variations and maize kernel traits.

However, the correlation among different levels is low because the flow of information from DNA to phenotype is a signal transduction and a yet unknown mechanism may regulate maize kernel size (Figure 1f).

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**Conflicts of interest**

This study did not involve human participants and/or animals. The authors have no conflict of interest to declare.

**Author contributions**

Z.F., J.T. and J.Y. designed and supervised this study. Z.F., Q.Z., J.W., R.T. and Y.L. performed the experiments. Z.F., H.L., Z.G., X.Z., J.Q. and W.L. analysed the data. Z.F. wrote the manuscript with input from all authors.

**REFERENCES**


