NEXT-GENERATION DNA SEQUENCING AND SNP GENOTYPING TECHNOLOGIES ENABLE THE RAPID DEVELOPMENT OF A MARKER-ASSISTED BREEDING PLATFORM FOR WATERMELON


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Modern watermelon (Citrullus lanatus ssp. lanatus) cultivars are products of intense breeding for increased sugar content, enlarged fruit, enhanced flavor, and decreased seed number. Domestication, selection for horticulturally important traits, and elite parent recycling have created population bottlenecks, dramatically narrowed genetic diversity among modern cultivars, and impeded forward genetic analyses and marker-assisted breeding (MAB) in elite x elite crosses. Moreover, limited genomic resources have been developed for watermelon, and previous analyses have been limited to unusual and exotic crosses. One of our goals was to develop the infrastructure needed for MAB in watermelon by targeting single nucleotide polymorphisms (SNPs) among modern cultivar alleles and developing the critical mass of SNP markers needed for genome-wide mapping and other applications in elite x elite crosses. First, several hundred simple sequence repeats (SSRs) were identified by reduced representation sequencing (RRS) of genomic DNA isolated from a single cultivar. Four hundred SSR markers were developed and screened for polymorphisms among 48 public and proprietary elite inbred lines. To thoroughly sample allelic diversity and identify common SNPs for applications in hybrid breeding programs, 18 elite inbred lines were selected for RRS using next-generation DNA sequencing technologies. Methylation-filtered genomic DNA libraries were produced and sequenced using next-generation technologies, assembled using MIRA, and mined for SNPs and other DNA polymorphisms using a custom SNP discovery pipeline. Genomic DNA sequences were produced by next-generation sequencing of RRS genomic DNA libraries, assembled using MIRA, and mined for SNPs and other DNA polymorphisms. Several thousand common SNPs were identified, filtered, and selected for validation and mapping using highly parallel SNP genotyping arrays. Three 1,536 SNP arrays were developed, yielded 3,400 validated SNPs, and enabled high-density genetic mapping of several hundred common SNPs and the assembly and orientation of complete linkage groups in several intraspecific populations. Next-generation DNA sequencing and SNP genotyping technologies mitigated long-standing technical problems and enabled the development of the infrastructure needed for the routine application of MAB approaches in elite x elite crosses in watermelon. We describe these and other MAB and genomics-assisted discovery strategies enabled by next-generation DNA sequencing and SNP genotyping technologies.