

RESEARCH PAPER

Underground heterosis for yield improvement in melon

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Abstract

Heterosis, the superiority of hybrids over their parents, is a major genetic force associated with plant fitness and crop yield enhancement. We investigated root-mediated yield heterosis in melons (*Cucumis melo*) by characterizing a common variety grafted onto 190 hybrid rootstocks, resulting from crossing 20 diverse inbreds in a diallel-mating scheme. Hybrid rootstocks improved yield by more than 40% compared with their parents, and the best hybrid yield outperformed the reference commercial variety by 65% under both optimal and minimal irrigation treatments. To characterize the genetics of underground heterosis we conducted whole genome re-sequencing of the 20 founder lines, and showed that parental genetic distance was no predictor for the level of heterosis. Through inference of the 190 hybrid genotypes from their parental genomes, followed by genome-wide association analysis, we mapped multiple quantitative trait loci for root-mediated yield. Yield enhancement of the four best-performing hybrid rootstocks was validated in multiple experiments with four different scion varieties. Our grafting approach is complementary to the common roots genetic approach that focuses mainly on variation in root system architecture, and is a step towards discovery of candidate genes involved in root function and yield enhancement.

Keywords: *Cucumis melo*, grafting, genome-wide association study (GWAS), half-diallel, heterosis, rootstock, whole-genome resequencing (WGS), yield.

Introduction

About 10 000 years have passed since humans shifted from being hunter-gatherers to establishing agricultural societies (Bellwood *et al.*, 2007). While agricultural productivity has evolved at an exponential scale, since then, human population growth and climate changes

pose substantial challenges to global food security (Godfray *et al.*, 2010; Wheeler and von Braun, 2013; Gerland *et al.*, 2014). Genetic improvement of crop plant yield is therefore more important than ever for addressing these challenges in a sustainable manner.

The challenge in genetic analysis of yield reflects the biological complexity of this trait, as yield is an outcome of the cumulative effects of multiple factors over time and across plant organs. From a genetic point of view, this complexity implies the action of multiple genes that interact with each other and with the environment, and explains the low heritability calculated for yield. Another complexity associated with the genetic architecture of yield is the prominent non-additive variance component for this trait. This deviation from additivity — also known as heterosis or hybrid vigor — is a major driver for yield improvement in crop plants (East, 1908; Shull, 1908). The impact of heterosis on agriculture is wide, and is estimated to globally cause 15–30% yield increases (Duvick, 2001). This impact is best demonstrated in corn breeding, in which a continuous linear yield improvement is ongoing for almost a century following the introduction of hybrid corn in the 1930s (Duvick, 2001; Troyer, 2006).

Empirical data in various species have shown that diverse genetic, molecular and physiological mechanisms are likely to explain heterosis, but we are still lacking a unifying theory that enables us to explain and predict heterosis of fitness-related traits, including biomass, growth rate and reproductive success (Lippman and Zamir, 2007; Chen, 2013; Birchler, 2015; Vasseur *et al.*, 2019). Several genetic hypotheses have been proposed to explain heterosis: (i) dominance: cumulative genome-wide dominance complementation that masks deleterious effects of non-shared recessive alleles; (ii) overdominance: also known as single-gene heterosis, a synergistic outperformance of heterozygous alleles at the same locus (Krieger *et al.*, 2010); (iii) pseudo-overdominance: a case of dominance that resembles overdominance because two recessive loci that complement each other are tightly-linked in repulsion (Li *et al.*, 2015); and (iv) epistasis: multi-locus inter-allelic interactions (Yu *et al.*, 1997; Li *et al.*, 2001).

Next-generation sequencing (NGS) technologies and the growing availability of whole-genome assemblies provide new tools to study heterosis. There is an ongoing effort to further explore and explain the underlying genetics and molecular basis of heterosis in model and crop plants (Huang *et al.*, 2016; Li *et al.*, 2016; Seymour *et al.*, 2016; J. Yang *et al.*, 2017; M. Yang *et al.*, 2017).

In parallel to these genetic studies on heterosis, there is a growing effort to improve plant productivity and adaptation through a partially overlooked factor, plant roots. The influence of root characteristics on whole-plant performance is shown in model and crop plants, and therefore root research is important for advancing plant biology and for the future of agriculture (Meister *et al.*, 2014; Rogers and Benfey, 2015). The challenge in root research is obvious: roots are underground and therefore less accessible for phenotypic characterization. A major part of the research is consequently directed to the development of phenotyping methods for root system architecture (RSA; Zhu *et al.*, 2011; Topp *et al.*, 2013; Rogers *et al.*, 2016). Genetic studies on roots are mostly focused on RSA variation, followed by testing the link between RSA and

whole-plant performance. Quantitative trait loci (QTL) for RSA traits were mapped in tomato (Ron *et al.*, 2013), soybean (Manavalan *et al.*, 2015), maize (Zurek *et al.*, 2015), rice (Zhao *et al.*, 2018) and other crop plants. In rice, a causative gene, *DEEPER ROOTING 1 (DRO1)*, affecting root growth angle, was cloned and shown to affect yield under drought stress (Uga *et al.*, 2013). Manifestation of heterosis in root development was also characterized in several studies in wheat (Wang *et al.*, 2006) and maize (Paschold *et al.*, 2010). However, while these studies and others are using advanced technologies to phenotype and genetically characterize RSA traits, the direct functional link to whole-plant performance remains challenging due to the inability to separate root effects from shoot effects.

Grafting is a common practice in fruit trees and several vegetable crops (mainly *Cucurbitaceae* and *Solanaceae*). The ability to separate and re-combine roots and shoots of different genotypes within or even across plant species has an increasing impact on plant research and agriculture (Gregory *et al.*, 2013; Goldschmidt, 2014; Albacete *et al.*, 2015). Grafting is an efficient tool to deliver tolerance to soil-borne pathogens or to improve abiotic stress tolerance (e.g. drought, salinity), through the use of tolerant rootstocks. It also plays an important role in physiological and developmental studies focused on signal movement across plant organs (Lifschitz *et al.*, 2006; Omid *et al.*, 2007; Shalit-Kaneh *et al.*, 2019). However, to date, the advantage of this experimental tool for genetic analyses of root function and direct effect on whole-plant performance is very limited, as reflected by the few published studies on QTL and rootstock traits (Estañ *et al.*, 2009; Gur *et al.*, 2011; Tandonnet *et al.*, 2018; Asins *et al.*, 2020).

Melon (*Cucumis melo*) is an economically important species of the *Cucurbitaceae* family. It is among the most important fleshy fruits for fresh consumption worldwide with 28 million tons produced globally in 2019 (<http://faostat3.fao.org/>). *Cucumis melo* is extremely diverse for phenotypic traits, and melons are cultivated in nearly all of the warmer regions of the world. Alongside the rich genetic resources available, the melon genome sequence was completed in 2012 (Garcia-Mas *et al.*, 2012) providing a solid anchor for advanced genomic research including recent whole-genome resequencing of more than 1000 diverse melon accessions (Zhao *et al.*, 2019).

In the current research we use grafting, which is a common commercial practice in melon and other cucurbit crops to separate root phenotypic variation from shoot effects, in order to specifically investigate, using a diverse diallel population, the mode of inheritance and impact of roots on yield variation and heterosis in melon.

Materials and methods

Plant material

Core melon panel

This research was centered on a core set of 25 diverse melon accessions (Supplementary Table S1) that were selected based on extensive

genotypic and phenotypic characterization of our broader genome-wide association study (GWAS) panel. The core set includes representatives of the two cultivated sub-species and the different horticultural groups in melon, as well as the broad phenotypic spectrum available for key traits, as described previously (Gur *et al.*, 2017). The core set was selected based on multiple criteria derived from the Gur *et al.* (2017) study: the initial tentative set ($n=40$) was constructed to represent all the different horticultural groups in the diverse collection (based on traditional classification). Phenotypic profiles were then used as the second primary factor; the preliminary core set was projected on the distribution of different traits to ensure that the phenotypic spectrum was well captured in the core panel (Gur *et al.* 2017). Following required adjustments and narrowing of the set to $n=30$, based on the first two steps, the final set was selected to meet the 25 target accessions, taking into account maximum polymorphism information content (PIC) value and uniform distribution on genetic diversity plots (PCA, Supplementary Fig. S1B). Using the same selection rationale, two smaller sets were defined within the 25 core set.

Creation of diverse, 25-way, diallel population

A multi-allelic population of 300 F_1 hybrids was built through a half-diallel crossing scheme between the 25 diverse founders (Fig. 1). Plants of the 25 parents were grown and intercrossed in the greenhouse at Newe-Ya'ar Research Center, Israel during the fall of 2017. We defined two subsets within the 25 founders set, where the smaller sets completely overlapped with the sets above them, and each corresponded to a half-diallel population specifically derived from its composition: *HDA10*–10 parental lines and 45 F_1 hybrids, and *HDA20*–20 parental lines and 190 F_1 hybrids (Supplementary Fig. S1; Table S1).

Field trials

Non-grafted yield trials

Yield trials were performed during the spring-summer seasons of 2018, 2019, and 2020, under standard growing conditions. Our main testing site was the open field at the Newe Ya'ar Research Center, Israel

(32°43'05.4"N 35°10'47.7"E). Replicated trials consisted of three plots of five plants per plot in a randomized complete block design (RCBD). The standard planting density was 0.5 m between plants in a row and 1.90 m between beds. Selective harvest was performed at maturity of each genotype by going through the field three times a week over four weeks (mid-June to mid-July). All fruits from each ripe plot were harvested. Number of fruits (FN) and total fruit weight (yield) per plot were collected at the field. Five representative ripe fruits were sampled from each plot for further analysis at the laboratory. Average fruit weight (AFW) was calculated on the sampled fruits by dividing total yield by FN as measured in the field. Concentrations of total soluble solids (TSS, measured in degrees Bx) were measured on flesh samples from each of the five fruits separately, using a hand-held refractometer (Atago Co. Ltd., Tokyo, Japan). Seeds were extracted from the sampled fruits, washed and dried, and average seed weight was calculated from a sample of 50 seeds per replicate (150 seeds per genotype).

Rootstock grafted yield trials

Each genotype (from either the *HDA10*, *HDA20*, parental lines or controls) was grafted as a rootstock with a common scion. Grafting for these large-scale experiments was performed in commercial nurseries ('Hishtil'—Ashkelon, Israel and 'Shorashim'—Ein-Habsor, Israel) under their standard grafting protocols. Briefly, rootstocks and scions were sown separately; approximately 21 days after sowing, seedlings from both rootstocks and scions were cut and grafted; plastic clips were used to attach the scion to the rootstock and promote efficient graft union development. Grafted plants were ready for transplanting in the field 7–10 d after grafting (Supplementary Fig. S2). The melon variety that was selected as the common scion for most parts of this project was 'Glory', a long shelf-life, high-yielding 'Galia'-type variety. In addition to the good field-holding capacity of the mature fruits, this variety is also characterized by uniform fruit setting; both were critical attributes for this project, in order to allow a single harvest of all yield. Each grafted entry was planted in five replicates with five plants per replicate (plot) in RCBD design. The standard planting density was 0.5

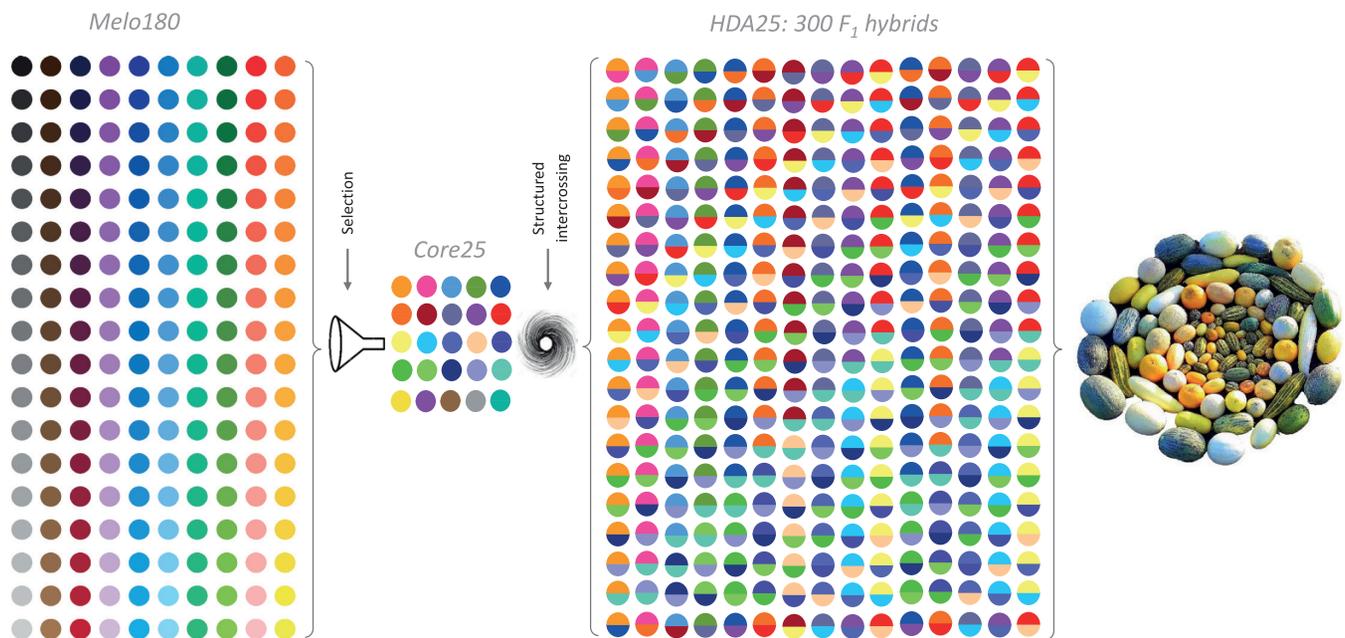


Fig. 1. The path for development of the *HDA25* population. *Melo180* is a diverse collection (Gur *et al.*, 2017). *HDA25* is half-diallel population developed from the 25 core founders. On the right are representative mature fruits from the *HDA25* population.

m between plants in a row and 1.90 m between beds. Drought stress treatment was applied by stopping the irrigation from the start of fruit setting throughout the season until the harvest. A single non-selective harvest was performed when at least ~70% of the fruits were ripe and 95% reached their maximal size. In each plot, all fruits were harvested, counted and weighed for total yield calculation. Average fruit weight (AFW) was calculated by dividing the total fruit weight by the total number of fruits (FN) per plot. A sample of three representative ripe fruits was taken from each plot for total soluble solids (TSS) measurements performed on each fruit separately. Rootstock-mediated vegetative biomass was measured on grafted plants 56 d after transplanting (at the peak of female flowering and fruit setting) when most of the measured biomass was vegetative. The whole canopy of each plant was cut above ground level and fresh weight was measured.

DNA preparation and genotyping

DNA was extracted using the GenElute™ Plant Genomic Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA). DNA quality and quantification were determined using a Nanodrop spectrophotometer ND-1000 (Nanodrop Technologies, Wilmington, DE, USA), electrophoresis on agarose gel (1.0%) and Qubit® dsDNA BR Assay Kit (Life Technologies, Eugene, OR, USA).

DNA of the 25 core accessions was shipped to the Genomic Diversity Facility at Cornell University (Ithaca, NY, USA) for whole genome resequencing (WGS). Each sample was sequenced on an Illumina HiSeq 2000/2500 platform as 150 bp paired-end reads that were mapped to the *C. melo* reference genome DHL92 v4.0 (Ruggieri *et al.*, 2018), available at <https://www.melonomics.net/melonomics.html#/download>. Single nucleotide polymorphism (SNP) calling was carried out using the Broad Institute's (USA) genome analysis toolkit (GATK ver. 3.7, McKenna *et al.*, 2010), initially creating a separate genomic variant calling file (gVCF) for each individual detailing its polymorphism versus the reference genome, and later running a SNP discovery within the population. The initial SNP set was composed of ~9 M SNPs that was filtered using TASSEL v.5.2.43 (Bradbury *et al.*, 2007) with the following criteria: (i) masking (as missing) scores with less than three reads per site, followed by the removal of sites with more than 50% missing data; and (ii) minor allele frequency (MAF) >0.1. The final SNP set consisted of 4 M SNPs. The whole-genome sequence alignment and derived HapMap from the 25 founders are now useful tools for detection of potential causative polymorphisms within candidate genes (Oren *et al.*, 2019).

Statistical analyses

JMP ver. 14.0.0 statistical package (SAS Institute, Cary, NC, USA) was used for statistical analyses. Mean comparisons were performed using the Fit Y by X function. GWA analysis was performed in TASSEL v.5.2.43 using the mixed-linear model (MLM) function. Distance matrix and Relatedness matrix of pairwise kinship (k matrix) were calculated in TASSEL from the filtered SNP dataset using the Centered_ IBS method (Endelman and Jannink, 2013). Stringent Bonferroni method was used to control for multiple comparisons in GWA. Best-parent Heterosis (BPH) was calculated as the deviation of the F₁ hybrid from its better parent (F₁-best-parent) and was expressed as absolute trait values or as Δ % from best parent.

Results

Construction of diverse diallel population in melon

A primary resource for our genetic research on melon (*Cucumis melo*) was a diverse collection, composed of hundreds

of accessions, which was built over the last 50 years in the Cucurbits Unit at Neve Ya'ar (Burger *et al.*, 2009). We recently performed a Genome-Wide Association Study (GWAS) using 180 representative accessions and through comprehensive phenotyping and whole-genome genotyping-by-sequencing (GBS)-based genotyping, demonstrated the effectiveness of this diversity panel for linkage-disequilibrium (LD) mapping of Mendelian fruit traits to candidate gene intervals (Gur *et al.*, 2017). Out of the 180 GWAS accessions, a core subset of 25 representative melon lines was selected based on integration of phenotypic and genotypic data; the core subset represents the two *Cucumis melo* sub-species and 11 horticultural groups. (Supplementary Table S1; Gur *et al.*, 2017). Through structured intercrossing of the 25 lines in all possible combinations, we developed a half-diallel population (HDA25) composed of 300 F₁ hybrids (Fig. 1). This multi-allelic structure is a suitable design to characterize the mode-of-inheritance of traits, including general and specific combining abilities (GCA and SCA) patterns, and to perform GWAS on heterotic traits, such as yield.

Above and underground yield heterosis in HDA10 population

To characterize yield variation and heterosis patterns, we first used a subset composed of 45 half-diallel F₁ hybrids derived from intercrossing 10 representative lines from our diverse collection (HDA10; Supplementary Table S1; Fig. S1). These hybrids, placed alongside their parents, were tested in an open-field replicated yield trial during the summer of 2017. Half-diallel is a balanced design that reflects the same allelic composition and proportions in the F₁ hybrids as in the set of parental lines, and therefore allows informative general comparisons between the hybrids and inbreds sets, in addition to specific comparisons within hybrid groups (i.e. triads—hybrid and its two parents). In this experiment, hybrid fruit yield was on average 73% higher compared with their parental lines (Fig. 2A). While variation in mode of inheritance of yield was observed across the 45 hybrid groups (Fig. 2B), the superiority of hybrids over their parents was prevalent, with 13 F₁ hybrids that showed significant best-parent heterosis (BPH). For example, HDA10_005 is an F₁ hybrid between a *C. callosus* line (Parent1, QME) and a *C. melo*, var *inodorous* line (Parent2, NA) that showed 90% best-parent yield heterosis (Fig. 2C).

In parallel to the conventional yield trial, we also tested whether yield variation and heterosis in melon can be derived from root effects *per se* and whether we can identify heritable variation for root-mediated effects. For this purpose, we took a grafting approach: the same germplasm set (45 HDA10 hybrids + 10 parents) were used as rootstocks grafted with a common commercial hybrid scion ('Glory', a long shelf-life 'Galia'-type hybrid). Such rootstock-grafting strategy allowed us to eliminate substantial aboveground variation across our germplasm and to perform genetic

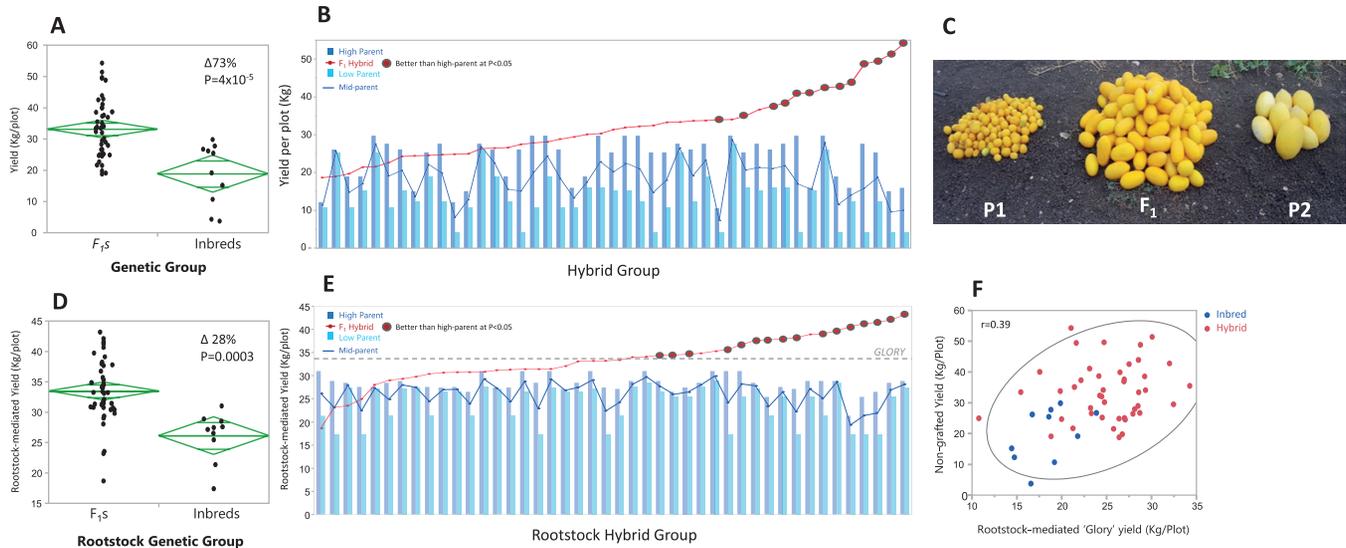


Fig. 2. Yield heterosis across *HDA10* population (45 F_1 hybrids and their 10 parental lines). (A) Yield comparison between inbreds and F_1 s. (B) Analysis of yield across 45 hybrid groups ordered in ascending manner by F_1 yield. (C) Example of heterotic hybrid (middle) alongside its parents. (D) Root-mediated yield comparison between inbreds and F_1 s. (E) Analysis of root-mediated yield across 45 hybrid groups ordered in ascending manner by F_1 yield. (F) Correlation between root-mediated yield (grafted) and yield of parallel genotypes in the non-grafted experiment, across the *HDA10* population.

analyses focusing on the exclusive effect of the underground portion (roots) on yield. ‘Glory’ grafted on itself was used as a control in this experiment. ‘Glory’ grafted with hybrid rootstocks yielded on average 28% more than parallel grafting with inbred rootstocks across the *HDA10* set (Fig. 2D). Furthermore, most hybrid rootstocks across this set mediated higher yields compared with their best-parents, and 16 hybrid rootstocks showed significant BPH ($P < 0.05$; Fig. 2E). Overall, the proportion of yield variation explained by root-mediated genetic effects (broad-sense heritability) in this experiment was 40% ($H^2=0.40$), a significant value that indicates a prominent contribution of roots to the variation in yield. Moderate correlation was calculated between the rootstock-mediated yield and yield of the parallel *HDA10* hybrids and parental lines in the non-grafted experiment ($r=0.39$, Fig. 2F), indicative of the independent aboveground variation components and the expected interactions between roots and scions.

Rootstock-mediated yield heterosis across *HDA20* population

Based on the positive results obtained on rootstock-mediated yield heterosis in the *HDA10* set, we extended the experiment and tested the wider *HDA20* set (190 half-diallel hybrids + 20 parents) as rootstocks grafted with the same common commercial hybrid, ‘Glory’, as scion. This set of 210 rootstock entries plus two controls (‘Glory’ grafted on itself and non-grafted ‘Glory’) were planted in replicated yield trials under optimal and minimal-irrigation conditions (referred to as ‘irrigated’ and ‘dry’ herein, respectively). (Supplementary Fig. S2A, B).

The dry field had on average 30% less yield than the irrigated field, and the correlation between the dry and irrigated trials was high ($R=0.71$, Supplementary Fig. S2C, D), supporting the significant genetic effect calculated for root-mediated yield variation ($H^2=0.48$). Further support for the significant genetic basis of the root effects is obtained from the correlation between the 2017 and 2018 grafted field experiments across the 55 *HDA10* genotypes (Supplementary Fig. S2E, F). Rootstock-mediated yield heterosis was apparent in both fields across the *HDA20* population, with 38% ($P=1.1 \times 10^{-8}$) and 56% ($P=1.8 \times 10^{-7}$) average yield increase of hybrids, compared with their inbred parents in the irrigated and dry fields, respectively (Fig. 3A, B).

The *HDA20* set can be viewed as 190 triads where each triad includes a hybrid and its two inbred parents; using this setup, we can define the mode of inheritance (additive and dominance components) within each triad, and draw patterns across the whole set. In this study, we used the stringent genetic definition of heterosis—the deviation of the hybrid from the high-parent (best-parent heterosis, BPH)—which is also the relevant definition from a breeding standpoint. The root-mediated yield of the 190 *HDA20* hybrids in the irrigated and dry experiments was, accordingly, partitioned to best-parent (BP) and heterotic (BPH) components (Fig. 4A, B). A prevalent root-mediated yield BPH is evident, with 130 out of the 190 hybrids in the irrigated field showing a certain level of positive over-dominance, and 79 out of them displaying significant BPH (at $P < 0.05$) and out-performing their best-parent at an average of 55% (Fig. 4A). The average BPH across all 190 hybrids was 26% ($P=4.9 \times 10^{-30}$) and 35% ($P=1.2 \times 10^{-19}$) in the irrigated and dry experiments, respectively.

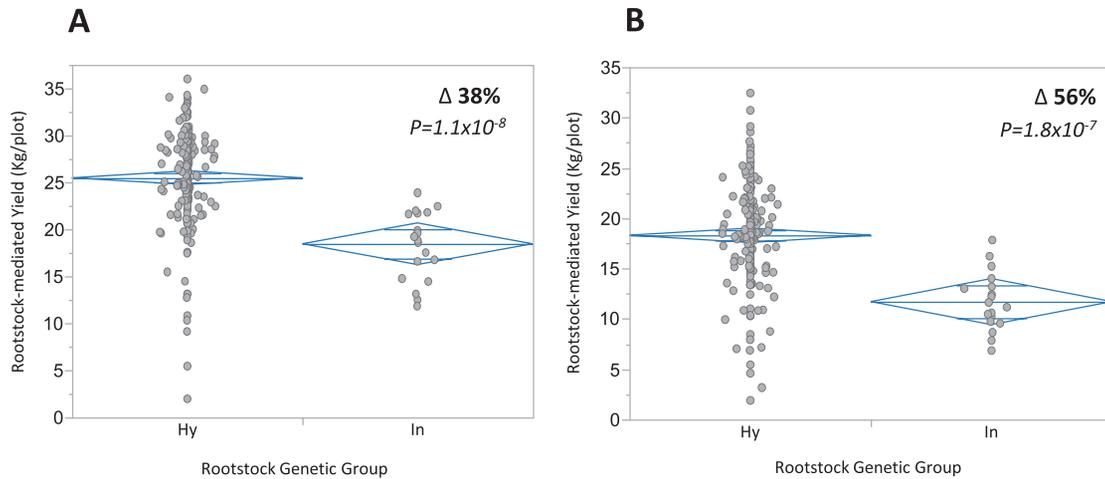


Fig. 3. Root-mediated yield comparison between F₁ hybrids and parental inbreds in the *HDA20* grafted rootstock yield trial. (A) Irrigated field. (B) Dry field.

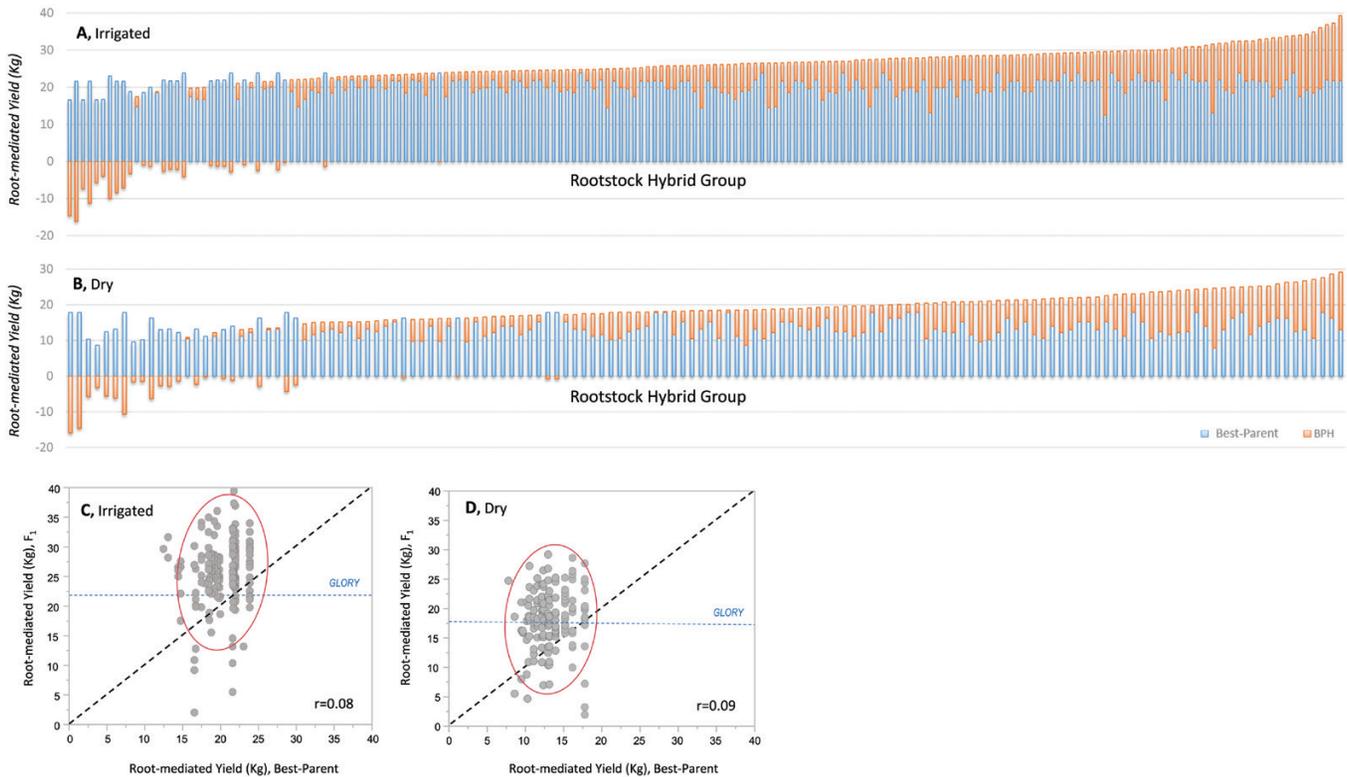


Fig. 4. Partition of yield of hybrids to parental and heterotic components. (A, B) Yield of the 190 *HDA20* hybrids in the irrigated and dry fields, presented by its components: blue bars are the best-parent (BP) yield for each hybrid group, and orange bars represent the deviation of hybrid from best-parent (best-parent heterosis; BPH). Hybrids are ordered in an ascending manner by their yield. Negative orange bars reflect hybrids that are lower than their best-parent. (C, D) Correlations between root-mediated yield of best-parent and F₁ hybrids across 190 *HDA20* triads. Dashed diagonal is $x=y$ ($BP=F_1$). Horizontal dashed blue lines are the yield of self-grafted ‘Glory’, the common scion variety.

Using the triads design, we could also test the broad relationship between parental and hybrid root-mediated yield performance across the diallel population. We show that there is no correlation between best-parents and hybrids root-mediated yield across the 190 hybrid triads in the irrigated and dry experiments ($R=0.08$ and $R=0.09$, respectively; Fig.

4C, D). This absence of correlation supports the observation that hybrid rootstock-mediated yield is independent of parental breeding value. It is apparent from these results that (over)-dominant deviation, a non-additive genetic component, is the major contributor to the root-mediated hybrid yield variation.

Mode-of-inheritance of root-mediated yield compared with other melon traits

It was previously shown that heterosis is more prevalent in fitness-related, reproductive traits (Lu *et al.*, 2003; Rocha *et al.*, 2004; Semel *et al.*, 2006; Flint-Garcia *et al.*, 2009). We therefore collected data on additional traits in a non-grafted replicated experiment of this population (*HDA20*, 210 genotypes) and compared the general mode of inheritance between the root-mediated (grafted) yield and three seed- and fruit-related traits measured on non-grafted plants: average fruit weight (AFW), average seed weight (ASW) and flesh sweetness (total soluble solids, TSS). The comparison was performed by calculating the correlations between parental means and F_1 hybrids across the 190 *HDA20* triads. While this correlation for root-mediated yield was essentially zero ($R=0.01$, Fig. 5A), for AFW, ASW, and TSS we found high positive correlations between hybrids and mid-parental performance ($R=0.83$, 0.92, and 0.80, for AFW, ASW, and TSS, respectively, Fig. 5B–D). We also show that the means of hybrids and mid-parents were not different in AFW, ASW, and TSS of non-grafted plants, compared with the 40% advantage of hybrids calculated for root-mediated yield (red triangles, Fig. 5A–D). Another visual way to demonstrate that non-additive, specific combining ability (SCA) is the prominent

variation component of root-mediated yield across the *HDA20* population, is through the comparison of duplicated heat maps of the 20×20 half-diallel matrices of root-mediated yield (Fig. 5E) and AFW (on the non-grafted experiment, Fig. 5F). In these plots, both dimensions are ordered by the average performance of each line across its hybrids (GCA) and the variation within rows or columns reflect the SCA. The uniform directional gradient apparent in AFW reflect the strong additive inheritance of this trait, while the mostly random distribution of high and low-performing hybrids in the root-mediated yield plot is indicative of non-additive inheritance. These analyses express the prominent additive component in the inheritance of the morphological and metabolic traits in melon, and demonstrate the fundamentally different mode of inheritance found for root-mediated yield.

Root-mediated effects on yield components and fruit quality traits in the *HDA20* population

To describe further the nature of root-mediated effects across the *HDA20* population, we dissected the total fruit yield to its components—number of fruits per plot (FN) and average fruit weight (AFW). FN of ‘Glory’ on a rootstock genotype-mean

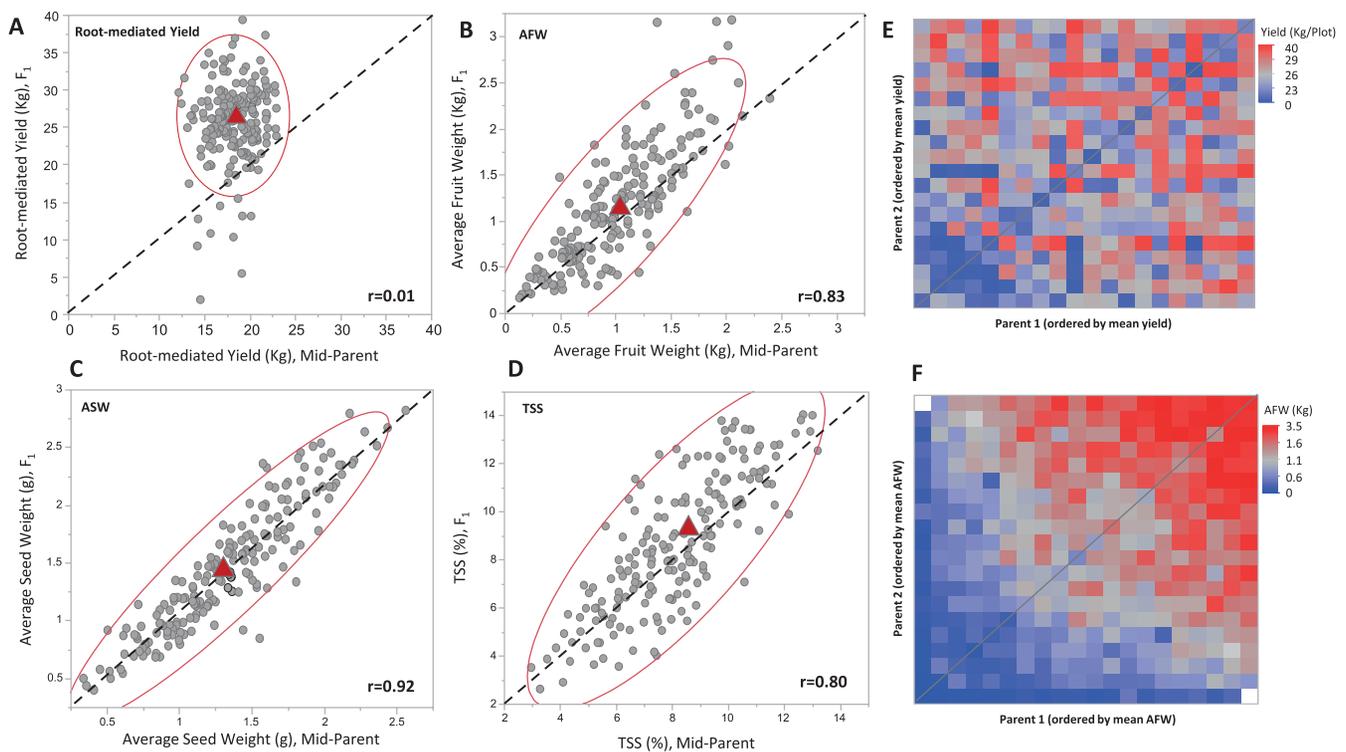


Fig. 5. Correlations between mid-parent and F_1 hybrid across 190 hybrid groups (*HDA20*). (A) Root-mediated yield (grafted). (B) Average fruit weight (AFW, non-grafted). (C) Average seed weight (ASW, non-grafted). (D) Total soluble solids (TSS, non-grafted). Red triangles represent the averages of mid-parent and F_1 s. (E, F) Duplicated heat maps of the 20×20 half-diallel matrices for root-mediated yield (E) and for AFW (F). Both axes are ordered by parental GCA. Diagonals are the performance of the parents *per se*.

basis ranged between 11 and 30 fruits per plot and AFW range was 0.70–1.20 kg per fruit. Surprisingly, both FN and AFW showed significant positive correlations with yield in the dry and irrigated experiments, and accordingly were also positively correlated with each other (Supplementary Fig. S3). This pattern of yield variation and relation between its components is in contrast to the common negative tradeoff observed between FN and AFW across natural melon diversity, as we show in our non-grafted *HDA10* population (Supplementary Fig. S4). To assess the root-mediated effects on ‘Glory’ fruit quality, we also measured total soluble solids (TSS) on 2100 fruits (10 fruits per genotype) across the grafted *HDA20* population in the irrigated experiment. TSS is highly correlated with sugar content in the fruit flesh, which is a major determinant of melon fruit quality. The effect of rootstock genotype on TSS variation was not significant ($H^2=0.07$) and accordingly, was not correlated with the wide variation and high heritability of this trait across the *HDA20* population in non-grafted plants (Supplementary Fig. S5). Taken together, we found that high-yielding rootstocks were associated with more fruits, which were also larger on average, and these effects on yield and its components were not associated with any compensatory effect on fruit sugar content.

Potential predictors of root-mediated yield heterosis

The significance of heterosis, as shown above, in explaining hybrid root-mediated yield variation in melon, provided an incentive to explore the genetic basis and underlying genes for this unique phenomenon and to develop predictive tools for effective breeding of heterotic yield-promoting rootstocks.

Root-mediated canopy biomass

We started by testing a potentially simple phenotypic predictor. Using the same common-scion grafting setup, we measured root-mediated variation in plant canopy biomass across the *HDA20* set, and tested whether it correlated with the root-mediated fruit yield variation. The rationale was that canopy vigor (biomass) is an easy-to-measure trait that can be phenotyped in a high-throughput and cost-effective manner using remote-sensing technologies. While we also found heterosis for root-mediated plant vegetative biomass (Fig. 6A), this trait was found to be a poor predictor and explained only 3% of the root-mediated yield variation (Fig. 6B).

Parental genetic distance

To test potential genetic predictors for root-mediated hybrid yield, we conducted whole-genome re-sequencing of the 25 founder lines and extracted ~4 000 000 informative SNPs that describe the genetic variation. We show that parental genetic distance, which corresponds with the level of heterozygosity in F_1 , is also a poor predictor, and explained only 8% of the root-mediated yield variation and 7% of BPH variation across the

190 *HDA20* F_1 hybrids (Fig. 6C, D). Accordingly, the type of the hybrid (*melo* and *agrestis*, inter or intra sub-specific) was also not predictive for rootstock performance. This lack of correlation between parental genetic distance or taxonomic classification and root-mediated hybrid yield suggests that yield heterosis is not confounded with relatedness or population structure, and that there is a good chance of identifying specific loci significantly associated with this trait.

Root-mediated yield QTL

To perform genome-wide association (GWA) analysis, we carried-out whole-genome resequencing of the 25 founder lines. A total of 587.6 Gb of raw sequences were produced with an average of 78.3 million reads, 23.5 Gb and 63× coverage per line (detailed statistics in Supplementary Table S2). Genome alignment resulted in ~4 000 000 informative SNPs describing the genetic variation across the core set. We then inferred the complete genotype (composed of ~4 M SNPs) for each of the 190 *HDA20* F_1 hybrids, from their 20 parental genomes. A filtered subset of 400 K uniformly spaced SNPs (at parental minor allele frequency (MAF)>0.25) was used for the GWA analyses. The complex genetic nature of root-mediated yield variation was supported by multiple significant associations that were identified across the genome (Fig. 7). In the irrigated experiment, we found significant SNPs on all chromosomes, and seven QTL (on six chromosomes) were also common to the dry experiment (Fig. 7B). Allelic effects of two QTL (q.RMY3.1 and q.RMY6.1) that were common to both environments are shown in Fig. 7C, D. Both QTL display heterotic inheritance, as the heterozygotes are associated with significant yield increase compared with homozygote genotypes in each SNP. While independently, q.RMY3.1 explained 23% and 25% of the genetic variation (dry, irrigated, respectively) and q.RMY6.1 explained 22% and 28% of the genetic variation (dry, irrigated, respectively) (Fig. 7C, D), joint haplotype of these SNPs significantly improved the model and explained 36% and 37% of the variation (dry, irrigated, respectively). F_1 hybrids that were heterozygous in both QTL were associated with higher root-mediated yield compared with those that were heterozygous at one locus or other homozygote combinations (Fig. 7E). The double heterozygous haplotype associated with 16% and 14% root-mediated yield increase over the *HDA20* population mean, in the dry and irrigated fields, respectively. This effect reflects the estimated response to selection of favorable genotypes at these loci.

Validation of selected hybrid rootstocks with multiple scions

Based on the large-scale analysis of rootstock performance under two environments, we were able to select four high-yielding hybrid rootstocks for further testing. Scion × rootstock interactions are common in grafted plants and therefore,

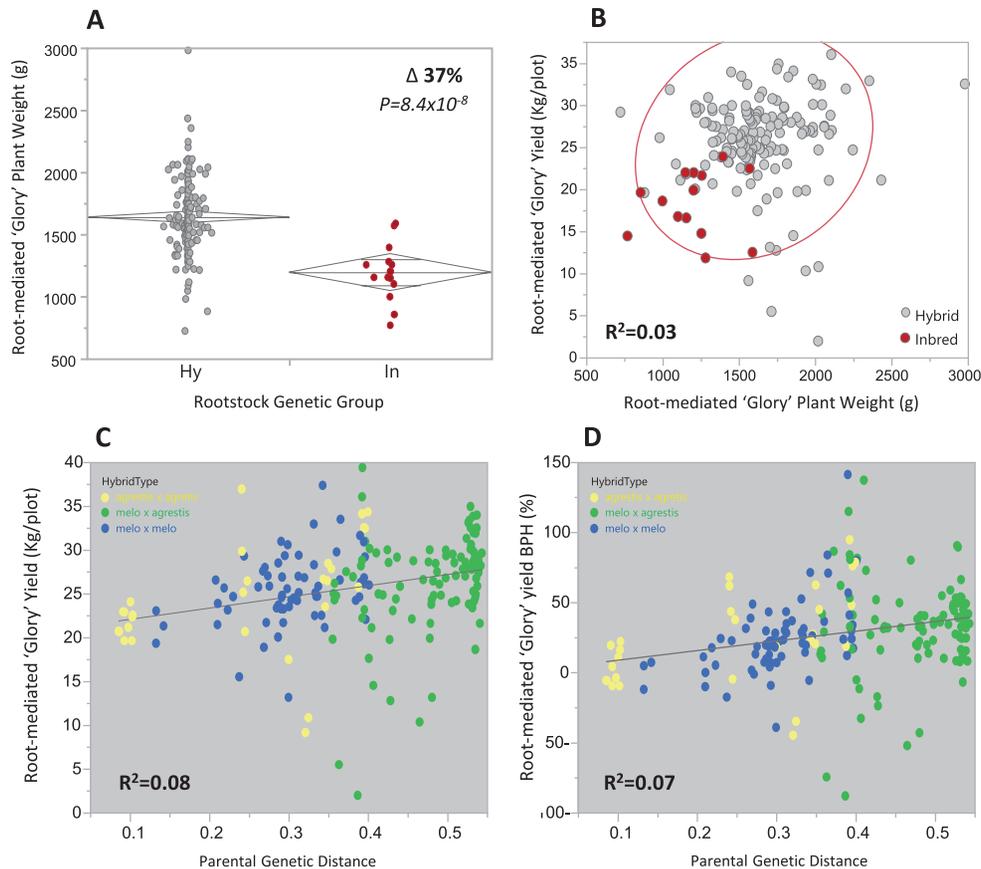


Fig. 6. Potential predictors of hybrid root-mediated yield. (A) Comparison of root-mediated young-plant vegetative biomass between *HDA20* hybrids and their inbred parents. (B) Correlation between root-mediated 'Glory' plant biomass and root-mediated 'Glory' fruit yield, across 156 hybrids + 13 inbred parents. (C) Correlation between parental genetic distance and root-mediated yield, across 190 *HDA20* hybrids. (D) Correlation between parental genetic distance and root-mediated yield BPH, across 190 *HDA20* hybrids.

we grafted the selected rootstocks with four scions that represent different melon variety types: 'Glory' – *reticulatus*, long shelf-life Galia type; 'Noy-Amid' – inodorous, yellow canary type; 'Hudson' – *reticulatus*, 'Ananas' type; and 'HDA005' – an experimental small-fruited (300 g) inter-sub-specific hybrid. The four scion varieties were used as non-grafted controls in addition to two other control rootstocks: 'Dulce' – a *reticulatus* inbred line and one of the parents in the *HDA20* set, and 'Tatsacabuto', an inter-specific *Cucurbita* hybrid rootstock used commercially in melon and watermelon fields. The results of the 28 scion × rootstock combinations from multiple field experiments, representing different locations, planting densities and irrigation regimes are summarized in Fig. 8A. Yield performance of the different combinations is presented as percentage difference from the corresponding non-grafted scion variety; in a unified analysis of this experiment, the selected hybrid rootstocks significantly increased yield ($P < 0.05$) compared with the control varieties by 11–19% (Fig. 8A, unified mean). While interactions between rootstock and scion, and between genotype and environment existed across the different combinations, we found a significant overall yield advantage ($P < 0.05$) mediated by our selected experimental

hybrid rootstocks over the commercial *Cucurbita* rootstock and the corresponding non-grafted scion varieties. We further tested two selected hybrid rootstocks the following year under two scions ('Glory and 'Noy-Amid'), in two irrigation regimes and two planting densities (Fig. 8B). The advantage of our experimental hybrids over the control rootstocks and self-grafted varieties was consistent in both scions and more prominent under standard planting density compared with wide spacing. These results that are based on yield analysis of more than 4500 grafted plants over the different experiments conducted with the selected rootstocks in both years, provide an important proof-of-concept for the potential of hybrid rootstocks as a possible alternative channel for yield improvement in melon.

Discussion

Fruit yield heterosis in melon is prevalent and controlled independently above and underground

Charles Darwin noted in 1876 that cross-pollinated F_1 hybrids are more vigorous and productive than their parents (Darwin, 1876). Hybrid vigor, later termed heterosis to discriminate it

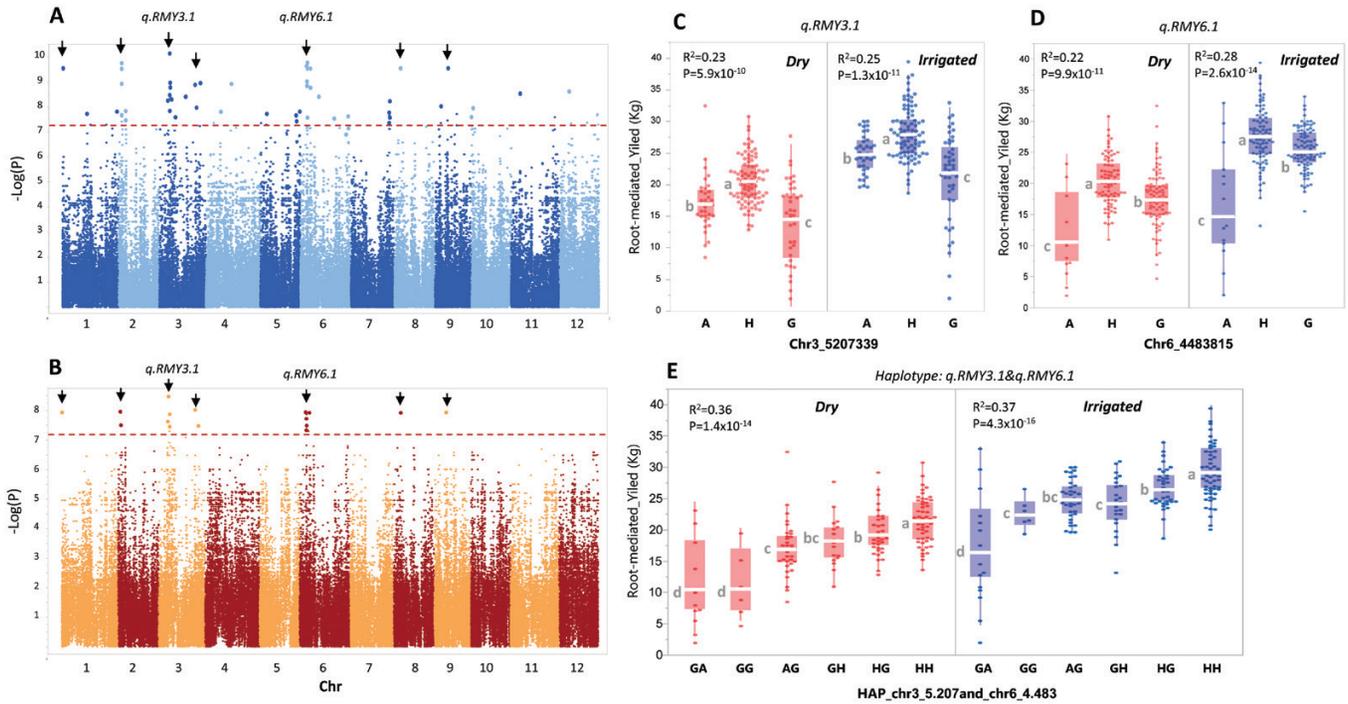


Fig. 7. GWAS of root-mediated yield across 190 *HDA20* hybrids. (A) Manhattan plot, irrigated field. (B) Manhattan plot, dry field. Arrows indicate significant SNPs that are common to the irrigated and dry experiments. (C) ANOVA for allelic effect of QTL on chromosome 3 (qRMY3.1). (D) ANOVA for allelic effect of QTL on chromosome 6 (qRMY6.1). (E) ANOVA for allelic effect of the combined haplotype of qRMY3.1 and qRMY6.1.

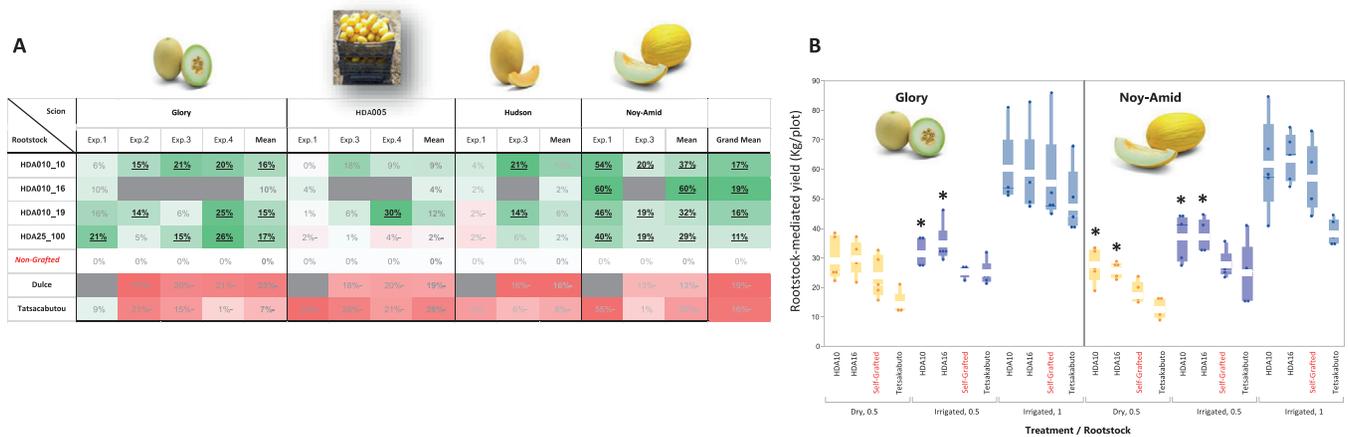


Fig. 8. Yield advantage of selected rootstocks across scions and growing conditions. (A) 2019 yield trials. Values in each cell are the average of five plots with 10 plants per plot, and are presented as $\Delta\%$ from the corresponding non-grafted variety. Significant values at $P < 0.05$ are in bold and underlined. Exp.1: Maoz-Haim, irrigated, 1.66 plants m^{-2} ; Exp.2: Newe-Ya'ar, dry, 2 plants m^{-2} ; Exp.3: Newe-Ya'ar, irrigated, 2 plants m^{-2} ; Exp.4: Newe-Ya'ar, irrigated, 1 plant m^{-2} . (B) 2020 yield trials. * indicate significant difference ($P < 0.05$) from the self-grafted controls.

from heterozygosity (Shull, 1948), is still intriguing geneticists and is commonly utilized for crop improvement (Duvick, 2001; Hochholdinger and Baldauf, 2018). While yield heterosis was extensively described in multiple plant species, so far it was investigated in a limited number of studies in melon, with variable conclusions regarding its magnitude and breeding impact (Katherine et al., 2011; Pouyesh et al., 2017; Napolitano et al., 2020).

In the current study, we initially show that as in other self and cross-pollinated crop plants, there is substantial yield heterosis also in melon. The average yield of the 45 diallel hybrids from our *HDA10* population was 73% higher compared with the average of their parents, and almost one-third of these hybrids displayed significant BPH (Fig. 2A, B). The yield heterosis was explained by combined effects on fruit number, average fruit weight and the tradeoff between them. An inherent drawback

of studying yield heterosis across such a diverse multi-parental melon population lay in the fact that the yield variation is potentially confounded by substantial variation in other morphological and developmental traits across the diversity. For example, variation in female sex expression type (monoecious or andromonoecious; Gur *et al.*, 2017), 50-fold fruit weight variation (60–3500 g; Fig. 5B) or substantial variation in earliness (85–120 days to maturity) were characterized across our population. These effects expand the overall phenotypic variation for multiplicative traits such as yield, and complicate the interpretation of genetic analyses. To dissect yield heterosis more effectively, we therefore took advantage of the fact that melon is amenable for grafting, and allows physical separation and re-assembly of root and shoot combinations. We focused our yield analysis on root-mediated effects by performing a common-scion rootstock experiment. While, as expected, the overall coefficient of variation (CV) of yield in the common-scion grafted experiment was less than a third of yield CV in the parallel non-grafted experiment (0.29 and 1.02, respectively), the broad sense heritability was very similar ($H^2 \sim 0.40$), confirming the effectiveness of this approach and the significant heritable contribution of roots to yield variation. We detected prominent yield heterosis both above (non-grafted) and underground (root-mediated), but the correlation between these setups was low (Fig. 2F), which makes sense, considering the substantial morphological and physiological aboveground variation that is only partly dependent on roots function, and the probable cross-talk between roots and shoots. The significant root-mediated effects that we describe here for yield variation and heterosis emphasize the essential, underestimated contribution of roots to the whole plant phenotype. It is important to note, however, that root-mediated effects were not common to all traits. A quantitative example for that is fruit TSS, for which we find substantial heritable variation across the 210 *HDA20* genotypes in non-grafted plants (3–16% Brix) but minor, non-significant, root-mediated effects in the common scion experiments (Supplementary Fig. S5). This indicates that fruit TSS is determined largely by above-ground (canopy) properties, including genetically controlled fruit metabolism (Burger and Schaffer, 2007).

Root-mediated yield variation is positively correlated with variation in both fruit number (FN) and average fruit weight (AFW)

Analysis of yield components across more than 7300 common-scion grafted rootstocks in the multi-allelic *HDA20* population revealed 3-fold range for FN and 1.7-fold for AFW (Supplementary Fig. S3A–D) with significant positive correlations of both traits with yield, and accordingly, positive correlation between these two components (Supplementary Fig. S3E, F). This pattern is in complete contrast to the significant negative tradeoff observed between AFW and FN across our non-grafted melon diversity, where increase in AFW is strongly

associated with decrease in FN ($R^2=0.75$, Supplementary Fig. S4). Tradeoff between yield components is a common pattern in plants (Nesbitt and Tanksley, 2001; Golan *et al.*, 2019; Gadri *et al.*, 2020) and may reflect evolution of developmental plasticity that promotes reproductive fitness stability. More generally, trade-off between size and number is common across biological systems and can be explained simply as a result of limited resources (Garland, 2014). The absence of negative tradeoff between AFW and FN in our rootstock experiments, expressed as parallel increase in both FN and AFW in high-yielding rootstocks, suggest that the rootstock variation is associated with modifications in availability of resources, or in alterations of sink-source relations in a way that is not interfering with the developmental program of the scion genotype. The morphological and physiological basis for the root-mediated effects on above-ground traits in our population are still to be studied, but these positive correlations between yield components imply that higher yield is associated with larger or more efficient root systems that most likely explore more soil and support enhanced resource availability.

Mode of inheritance of reproductive versus morphological or metabolic traits in melon

We show here that underground yield heterosis is a prominent attribute in melon (Figs 2D, E; 3) and that most of the root-mediated yield variation across 190 diverse *HDA20* hybrids can be explained by non-additive genetic components (Fig. 4). Comparisons with the mode of inheritance of AFW, ASW and TSS, measured on non-grafted plants across the same *HDA20* population (Fig. 5), indicates that heterosis in melon is more prevalent in reproductive traits compared with non-reproductive (morphological or metabolic) traits. This observation confirms the similar phenomena previously described in maize (Flint-Garcia *et al.*, 2009), tomato (Semel *et al.*, 2006) and mice (Rocha *et al.*, 2004). This fundamental difference in mode-of-inheritance between trait categories, that is consistent across diverse taxonomic groups, indicates a possible evolutionary role of this pattern. Our results expand the perspective on this, as we show here that even the exclusive effect of root variation on whole-plant performance maintains the prominent heterotic mode-of-inheritance of total fruit yield and canopy biomass across natural melon diversity.

Prediction of root-mediated yield heterosis

Heterosis, the positive deviation of hybrid from its parental mean is both a desirable and challenging genetic property for plant breeders. Predicting and maximizing the heterotic response in F_1 hybrids is a challenge, as parental performance *per se* are not necessarily informative. The development of prediction tools or breeding strategies to maximize the chances for producing successful crosses is therefore a key objective in hybrid breeding (Bernardo, 1994; Zhao *et al.*, 2015). We show

here that root-mediated yield of melon hybrids is superior, but independent of their parental performance *per se* (Figs 4, 5A), and therefore implementation of high-throughput indirect selection or prediction methods is important for efficient rootstock breeding. Root-mediated early-stage vegetative canopy biomass was not predictive as a potential indirect selection trait. Parental genetic distance was also poorly correlated with root-mediated hybrids yield. However, our GWA results (Fig. 7) indicate that QTL or genomic selection strategies can be effective for accelerating rootstock breeding. Haplotype of two QTL that were consistent across the irrigated and dry experiments, explained 36% of the root-mediated yield variation, and the favorable haplotype (heterozygote at both loci) was associated with average yield increase of 15%, compared with the *HDA20* population mean.

Supported by the multiple QTL that were identified in the current study, we suggest that root-mediated yield heterosis in melon is explained by a combination of dominant and epistatic genome-wide effects. This polygenic hypothesis, where favourable dominant QTL alleles are contributed by both parents in hybrids or segregating bi-parental populations, is the focus of ongoing work. While contribution of specific over-dominant loci cannot be excluded, it is likely that most of the root-mediated heterosis is a result of these prevalent polygenic mechanisms. Quantitative genetics approaches are therefore the right path to further dissect this trait and develop effective genomic prediction tools.

Breeding implications

World population growth and global climate change are forming major challenges to our civilization (Godfray *et al.*, 2010; Wheeler and von Braun, 2013). Agriculture, among other disciplines, plays a key role in dealing with these challenges (Garnett *et al.*, 2013) and one of the important channels of action for improving yields of crop plants in a sustainable manner is through genetic research and breeding. Heterosis is a well-established genetic mechanism for yield enhancement in crop plants. While parental genetic distance *per se* is not necessarily a robust predictor for the level of heterosis in F_1 hybrids, as shown here and by others (Huang *et al.*, 2015; M. Yang *et al.*, 2017; Kaushik *et al.*, 2018), it is a consensus that stronger heterotic effects are expected in hybrids by crossing diverse rather than closely related parents. Commercial melon breeding is commonly performed within market-defined narrow germplasm pools, which on one hand ensures strict maintenance of fruit-related varietal characteristics, but on the other hand inhibits the ability to perform wide crosses and explore the full potential of heterosis for productivity traits. By focusing our yield enhancement research effort on rootstocks, we essentially bypassed this barrier, as the above and underground genetic actions are performed independently. We show here that melon hybrid rootstocks significantly outperform inbreds and that selected melon hybrids, used as

rootstocks grafted with a commercial melon variety, increase yield across scions and environments without any visible negative effect on fruit quality. The ability to implement focused and autonomous breeding for rootstocks to efficiently introduce beneficial genetic properties to roots in species amenable for grafting, is a powerful, currently underutilized approach to improve crop performance under optimal and stress conditions. Mapping root-mediated heterotic yield QTL in a multi-allelic population is a first step towards focused QTL analysis in bi-parental populations and development of marker-assisted selection protocols. Using hybrid breeding methodologies, rootstock breeding can be an effective alternative channel for development of stress-tolerant and high-yielding varieties in crop species that are suitable for grafting, such as *Cucurbitaceae* and *Solanaceae*.

Complementary scheme in root genetics

Root biology is receiving increased attention in recent years as a potential channel to improve plant productivity under optimal and stress conditions. However, most of the genetic research in model and crop plants is taking an inherent approach with initial focus on analysis of root development and variation in root system architecture (Bray and Topp, 2018; Zhao *et al.*, 2018; Jia *et al.*, 2019; Wachsmann *et al.*, 2020), rather than direct analysis of root functional variation. Here, we propose a complementary scheme; using grafting, we directly characterize variation in root function and its effect on whole plant performance in the field to study the genetics of root-mediated yield variation. The combination of a crop plant amenable for grafting, such as melon, with rich genetic and genomic resources, is a powerful platform for applied root genomics and for exploring the interactions between roots and shoots. We therefore believe that a forward genetics approach is a first step towards discovery of candidate genes involved in root function, that show proven effects on yield. The current research expands the view on genetic properties of heterosis in plants by highlighting the contribution of roots to yield heterosis.

Supplementary data

The following supplementary data are available at [JXB online](#).

Table S1. List of 25 founder lines that compose the melon core subset.

Table S2. Properties of whole-genome resequencing of the 25 core accessions.

Fig. S1. Structure of the core subsets and *HDA* populations.

Fig. S2. Description of *HDA20* grafted-rootstock yield trials in summer 2018 (irrigated and dry).

Fig. S3. Correlations between root-mediated yield and its components—number of fruits per plant (FN) and average fruit weight (AFW), in the *HDA20* population in the irrigated and dry fields.

Fig. S4. Correlation between average fruit weight (AFW) and fruit number (FN) across 45 *HDA10* F₁ hybrids and their 10 parents.

Fig. S5. Correlation for TSS between the rootstock-mediated 'Glory' and non-grafted experiments across the *HDA20* population.

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Author contributions

AG conceived the research plan; AG and AD designed the experiments; AD, JB, and AG developed plant genetic materials; AD, IH, EO, GT, AM, TI, and AG performed the experiments and collected the data; AG and AD analyzed the results; AAS, YT, and ESB provided genomic support; AG wrote the manuscript. All authors discussed the results and approved the manuscript.

Data availability

The data supporting the findings of this study are available within the paper and within its supplementary data published online. All raw sequences from WGS of the 25 melon accessions are openly available in NCBI BioProject PRJNA726743 at https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA726743&o=acc_%3Aa.

References

- Albacete A, Martínez-Andújar C, Martínez-Pérez A, Thompson AJ, Dodd IC, Pérez-Alfocea F.** 2015. Unravelling rootstock×scion interactions to improve food security. *Journal of Experimental Botany* **66**, 2211–2226.
- Asins MJ, Raga MV, Torrent D, Roca D, Carbonell EA.** 2020. QTL and candidate gene analyses of rootstock-mediated tomato fruit yield and quality traits under low iron stress. *Euphytica* **216**, 1–19.
- Bellwood P, Gamble C, Le Blanc SA, Pluciennik M, Richards M, Terrell JE.** 2007. First farmers: the origins of agricultural societies. *Cambridge Archaeological Journal* **17**, 87–109.
- Bernardo R.** 1994. Prediction of maize single-cross performance using RFLPs and information from related hybrids. *Crop Science* **34**, 20–25.
- Birchler JA.** 2015. Heterosis: the genetic basis of hybrid vigour. *Nature Plants* **1**, 15020.
- Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES.** 2007. TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* **23**, 2633–2635.
- Bray AL, Topp CN.** 2018. The quantitative genetic control of root architecture in maize. *Plant & Cell Physiology* **59**, 1919–1930.
- Burger Y, Paris HS, Cohen R, Katzir N, Tadmor Y, Lewinsohn E, Schaffer AA.** 2009. Genetic diversity of *Cucumis melo*. *Horticultural Reviews* **36**, 165–198.
- Burger Y, Schaffer AA.** 2007. The contribution of sucrose metabolism enzymes to sucrose accumulation in *Cucumis melo*. *Journal of the American Society for Horticultural Science* **132**, 704–712.
- Chen ZJ.** 2013. Genomic and epigenetic insights into the molecular bases of heterosis. *Nature Reviews. Genetics* **14**, 471–482.
- Darwin C.** 1876. *The effects of cross and self fertilisation in the vegetable kingdom*. London: Murray.
- Duvick DN.** 2001. Biotechnology in the 1930s: the development of hybrid maize. *Nature Reviews. Genetics* **2**, 69–74.
- East EM.** 1908. Inbreeding in corn. In *Reports of the Connecticut Agricultural Experiment Station* 419–428.
- Endelman JB, Jannink JL.** 2013. Shrinkage estimation of the realized relationship matrix. *G3* **2**, 1405–1413.
- Estañ MT, Villalta I, Bolarín MC, Carbonell EA, Asins MJ.** 2009. Identification of fruit yield loci controlling the salt tolerance conferred by solanum rootstocks. *Theoretical and Applied Genetics* **118**, 305–312.
- Flint-Garcia SA, Buckler ES, Tiffin P, Ersoz E, Springer NM.** 2009. Heterosis is prevalent for multiple traits in diverse maize germplasm. *PLoS One* **4**, e7433.
- Gadri Y, Eshed Williams L, Peleg Z.** 2020. Tradeoffs between yield components promote crop stability in sesame. *Plant Science* **295**, 110105.
- Garcia-Mas J, Benjak A, Sanseverino W, et al.** 2012. The genome of melon (*Cucumis melo* L.). *Proceedings of the National Academy of Sciences, USA* **109**, 11872–11877.
- Garland T Jr.** 2014. Trade-offs. *Current Biology* **24**, R60–R61.
- Garnett T, Appleby MC, Balmford A, et al.** 2013. Sustainable intensification in agriculture: premises and policies. *Science* **341**, 33–34.
- Gerland P, Raftery AE, Sevčíková H, et al.** 2014. World population stabilization unlikely this century. *Science* **346**, 234–237.
- Godfray HC, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C.** 2010. Food security: the challenge of feeding 9 billion people. *Science* **327**, 812–818.
- Golan G, Ayalon I, Perry A, Zimran G, Ade-Ajayi T, Mosquna A, Distelfeld A, Peleg Z.** 2019. GNI-A1 mediates trade-off between grain number and grain weight in tetraploid wheat. *Theoretical and Applied Genetics* **132**, 2353–2365.
- Goldschmidt EE.** 2014. Plant grafting: new mechanisms, evolutionary implications. *Frontiers in Plant Science* **5**, 727.
- Gregory PJ, Atkinson CJ, Bengough AG, Else MA, Fernández-Fernández F, Harrison RJ, Schmidt S.** 2013. Contributions of roots and rootstocks to sustainable, intensified crop production. *Journal of Experimental Botany* **64**, 1209–1222.
- Gur A, Semel Y, Osorio S, et al.** 2011. Yield quantitative trait loci from wild tomato are predominately expressed by the shoot. *Theoretical and Applied Genetics* **122**, 405–420.
- Gur A, Tzuri G, Meir A, Sa'ar U, Portnoy V, Katzir N, Schaffer AA, Li L, Burger J, Tadmor Y.** 2017. Genome-wide linkage-disequilibrium mapping to the candidate gene level in melon (*Cucumis melo*). *Scientific Reports* **7**, 9770.
- Hochholdinger F, Baldauf JA.** 2018. Heterosis in plants. *Current Biology* **28**, R1089–R1092.
- Huang X, Yang S, Gong J, et al.** 2015. Genomic analysis of hybrid rice varieties reveals numerous superior alleles that contribute to heterosis. *Nature Communications* **6**, 6258.
- Huang X, Yang S, Gong J, et al.** 2016. Genomic architecture of heterosis for yield traits in rice. *Nature* **537**, 629–633.
- Jia Z, Liu Y, Gruber BD, Neumann K, Kilian B, Graner A, von Wirén N.** 2019. Genetic dissection of root system architectural traits in spring barley. *Frontiers in Plant Science* **10**, 1–14.

- Katherine A, Barros DA, Henrique G, Nunes DS, De Queiróz MA, Welk E, Pereira L.** 2011. Diallel analysis of yield and quality traits of melon fruits. *Crop Breeding and Applied Biotechnology* **11**, 313–319.
- Kaushik P, Plazas M, Prohens J, Vilanova S, Gramazio P.** 2018. Diallel genetic analysis for multiple traits in eggplant and assessment of genetic distances for predicting hybrids performance. *PLoS One* **13**, e0199943.
- Krieger U, Lippman ZB, Zamir D.** 2010. The flowering gene *SINGLE FLOWER TRUSS* drives heterosis for yield in tomato. *Nature Genetics* **42**, 459–463.
- Li D, Huang Z, Song S, et al.** 2016. Integrated analysis of phenome, genome, and transcriptome of hybrid rice uncovered multiple heterosis-related loci for yield increase. *Proceedings of the National Academy of Sciences, USA* **113**, E6026–E6035.
- Li X, Li X, Fridman E, Tesso TT, Yu J.** 2015. Dissecting repulsion linkage in the dwarfing gene *Dw3* region for sorghum plant height provides insights into heterosis. *Proceedings of the National Academy of Sciences, USA* **112**, 11823–11828.
- Li ZK, Luo LJ, Mei HW, et al.** 2001. Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. *Genetics* **158**, 1737–1753.
- Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, Amsellem Z, Alvarez JP, Eshed Y.** 2006. The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. *Proceedings of the National Academy of Sciences, USA* **103**, 6398–6403.
- Lippman ZB, Zamir D.** 2007. Heterosis: revisiting the magic. *Trends in Genetics* **23**, 60–66.
- Lu H, Romero-Severson J, Bernardo R.** 2003. Genetic basis of heterosis explored by simple sequence repeat markers in a random-mated maize population. *Theoretical and Applied Genetics* **107**, 494–502.
- Manavalan LP, Prince SJ, Musket TA, et al.** 2015. Identification of novel QTL governing root architectural traits in an interspecific soybean population. *PLoS One* **10**, e0120490.
- McKenna A, Hanna M, Banks E, et al.** 2010. The genome analysis toolkit: a mapreduce framework for analyzing next-generation dna sequencing data. *Genome Res* **20**, 1297–1303.
- Meister R, Rajani MS, Ruzicka D, Schachtman DP.** 2014. Challenges of modifying root traits in crops for agriculture. *Trends in Plant Science* **19**, 779–788.
- Napolitano M, Krishnamurthy S, Albertini E.** 2020. Exploring heterosis in melon (*Cucumis melo* L.) hybrids. *Plants* **9**, 282.
- Nesbitt TC, Tanksley SD.** 2001. *fw2.2* directly affects the size of developing tomato fruit, with secondary effects on fruit number and photosynthate distribution. *Plant Physiology* **127**, 575–583.
- Omid A, Keilin T, Glass A, Leshkowitz D, Wolf S.** 2007. Characterization of phloem-sap transcription profile in melon plants. *Journal of Experimental Botany* **58**, 3645–3656.
- Oren E, Tzuri G, Vexler L, et al.** 2019. The multi-allelic *APRR2* gene is associated with fruit pigment accumulation in melon and watermelon. *Journal of Experimental Botany* **70**, 3781–3794.
- Paschold A, Marcon C, Hoecker N, Hochholdinger F.** 2010. Molecular dissection of heterosis manifestation during early maize root development. *Theoretical and Applied Genetics* **120**, 383–388.
- Pouyesh A, Lotfi M, Ramshini H, Karami E, Shamsitabar A, Armiyoun E.** 2017. Genetic analysis of yield and fruit traits in cantaloupe cultivars. *Plant Breeding* **136**, 569–577.
- Rocha JL, Eisen EJ, Siewerdt F, Van Vleck LD, Pomp D.** 2004. A large-sample QTL study in mice: III. Reproduction. *Mammalian Genome* **15**, 878–886.
- Rogers ED, Benfey PN.** 2015. Regulation of plant root system architecture: implications for crop advancement. *Current Opinion in Biotechnology* **32**, 93–98.
- Rogers ED, Monaenkova D, Mijar M, Nori A, Goldman DI, Benfey PN.** 2016. X-ray computed tomography reveals the response of root system architecture to soil texture. *Plant Physiology* **171**, 2028–2040.
- Ron M, Dorrity MW, de Lucas M, Toal T, Hernandez RI, Little SA, Maloof JN, Kliebenstein DJ, Brady SM.** 2013. Identification of novel loci regulating interspecific variation in root morphology and cellular development in tomato. *Plant Physiology* **162**, 755–768.
- Ruggieri V, Alexiou KG, Morata J, et al.** 2018. An improved assembly and annotation of the melon (*Cucumis melo* L.) reference genome. *Scientific Reports* **8**, 8088.
- Semel Y, Nissenbaum J, Menda N, Zinder M, Krieger U, Issman N, Pleban T, Lippman Z, Gur A, Zamir D.** 2006. Overdominant quantitative trait loci for yield and fitness in tomato. *Proceedings of the National Academy of Sciences, USA* **103**, 12981–12986.
- Seymour DK, Chae E, Grimm DG, Martín Pizarro C, Habring-Müller A, Vasseur F, Rakitsch B, Borgwardt KM, Koenig D, Weigel D.** 2016. Genetic architecture of nonadditive inheritance in *Arabidopsis thaliana* hybrids. *Proceedings of the National Academy of Sciences, USA* **113**, E7317–E7326.
- Shalit-Kaneh A, Eviatar-Ribak T, Horev G, Suss N, Aloni R, Eshed Y, Lifschitz E.** 2019. The flowering hormone florigen accelerates secondary cell wall biogenesis to harmonize vascular maturation with reproductive development. *Proceedings of the National Academy of Sciences, USA* **116**, 16127–16136.
- Shull GH.** 1908. The composition of a field of maize. *Journal of Heredity* **4**, 296–301.
- Shull GH.** 1948. What is “heterosis”? *Genetics* **33**, 439–446.
- Tandonnet JP, Marguerit E, Cookson SJ, Ollat N.** 2018. Genetic architecture of aerial and root traits in field-grown grafted grapevines is largely independent. *Theoretical and Applied Genetics* **131**, 903–915.
- Topp CN, Iyer-Pascuzzi AS, Anderson JT, et al.** 2013. 3D phenotyping and quantitative trait locus mapping identify core regions of the rice genome controlling root architecture. *Proceedings of the National Academy of Sciences, USA* **110**, E1695–E1704.
- Troyer AF.** 2006. Adaptedness and heterosis in corn and mule hybrids. *Crop Science* **46**, 528–543.
- Uga Y, Sugimoto K, Ogawa S, et al.** 2013. Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions. *Nature Genetics* **45**, 1097–1102.
- Vasseur F, Fouqueau L, De Vienne D, Nidelet T.** 2019. Non-linear phenotypic variation uncovers the emergence of heterosis in *Arabidopsis thaliana*. *PLoS Genetics* **17**, e3000214.
- Wachsman G, Zhang J, Moreno-Risueno MA, Anderson CT, Benfey PN.** 2020. Cell wall remodeling and vesicle trafficking mediate the root clock in *Arabidopsis*. *Science* **370**, 819–823.
- Wang Z, Ni Z, Wu H, Nie X, Sun Q.** 2006. Heterosis in root development and differential gene expression between hybrids and their parental inbreds in wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* **113**, 1283–1294.
- Wheeler T, von Braun J.** 2013. Climate change impacts on global food security. *Science* **341**, 508–513.
- Yang J, Mezouk S, Baumgarten A, Buckler ES, Guill KE, McMullen MD, Mumm RH, Ross-Ibarra J.** 2017. Incomplete dominance of deleterious alleles contributes substantially to trait variation and heterosis in maize. *PLoS Genetics* **13**, e1007019.
- Yang M, Wang X, Ren D, Huang H, Xu M, He G, Deng XW.** 2017. Genomic architecture of biomass heterosis in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **114**, 8101–8106.
- Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, Saghai Maroof MA.** 1997. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proceedings of the National Academy of Sciences, USA* **94**, 9226–9231.
- Zhao G, Lian Q, Zhang Z, et al.** 2019. A comprehensive genome variation map of melon identifies multiple domestication events and loci influencing agronomic traits. *Nature Genetics* **51**, 1607–1615.
- Zhao Y, Mette MF, Reif JC.** 2015. Genomic selection in hybrid breeding. *Plant Breeding* **134**, 1–10.
- Zhao Y, Zhang H, Xu J, et al.** 2018. Loci and natural alleles underlying robust roots and adaptive domestication of upland ecotype rice in aerobic conditions. *PLoS Genetics* **14**, e1007521.
- Zhu J, Ingram PA, Benfey PN, Elich T.** 2011. From lab to field, new approaches to phenotyping root system architecture. *Current Opinion in Plant Biology* **14**, 310–317.
- Zurek PR, Topp CN, Benfey PN.** 2015. Quantitative trait locus mapping reveals regions of the maize genome controlling root system architecture. *Plant Physiology* **167**, 1487–1496.