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ASSOCIATION BETWEEN SRAP-BASED GENETIC DISTANCE AND HETEROSIS FOR FRUIT WEIGHT AND HARVEST TIME IN TOMATO

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SUMMARY

Accurate heterosis prediction is essential for tomato (*Solanum lycopersicum* L.) hybrid breeding. This study evaluated heterotic effects and parental genetic distance (GD) using open reading frames (ORF)-targeted sequence-related amplified polymorphism (SRAP) markers, assessing their correlation with agronomic traits. Six parental genotypes' crossing used a North Carolina II design, with F1 hybrids evaluated in a randomized complete block design. Hybrid H_2 exhibited the highest heterosis for fruit weight, achieving 181% mid-parent heterosis (MPH) and 173.3% best-parent heterosis (BPH). Maximum GD (Nei = 0.183) occurred between P1 and P5. Notably, GD correlated significantly and positively with fruit weight per plant (MPH: $r = 0.713$; BPH: $r = 0.742$) but negatively with harvest time (MPH: $r = -0.733$; BPH: $r = -0.742$). These findings suggest that SRAP-derived GD is a reliable predictor for fruit weight and harvesting time in tomato breeding.

Keywords: Tomato (*S. lycopersicum* L.), heterosis, genetic distance, correlation, SRAP, yield-related traits

Key findings: This study indicates that using SRAP-based genetic distance can help choose parents to create tomato (*S. lycopersicum* L.) hybrid with high productivity and early maturity.

INTRODUCTION

Conventional breeding is often slow and resource-intensive, challenging the need for rapid food production (Abu and Yimer, 2023). In tomato (*Solanum lycopersicum* L.), hybrid development is the primary strategy, yet traditional evaluation of many physical traits across generations remains costly and labor-intensive (Moraes *et al.*, 2023). Heterosis—the

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superior performance of F1 hybrids over their parents—is largely an outcome of heterozygosity from crossing genetically distant cultivars (Begna, 2021; Wu *et al.*, 2021; Kozlova *et al.*, 2024).

Genetic distance (GD), estimated using molecular markers, serves as a predictive tool for heterosis by quantifying allelic divergence (Marcón *et al.*, 2019). Studies in tobacco, rice, and other crops show that hybrid performance generally increases with GD up to an optimal threshold (Tomkowiak *et al.*, 2020; Sreewongchai *et al.*, 2021; Kınay *et al.*, 2024). Among markers, sequence-related amplified polymorphism (SRAP) is a polymerase chain reaction (PCR)-based dominant marker widely used due to its simplicity, cost-effectiveness, high reproducibility, and ability to amplify large genomic fragments.

Even though SRAP is a dominant marker with difficulty finding heterozygous loci, it can focus on open reading frames (ORFs). The ORF allows it to cover a wide range of the genome, making it useful for studying genetic diversity in different plant species. SRAP markers capture functional genetic variations linked to phenotypic traits (Li and Quiros, 2001; Yi *et al.*, 2021).

Despite its potential, the correlation between GD and heterosis remains inconsistent across crops, including rapeseed, wheat, and cotton (Tian *et al.*, 2017; Nie *et al.*, 2019; Geng *et al.*, 2021). In tomatoes, previous studies using randomly amplified polymorphic DNA (RAPD) markers yielded inconclusive results (El-Aziz *et al.*, 2016), and research utilizing SRAP markers remains limited. This study aimed to clarify the relationship between SRAP-derived GD and heterotic effects on yield-related traits in tomatoes.

MATERIALS AND METHODS

Genetic material and procedure

The study began from July to November 2022 in Getasan, Semarang Regency, Central Java, Indonesia, during the dry season, and at the Laboratory of Genetics and Plant Breeding, Faculty of Agriculture, Gadjah Mada University. The plant material included six tomato (*S. lycopersicum* L.) parental lines from inbred lines. This comprised three female lines (MA131-22-2-1/P1, MA131-6-3/P2, and GM2/P3) and three male genotypes (MA131-6-1-1/P4, MA131-6-2-1/P5, and LA175-1-2/P6), along with their nine F1 hybrids developed using the North Carolina II mating design. The parental line coded MA 131 resulted from crosses involving the same parents but exhibiting different characteristics.

Cultivation procedures included plot preparation, crop management, and plant protection. Plots (6 m × 1 m; 30–40 cm high) preparation consisted of 60-cm spacing between plots covered with black-silver plastic mulch and perforated at 50 cm × 50 cm spacing. Basal fertilization during plot preparation used chicken manure (1.5 t ha^{-1}) and NPK fertilizer (100 kg ha^{-1}). Conducting pest and disease control occurred twice weekly using fungicides (mancozeb and dimethomorph) and insecticide (profenofos) for caterpillars and whiteflies (*Bemisia tabaci*). Weed control proceeded mechanically and chemically (glyphosate). Supplemental NPK application was weekly by drenching, adjusted from vegetative (0.4:0.4:0.3 g $plant^{-1}$) to generative stages (0.4:0.6:0.6 g $plant^{-1}$).

The hybrid tomatoes bore designations as H1 (P1×P4), H2 (P1×P5), H3 (P1×P6), H4 (P2×P4), H5 (P2×P5), H6 (P2×P6), H7 (P3×P4), H8 (P3×P5), and H9 (P3×P6). The morphological and yield-related assessments ensued on parental genotypes and hybrids. The data recorded on traits included leaf length (LL, cm), leaf width (LW, cm), first branch height (FBH, cm), stem diameter (SD, mm), plant height (PH, cm), flowering time (FT, days), harvest time (HT, days), and fruit set (FS, %). Other traits recorded were the number of flowers (NFL), number of fruits (NFR), fruit length (FL, cm) by a digital caliper, fruit diameter (FD, cm), individual fruit weight (FW, g), and fruit weight per plant (FWP, g). Measuring fruit flesh thickness (FFT, mm), fruit firmness (FF, N) by penetrometer with a 5-mm probe, and total soluble solids (TSS, brix), as determined using a refractometer, succeeded.

Table 1. Primers used in SRAP analysis.

	Forward primer sequence (5′ – 3′)		Reverse primer sequence (5′ – 3′)
Me1	TGAGTCCAAACCGGATA	Em2	GACTGCGTACGAATTTGC
Me2	TGAGTCCAAACCGGAGC	Em3	GACTGCGTACGAATTGAC
Me3	TGAGTCCAAACCGGAAT	Em4	GACTGCGTACGAATTTGA
Me5	GACTGCGTACGAATTAAC	Em5	GACTGCGTACGAATTAAC
Me6	TGAGTCCAAACCGGTAG	Em6	GACTGCGTACGAATTGCA
		Em7	GACTGCGTACGAATTCAA
		Em8	TGAGTCCAAACCGGACT
		Em9	GACTGCGTACGAATTCAG
		Em10	GACTGCGTACGAATTCAT

The experiment followed a randomized complete block design with three replications. Each block included 16 plots (nine hybrids, six parents, and one check variety), with 24 plants per plot. Three representative plants per genotype entailed sampling for data collection.

DNA extraction

Genomic DNA extraction came from fresh young leaves of parental lines using a modified CTAB protocol (Doyle and Doyle, 1990), supplemented with 2% β-mercaptoethanol and 2% polyvinylpyrrolidone (PVP). The DNA concentration and purity (A260/280 ratio; range 1.8 to 2.0) assessment used a BioDrop Duo+UV/Vis spectrophotometer. For PCR amplification, the DNA stock solutions received dilution to a final concentration of 10 ng·µL^{-1}.

SRAP marker analysis

Nineteen SRAP primer combinations (Table 1) reached analysis with 12.5 µL reaction mixture containing 6.25 µL PowerPol2× PCR Mix (ABclonal), 3.25 µL nuclease-free water, 0.25 µL forward primer, 0.25 µL reverse primer, and 2.5 µL DNA template. Performing amplification continued in a T100™ thermal cycler (Bio-Rad, USA) following the procedure of Li and Quiros (2001). The initial denaturation was at 94 °C for 5 min, followed by five cycles of 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 1 min. Subsequently, 35 cycles took place at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 8 min. The PCR products underwent separation on 1.5% agarose gels (100 V, 400 mA for 75 min) in 1x TBE buffer before visualization under UV illumination.

Data analysis

Heterosis calculation

Mid-parent heterosis (MPH) and best-parent heterosis (BPH) calculations were as follows:

MPH = (F1−MP)/MP×100%; BPH = (F1−BP)/BP×100%, where F1 is the average of the hybrid, MP = (PA + PB)/2 is the average of the two parents, PA and PB are the values of each parent, and BP is the value of the best parent (Geng *et al.*, 2021).

Genetic analysis

SRAP fragments scoring as binary data were 1 = presence and 0 = absence. Polymorphic information content (PIC) determination used the iMEC software (Amiryousefi *et al.*, 2018). Genetic distance among the parental genotypes succeeded in their estimation using Nei's genetic distance. Pearson's correlation coefficients between GD and heterosis received analysis using R-Studio version 4.3.2.

Nei's genetic distance (D) = $-ln\,(I)$

Where I represent Nei's genetic identity, defined as

$$I = \frac{\Sigma\ xi.yi}{\sqrt{(\Sigma xi^{\,2})\,(\Sigma yi^{2})}}$$

Where xi = frequency of the *i*-th allele in the genotype X, and yi = frequency of the *i*-th allele in the genotype Y (Nei, 1972).

Pearson's correlation coefficient (r) underwent calculations for n pairs of observations using the standard formula (Profillidis and Botzoris, 2019):

$$r_{XY} = \frac{n \cdot \sum_{i=1}^{n} x_i \cdot y_i - \left(\sum_{i=1}^{n} x_i\right) \cdot \left(\sum_{i=1}^{n} y_i\right)}{\sqrt{n \cdot \sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2} \cdot \sqrt{n \cdot \sum_{i=1}^{n} y_i^2 - \left(\sum_{i=1}^{n} y_i\right)^2}}$$

Where xi and yi represent the individual data points for variables X and Y, respectively.

RESULTS AND DISCUSSION

SRAP profile

Nineteen SRAP primer combinations produced 319 loci, with 84.34% (272 loci) polymorphism in tomato (*S. lycopersicum* L.) populations (Table 2). The average loci per combination (16.8) was higher than as reported by Emsaed *et al.* (2024) (9.67) and Alzahib *et al.* (2021) (7.86). Although Al-Shaye *et al.* (2018) observed more average loci (27.4), the polymorphism rate in this study (84.34%) exceeded their 64.74%. This higher polymorphism likely resulted from optimized PCR cycles and electrophoresis settings, which enhanced band separation. Variations in amplification sustained influences from species, genetic background, and the number and primer combinations selected.

The Me5 × Em9 combination was most effective, producing 27 fully polymorphic bands (100–2600 bp), indicating it was excellent at identifying genetic differences among tomato genotypes. Conversely, the Me2 × Em2 showed a lower efficiency, with only 11 loci (100–1750 bp) and 54.55% polymorphism, reflecting lower amplification efficiency and limited discriminatory power. In contrast, Emsaed *et al.* (2024) found 100% polymorphism for the Me2 × Em2 in different hybrids, suggesting that cultivar types significantly influence locus number and polymorphism. The average PIC value (0.383) shows the amplified loci.

Table 2. SRAP primer profile.

Primer combination	TNB	NPB	% polymorphism	Size (bp)	PIC
Me1xEm2	20	15	75	140-1725	0.431
Me1xEm3	16	15	93.75	100-2000	0.385
Me1xEm4	13	13	100	150-1500	0.375
Me1xEm8	17	16	94.12	100-2000	0.377
Me1xEm9	14	9	64.29	120-2100	0.375
Me1xEm10	20	17	85	100-3000	0.412
Me2xEm2	11	6	54.55	100-1750	0.375
Me2xEm3	18	14	77.78	130-1600	0.378
Me2xEm4	19	17	89.47	100-2600	0.384
Me2xEm10	16	13	81.25	100-1500	0.378
Me3xEm5	17	16	94.12	100-1800	0.375
Me3xEm6	15	13	86.67	180-1800	0.390
Me5xEm2	17	13	76.47	150-2625	0.379
Me5xEm4	14	12	85.71	150-1800	0.375
Me5xEm6	16	13	81.25	175-1500	0.377
Me5xEm7	19	16	84.21	120-1900	0.380
Me5xEm9	27	27	100	100-2600	0.378
Me6xEm3	13	11	84.62	180-1600	0.375
Me6xEm4	17	16	94.12	180-2900	0.378
Mean	16.789	14.316	84.336		0.383

Note: TNB: Total number of bands, NPB: Number of polymorphic bands, PIC: polymorphic information content.

Table 3. Genetic distance estimation among the parental genotypes using SRAP markers.

♂ / ♀	GD max			GD avg		
	P4	P5	P6	P4	P5	P6
P1	0.173	0.183	0.133	0.137	0.127	0.090
P2	0.141	0.164	0.150	0.084	0.085	0.105
P3	0.150	0.163	0.118	0.131	0.129	0.099

Note: GD max: maximum genetic distance, GD avg: average genetic distance.

Such discrepancies in the number of loci and the polymorphism percentage could refer to differences in the cultivar types and plant material used. The SRAP primers function by randomly pairing forward and reverse sequences that target different regions of ORFs in *Brassica* genotypes (Li and Quiros, 2001). The forward primer typically anneals to G–C-rich coding sequences, whereas the reverse primer anneals to A–T-rich promoters, introns, and spacers, thereby providing broad genomic coverage (Li and Quiros, 2001; Yi *et al.*, 2021). By comparing with other marker systems (RAPD, ISSR, SSR, and AFLP), the SRAP has been evidently more effective in distinguishing closely related genotypes because of its specificity in targeting ORF regions in *Zea mays* inbred lines (El-Azeem *et al.*, 2015).

Genetic distance in the parental genotypes

According to the results, the greatest GD was evident between parental genotypes P1 and P5 (GD = 0.183), followed by P1 and P4 (0.173) (Table 3). Hybridization between parental genotypes with greater genetic divergence tended to be most effective and advantageous in hybrid breeding, as it often results in hybrids with higher yield potential, improved disease resistance, and enhanced adaptability (Marcón *et al.*, 2019; Tomkowiak *et al.*, 2020). In the presented study, GD determination depended on the highest values of the coefficient matrix between two parental lines. Since heterosis over mid- and better parents stems from genetic variation, these divergent parental lines could produce significant heterotic effects, similar to findings in rice and other crops (Sreewongchai *et al.*, 2021; Wu *et al.*, 2021). Accordingly, the crosses between parental P1×P4 and P1×P5 represent the most promising combinations for developing superior tomato hybrids.

Heterosis in F1 hybrids

The F1 hybrids exhibited wide variability in heterotic performance, with mid-parent heterosis (MPH) ranging from -44.75% to 181.99% and best-parent heterosis (BPH) from -50.62% to 173.03% (Tables 4 and 5). Significant heterotic effects appeared across morphological, yield, and quality traits, offering considerable potential for selecting superior tomato combinations. For fruit weight per plant, several hybrids substantially surpassed their best parents, while others performed below the parental average, highlighting the complex nature of trait inheritance in this population. This wide variation in hybrids for MPH and BPH highlights their significant heterotic effects within the population, offering considerable potential for selecting superior hybrids.

Following the classification by Bennett *et al.* (2014), which categorizes heterosis as low (0%), medium (5%–10%), and high (>15%), hybrids H1, H2, H3, and H8 displayed low to medium heterosis (0.41%–9.11%) for leaf length (LL) and width (LW). In hybrid tomatoes, the hybrids generally showed a decrease in first branch height (FBH) and an increase in total plant height (PH) relative to the parental genotypes. The standard deviation for these traits remained comparable to parental values, indicating that variability did not expand disproportionately in the hybrids. Furthermore, hybrids demonstrated improved performance in flowering time (FT) and harvest time (HT), indicating a trend toward earliness.

Most hybrids showed consistently high MPH and BPH values (10.25%–181.99%) for yield-related traits, particularly fruit weight per

Table 4. Mid-parent and best-parent heterotic effects for observed characters.

Hybrids		LL	LW	FBH	PH	SD	FWP	FT	HT	FS	NFL	NFR
H1	MPH	7.83±3.06	7.30±4.89	-11.67±1.71	4.88±6.33	-4.08±1.06	163.64±5.89	-9.03±2.72	-3.80±0.33	41.79±7.80	-4.10±0.47	15.64±1.00
	BPH	0.41±7.49	0.65±5.96	-13.71±3.15	1.93±5.41	-8.45±1.54	158.84±5.25	-12.08±2.69	-3.96±0.58	38.27±8.91	-8.35±0.80	12.82±1.53
H2	MPH	4.90±3.48	8.03±3.65	-20.08±3.18	8.06±7.25	-4.09±1.05	181.99±55.4	-13.04±2.23	-4.59±0.33	38.17±9.78	-0.62±0.62	18.84±1.26
	BPH	0.31±4.84	2.48±5.14	-23.8±5.31	1.26±7.01	-10.50±1.53	173.03±51.87	-13.33±2.11	-5.21±0.33	23.61±14.15	-5.56±0.95	12.33±1.75
H3	MPH	7.96±2.72	3.63±2.11	-8.84±4.17	2.27±5.36	-1.11±1.05	91.43±18.8	-15.23±2.27	-3.80±0.53	51.69±11.16	2.54±0.46	19.07±1.19
	BPH	5.81±4.37	1.73±4.20	-20.37±4.65	2.18±4.71	-9.47±1.54	70.13±15.65	-16.34±3.48	-3.96±0.58	50.77±14.68	-6.20±0.54	13.79±1.65
H4	MPH	1.07±1.69	-1.59±1.47	-13.45±2.14	-7.36±3.88	-10.69±1.21	39.33 ±19.69	1.05±2.65	-1.30±0.53	10.62±7.63	-4.46±0.81	-0.37±1.02
	BPH	-4.50±0.66	-6.17±0.81	-13.75±2.22	-10.26±2.25	-15.28±1.36	30.88±13.87	-2.03±2.36	-2.88±0.96	2.70±12.12	-8.69±0.90	-8.74±1.45
H5	MPH	0.77±1.96	4.00±2.82	-6.52±2.90	4.91±5.59	-3.62±1.28	112.34±20.13	-7.38±3.53	-3.07±1.81	16.47±16.82	10.36±1.06	9.05±1.77
	BPH	-2.19±1.85	0.31±2.81	-8.51±5.08	-2.00±5.35	-3.87±1.42	102.17±11.86	-8.00±3.51	-3.85±1.92	14.69±19.79	4.88±1.18	8.07±1.98
H6	MPH	-7.02±0.99	-4.93±0.97	-12.19±3.92	-5.36±4.66	-2.10±0.77	133.49±19.83	0.33±3.17	-3.58±1.80	19.06±10.89	14.24±0.87	15.79±1.37
	BPH	-7.45±0.99	-5.05±0.98	-21.47±4.32	-5.75±3.99	-10.89±1.01	123.94±16.05	-1.31±4.18	-5.13±1.94	7.41±14.04	4.50±0.76	13.45±1.66
H7	MPH	3.51±2.72	7.33±2.88	3.55±5.01	4.32±8.03	-2.60±1.60	78.55±78.38	0.70±3.95	-1.64±1.46	-5.44±10.47	1.45±0.80	6.77±1.18
	BPH	-0.54±2.22	2.46±2.61	-1.96±5.69	0.89±10	-3.47±1.58	69.25±73.12	-2.04±3.65	-2.29±1.45	-10.64±9.49	-16.35±1.03	-10.78±1.61
H8	MPH	6.01±1.69	9.11±1.58	6.11±6.48	6.17±5.17	-1.76±0.55	14.74±22.25	4.38±0.97	-2.12±1.74	0.32±8.79	-8.92±0.61	-13.25±0.88
	BPH	4.68±1.53	5.38±1.57	-1.86±7.77	5.71±4.73	-9.61±0.48	10.25 ±14.33	3.33±1.05	-2.28±1.76	-3.03±14.90	-25.25±0.95	-31.96±1.68
H9	MPH	-2.69±1.28	2.13±1.22	3.22±2.93	0.94±8.22	-1.53±1.57	-2.59±16.84	0.00±2.38	3.29±1.40	16.20±14.86	1.36±0.18	3.99±1.11
	BPH	-3.93±1.36	2.13±1.24	-12.27±3.58	-4.96±9.50	-11.08±1.56	-7.43±12.08	-1.96±3.65	2.61±1.33	6.65±13.82	-19.38±0.35	-17.76±1.73

Note: LL = leaf length, LW = leaf width, FBH = first branch height, SD = stem diameter, PH = plant height, FWP = fruit weight per plant, FT = flowering time, HT = harvest time, FS = fruit set, NFL = number of flowers, and NFR = number of fruits.

plant, except for hybrid H9. Reproductive traits also displayed positive responses; specifically, H5 and H6 recorded positive MPH and BPH for fruit set (FS), the number of flowers (NFL), and the number of fruits (NFR). Interestingly, while hybrids H1, H2, and H3 expressed no heterosis for flower number, they exhibited positive heterosis for fruit set and fruit number, suggesting higher reproductive efficiency.

In contrast to yield traits, negative heterosis was prevalent for fruit quality parameters, including fruit length, diameter, firmness, and individual fruit weight (Table 5). However, specific combinations like H2, H3, and H5 showed positive values for total soluble solids (TSS), and H1 through H4 exhibited positive heterosis for fruit flesh thickness (FFT). The highest overall response was notable for fruit weight per plant, with mean MPH and BPH values of 90.32% and 81.23%, respectively. According to Aisyah *et al.* (2016), heterotic effects >20% in yield traits of self-pollinated crops like tomatoes justify their advancement into hybrid cultivars. This gained support from Yu *et al.* (2021) and Tamta and Singh (2017), who emphasized that fruit weight and fruit number are the primary determinants of productivity in heterosis breeding.

Hybrids H1 (P1×P4), H2 (P1×P5), and H3 (P1×P6) emerged as the most promising, expressing moderate to high heterosis for most observed traits. These three hybrids share the common female parent P1, underscoring its value as a promising parent for future breeding. These results align with the high genetic distances (GD) observed (P1×P5 = 0.183; P1×P4 = 0.173), confirming that parental divergence is a reliable predictor of hybrid vigor, as demonstrated in rice by Zhang *et al.* (2010). Genetic distance between parental genotypes attained wide acknowledgment as a principal factor influencing heterosis, as increased genetic divergence frequently results in enhanced hybrid performance.

Table 5. Mid-parent and best-parent heterotic effects for fruit quality traits.

Hybrids		FL	FD	FW	FFT	FF	TSS
H1	MPH	0.70±0.95	2.59±0.63	11.14±11.41	21.44±0.45	-21.50±1.95	3.11±0.10
	BPH	-1.02±0.62	-14.03±0.79	-23.96±13.81	16.56±0.48	-33.62±2.96	-2.98±0.11
H2	MPH	-8.61±2.14	1.10±2.67	27.15±24.82	13.71±0.19	-32.16±1.35	8.85±0.23
	BPH	-14.35±2.46	-16.17±2.83	-7.67±25.27	6.80±0.19	-45.53±2.12	4.53±0.23
H3	MPH	-4.37±1.97	1.99±1.48	22.32±14.62	10.24±0.22	-13.04±1.31	4.08±0.11
	BPH	-5.42±1.91	-8.17±1.71	-1.60±18.69	6.39±0.20	-21.34±2.01	2.69±0.14
H4	MPH	-6.15±0.84	-7.13±1.09	-44.75±7.86	2.33±0.22	-6.04±2.62	-0.94±0.13
	BPH	-7.44±0.74	-14.68±1.00	-44.79±11.02	0.92±0.29	-14.86±3.49	-1.00±0.16
H5	MPH	-8.08±1.04	-1.46±2.44	-11.86±8.92	-8.21±0.24	-8.49±5.21	2.33±0.21
	BPH	-13.58±1.71	-10.55±2.54	-20.00±9.67	-15.97±0.23	-21.68±5.50	0.24±0.23
H6	MPH	1.91±1.23	5.85±1.44	4.21±11.11	12.34±0.17	6.89±3.43	-6.87±0.09
	BPH	1.14±1.29	5.42±1.55	-16.33±9.99	5.58±0.14	4.26±3.80	-13.43±0.11
H7	MPH	-6.37±1.79	-3.08±2.95	-26.89±12.70	-10.23±0.14	-26.18±1.41	-6.33±0.03
	BPH	-10.37±1.82	-6.32±3.01	-29.50±15.13	-15.48±0.14	-44.80±0.46	-13.94±0.03
H8	MPH	-13.74±1.22	-9.20±0.59	-24.11±3.50	-3.16±0.93	-31.14±2.93	-0.45±0.16
	BPH	-21.16±1.89	-13.33±1.14	-28.79±1.52	-7.27±0.93	-50.62±3.41	-6.71±0.15
H9	MPH	-5.00±4.10	7.18±3.75	15.46±24.10	-14.81±0.36	-31.91±1.40	-0.16±0.12
	BPH	-8.50±4.15	12.82±3.71	-4.67±23.09	-16.13±0.36	-46.22±1.03	-1.36±0.11

Note: FL = fruit length, FD = fruit diameter, FW = individual fruit weight, FFT = fruit flesh thickness, FF = fruit firmness, and TSS = total soluble solids.

Although positive heterosis is sought for yield, negative heterosis is often desirable for traits where a reduction is beneficial, such as shorter harvest cycles or disease susceptibility. Labroo *et al.* (2021) emphasized that negative heterosis provides a significant advantage for early-maturing cultivars, with the same also observed in this study for the traits of flowering time and harvest time. These results were also greatly analogous to the findings of Tamta and Singh (2017), who reported that heterosis in hybrids for days to first harvest can surpass the parental genotype performance in tomatoes.

Gene interaction and allelic variations manage the crop’s hybrid performance. Within breeding populations, both additive and non-additive genetic effects contribute to trait expression, with their relative importance based on the specific trait and the genotypes’ genetic background. For example, in tomatoes, the hybrids exhibited substantial heterosis for traits such as total soluble solids, plant height at flowering, and the number of flowers per cluster, driven primarily by non-additive effects, although additive effects also play a significant role (Birchler *et al.*, 2014). Moreover, Paril *et al.* (2023) highlighted that heterosis arises from both genetic and epigenetic mechanisms, most of which remain only partially understood. Furthermore, they observed the epistatic effects in hybrids on grain yield. This epistasis operates during post-transcriptional modifications, such as RNA methylation. Genes associated with heterosis exhibit phenotypic plasticity. This plasticity provides evidence of the genetic contribution to hybrid stability across different environmental conditions. Although heterosis is a polygenic trait, a single gene can still produce a heterosis effect.

Correlation between genetic distance and MPH/BPH

The maximum genetic distance (GD) showed a positive correlation with fruit weight per plant (FWP) (r = 0.713 for MPH; r = 0.742 for BPH), indicating that crosses between more divergent parental genotypes generally produce greater heterosis over mid- and better parents. This relationship emphasizes the prospect of using GD to enhance the hybrid vigor for fruit weight, a key trait influencing fruit yield and market value. This aligns with observations in eggplant and other crops (Rajan *et al.*, 2023; Würschum *et al.*, 2023), where yield and heterosis gave a positive association with GD up to an optimal threshold. Conversely, harvest time (HT) showed a significant negative correlation with both MPH (r = -0.733) and BPH (r = -0.685). This suggests that greater genetic divergence signified an association with earlier maturation, a highly beneficial attribute for breeding programs targeting early-harvesting cultivars.

The emergence of these correlations is rooted in molecular mechanisms. Although SRAP markers target loci across the genome, their predictive strength in this study likely stems from sampling functional genomic regions that control the measured traits. Yield-related traits are polygenic, with linkage to the epistasis hypothesis, where non-additive interactions between alleles across multiple loci generate heterosis. During meiosis, recombination facilitates the combination of favorable alleles, ideally resulting in an optimal hybrid phenotype. High GD often reflects differences in coding sequences, regulatory open reading frames (ORFs), and allelic variants, which alter gene expression timing and protein activity. ORFs are critical as they regulate developmental timing, including flowering initiation, hormone signaling, and fruit senescence (Paril *et al.*, 2023; Shiraki *et al.*, 2023). Therefore, the variation detected by SRAP markers in these functional regions reflects biological variation directly influencing heterotic effects.

Past investigations into the GD-heterosis link have yielded mixed results. As noted by Zhang *et al.* (2010), while numerous genetic differences exist between parents, not all show linkage to yield-related traits. In this study, although SRAP markers are dominant, their focus on ORF regions provides a more reliable tool for assessing specific agronomic traits than strictly random markers. SRAP markers used in this study are dominant, revealing their measurement by visible PCR bands that show dominant alleles in crops. As a result, SRAP markers were non-specific to particular traits but remained as a reliable and practical tool for assessing particular traits.

However, weak correlations observed for other traits are consistent with findings in cotton, where GD was not a consistent predictor. These inconsistencies may arise from limited genome coverage, epistatic interactions among quantitative trait loci (QTL), the absence of linkage between markers and the trait-regulating genes, or environmental influences (El-Refaee *et al.*, 2016; Geng *et al.*, 2021). Furthermore, the predictive value of GD derived from molecular markers can vary depending on its formulation, the type of markers used, the reproductive system of the crop, and target traits. Overall, while GD provides a valuable preliminary approach for identifying promising parental combinations, its predictive power remains trait- and context-dependent. Integrating complementary marker systems and genomic tools will be essential for more precise heterosis prediction in future tomato breeding efforts.

CONCLUSIONS

Tomato (*S. lycopersicum* L.) hybrids H1, H2, H3, and H6 demonstrated moderate to high heterotic improvements in key agronomic traits, including fruit weight, fruit set, and flesh thickness. The maximum genetic distance (GD), identified using SRAP markers between parental genotypes P1 (MA131-22-2-1) and P5 (MA131-6-2-1), aligned with the significant heterotic expression observed in hybrid H2. Notably, GD exhibited a strong positive correlation with fruit weight per plant and a significant negative correlation with harvest time. These findings indicate that SRAP-derived genetic distance is a valuable tool for predicting hybrid vigor, particularly for yield and earliness in tomato breeding. However, its predictive reliability across different breeding contexts requires further validation. Future integration with high-throughput markers, such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs), may enhance the precision of heterosis prediction.

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