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Generation Mean Analysis for Resistance to Gummy Stem Blight (GSB) Disease in Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]

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Abstract

Gummy stem blight (GSB), caused by the fungal pathogen *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*), has emerged as a critical biotic constraint in watermelon cultivation, leading to significant yield reductions across major watermelon producing regions. To address this challenge, a six-generation mean analysis study was conducted to identify the genetics of Gummy Stem Blight (GSB) resistance in watermelon which is critical initial step to identify breeding strategies. Findings from joint scaling test revealed that the additive-dominance model alone was insufficient to explain resistance inheritance. This highlights the complex epistasis role of gene interactions in shaping GSB resistance in watermelon. The Perfect Fit Solution analysis revealed substantial contributions from additive [d], dominance [h], additive × additive epistatic [i] and dominance × dominance epistatic effects in governing resistance to GSB disease in crosses C1, C2 and C3. The negative values observed for additive [d], dominance [h] and additive × additive [i] effects suggested a genetic inclination toward the reducer parent, correlating with improved disease resistance. This directional shift underscores the role of specific gene interactions in enhancing resistance traits against GSB. The significance of additive [d], additive × additive [i] and additive × dominance [j] effects indicate that resistance could be genetically fixed and efficiently leveraged in elite breeding lines. Furthermore, moderate to high narrow-sense heritability in these crosses pointed to the predominance of additive genetic variance, emphasizing the central contribution of additive gene action to disease resistance. The significant additive effects and high additive variance and moderate to high narrow-sense heritability indicate that practicing early-generation selection would be effective in capturing and fixing GSB resistance in segregating populations. Collectively, this genetic framework highlights how targeted selection approaches, informed by additive and epistatic interactions, could refine resistance in future breeding lines, advancing strategies to combat GSB in watermelon crops.

Keywords : Generation mean analysis, GSB disease, Gene effects, Epistasis, Additive genetic variance

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai], a globally important crop in cucurbit agriculture, is renowned for its nutrient-dense composition rich in lycopene, citrulline and vitamins A and C. These compounds are associated with antioxidant properties, cardiovascular health promotion and anti-inflammatory effects, as evidenced by recent studies (Meghwar *et al*., 2024). Valued both agriculturally and nutritionally, watermelon underscores its dual role in supporting food systems and human wellness (Ashoka *et al.,* 2021). India cultivates watermelon across 125,450 hectares, producing 3.59 million metric tons annually at an average yield of 28 metric tons/ha which is lower

than the global average of 33.28 metric tons/ha (Anonymous, 2024). In India, Karnataka distinguishes itself as a leading producer, yielding 177,870 metric tons annually from 4,470 hectares (39.78 metric tons/ ha), a success attributed to introduction of new high yielding varieties suitable for cultivation in different agro climatic niches and precision farming methods (Soni *et al*., 2024). Despite these advancements, watermelon is susceptible to various biotic challenges. Pathogen attacks are a major barrier to achieving higher yields, often resulting in severe damage and in some cases, complete economic losses. Diseases such as Downy mildew, Powdery mildew, cucumber mosaic virus, bud necrosis virus, Bacterial fruit blotch, Anthracnose and Fusarium wilt all contribute to these losses. In recent years, Gummy Stem Blight (GSB) has emerged as a particularly devastating disease, especially in humid tropical and subtropical regions, where it continues to inflict considerable economic damage (Mahapatra *et al.,* 2022). Gummy Stem Blight (GSB) caused by the fungal pathogen *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*), poses significant risks to crop productivity and quality. This disease undermines yield potential, highlighting the urgent need for integrated disease management strategies in watermelon cultivation.

This GSB pathogen thrives well with temperatures ranging from 16-28°C and high humidity (>85%), exploiting prolonged leaf wetness to initiate infection. Surviving on infested crop debris, volunteer plants, susceptible weeds and infected seeds, the fungus disseminates *via* wind-borne ascospores for long-distance spread or splashing water (rain/overhead irrigation) for short-distance transmission. Early infection manifests as necrotic lesions on leaves and stems, followed by systemic invasion of the vascular system. The pathogen obstructs xylem vessels, triggering sudden wilting, rapid desiccation and plant death culminating in yield losses of 50-70 per cent under conducive conditions and it can reach up to 100 per cent (Keinath, 2011 and Mahapatra *et al*., 2022). Its polycyclic nature and resilience exacerbate existing yield gaps, particularly in regions like India, where productivity already lags global standards (Soni *et al*., 2024). Addressing gummy stem blight (GSB) through integrated strategies is essential to safeguard both the nutritional and economic value of watermelon, while promoting sustainability in cucurbit agriculture. Among these strategies, resistance breeding stands out as an environmentally friendly and cost-effective method for managing GSB. However, the successful implementation of this approach requires a thorough understanding of the inheritance pattern of resistance, which is crucial for designing an effective and targeted breeding program.

Early genetic studies of inheritance of gummy stem blight resistance in watermelon proposed a simplified model of monogenic control, with Norton (1979) linking resistance in the wild accession PI 189225 to a single recessive gene (*db*). Segregation patterns observed in F_2 and back cross populations aligned with Mendelian ratios, reinforcing the hypothesis of a simple genetic basis for the trait. However, subsequent research unveiled contradictions. Gusmini *et al.* (2017) observed complex ratios in crosses involving resistant PI lines of watermelon resistant to GSB, suggesting polygenic or epistatic interactions. Genomic advances further complicated the narrative when Ren *et al.* (2020) and Lee *et al.* (2021) identified major QTLs (*Qgsb8.1*, *qSB8.1*) on chromosome 8, flanked by minor loci, implicating a polygenic framework of GSB resistance. The monogenic simplicity versus polygenic complexity reflect the influence of genetic backgrounds, environmental plasticity and pathogen strain diversity, highlighting the need for identification of inheritance pattern of GSB resistance with different genetic background.

Generation mean analysis (GMA), a classical biometrical approach, offers a robust framework to unravel gene action by partitioning genetic variance into additive (*d*), dominance (*h*) and epistatic components (*i, j, l*) (Mather & Jinks, 1982; Falconer & Mackay, 1996 and Gawai *et al.,* 2024). Generation mean analysis (GMA) quantifies gene interactions

based on phenotypic data derived from six generations: P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2. This method has resolved ambiguities in diverse crops, from dry bean (Jung *et al.,* 2003) to cucumber (Amand and Wehner, 2001), where epistasis and environmental noise obscured inheritance patterns.

Genetic architecture and mode of inheritance of gummy stem blight resistance can be unravelled using first and second-degree statistics. First degree statistics, which account for additive, dominance and digenic interaction effects, may underestimate gene effects due to the mutual cancellation of positive and negative additive effects and ambi directional dominance (Jayasekara & Jinks 1976 and Jinks 1981). Similarly, second degree statistics can also under estimate effects when individual gene contributions are very low (<1.0). Using both statistical approaches helps distinguish whether under estimation is due to internal cancellation of large or small effect ambi directional dominant genes or simply smaller gene effects (Kearsey and Pooni, 1996). Most researchers tend to use either one or the other method, rarely both. Hence, the objective of this study is to decipher the mode of inheritance of gummy stem blight resistance in watermelon using generation mean analysis, based on a six-generation means from four watermelon populations. By analyzing six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2), we quantified the relative roles of additive, dominance and epistatic effects, providing a foundation for strategic resistance breeding. This work not only addresses a critical production constraint but also revitalizes classical biometrical methods in an era dominated by genomic tools, emphasizing their enduring relevance in resolving complex agricultural challenges.

Material and Methods

Generation and Evaluation of Genetic Materials

The development of planting materials commenced during the *kharif* 2023 season at the Pathology Farm, Noble Seeds Pvt. Ltd., Doddaballapura, Karnataka which is situated at 13° 29'N latitude and 77° 24'E longitude. Selective crosses were attempted between the resistant parent CL 0732 and susceptible lines CL 0695, CL 0699, CL 0033 and CL 0413 under protected conditions. To ensure genetic fidelity, designated male and female flowers were bagged 24 hours prior to anthesis, followed by hand pollination the following morning using pollen collected from the specified male parents. The resultant F_1 hybrids were grown in *rabi* 2023 alongside parental lines and subjected to molecular screening with the KASP marker *GSB_ClCG07* to validate gummy stem blight resistance introgression. Confirmed F_1 hybrids were subsequently self-pollinated to generate F_2 populations and simultaneously backcrossed with both the parental lines (P_1 and P_2) to produce BC_1P_1 and BC_1P_2 generations. Adequate seed of each parent and the F_1 seed was also reserved for evaluation alongside the F_2 and backcross generations.

The field screening for the generation mean analysis was conducted during early summer 2024 in a sick plot (a field purposely inoculated with the GSB pathogen to ensure consistent disease pressure) at the research facility of Noble Seeds Pvt. Ltd., Thondebhavi, Chikkaballapura District, Karnataka (13° 49'N, 77° 52'E). The experiment consisted of six generations, namely P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 of four cross combinations. Seeds of each genotype were raised in nursery with all plant protection measures. Twenty days old seedlings were transplanted to experimental field with a spacing of 1.5m between rows and 0.45m between plants. The gummy stem blight disease data was recorded from parental genotypes, F_1s, backcross generations and F_2 populations by employing a standardised rating scale of 0 (resistant) to 9 (highly susceptible) (Table 1) (Gusmini *et al*., 2005).

Statistical Analysis

The mean and variance of GSB incidence were computed for each generation using individual plant-level data, employing established statistical methods outlined by Singh and Chaudhary (2004).

$$\bar{X} = \frac{\sum X_i}{n}$$

Table 1

Disease assessment scale for testing resistance to gummy stem blight in watermelon given by Gusmini *et al*. (2005)

Rating	Description of symptoms
0	No symptoms
1	Yellowing on leaves
2	Moderate symptoms on leaves only
3	Slight symptoms on leaves only
4	Severe symptoms on leaves
5	Some leaves dead
6	Moderate symptoms on leaves with necrosis also on petioles and stem
7	Slight symptoms on leaves with necrosis also on petioles and stem
8	Severe symptoms on leaves with necrosis also on petioles and stem
9	Plant dead

Where,

x_i = observed value of the trait

x = mean of the trait

n = number of observations

Variance was calculated separately for each generation to determine generation-specific variability.

$$s^2 = \frac{\sum(x_i - \bar{x})^2}{n-1}$$

Where,

x_i = observed value of the trait

x = mean of the trait

n = number of observations

Estimation of Components of Mean

The weighted least squares method, as outlined by Cavalli (1952) and detailed by Mather and Jinks (1982), was applied to estimate generation mean parameters. The adequacy of the additive-dominance (A-D) model was evaluated using a χ^2 test to assess goodness of fit between the observed means of six generations and those predicted by the model parameters: the general mean [m], additive effects [a] and dominance effects [d] (Table 2). A statistically acceptable fit validated the model's adequacy, while a poor fit indicated its insufficiency (Cavalli, 1952; Mather and Jinks 1982). In cases where the A–D model failed, a full model incorporating digenic epistatic parameters like additive × additive [i], additive × dominance [j] and dominance × dominance [l] was employed to achieve a perfect fit (Table 2). The significance of these parameters ([a], [d], [i], [j], [l]) was then tested using Student's *t*-test (Mather and Jinks 1982).

Table 2

Expected generation means under additive-dominance and digenic epistasis models

Generation mean	Additive-dominance model			Digenic interaction model					
	m	(d)	(h)	m	(d)	(h)	(i)	(j)	(l)
P_1	1	1	0	1	1	0	1	0	0
P_2	1	-1	0	1	-1	0	1	0	0
F_1	1	0	1	1	0	1	0	0	1
F_2	1	0	0.5	1	0	0.5	0	0	0.25
B_1	1	0.5	0.5	1	0.5	0.5	0.25	0.25	0.25
B_2	1	-0.5	0.5	1	-0.5	0.5	0.25	-0.25	0.25

Where, m = Mean; (i) = additive × additive effect; (d) = additive effect (j) = additive × dominance effect; (h) = dominance effect; (l) = dominance × dominance effect

The six equations were weighted by the inverse of their mean variances and merged into three composite normal equations to estimate the three parameters. These simultaneous equations were resolved using matrix inversion techniques, following Cavalli's (1952) approach. This framework was methodically implemented across all model-fitting stages.

$$M = J^{-1}. S$$

Where,

M = column vector of the estimates of parameters

S = matrix of sector (right hand size)

J = information matrix

J^{-1} = inverse of information matrix (variance covariance matrix)

The expected generation means were subsequently computed using the inverse matrix. The goodness of fit was evaluated by calculating chi-square (χ^2) values with the following formulas.

$$\chi^2(n-p) = \sum_{1}^{n} (Oi - Ei)^2 \; x\, Wi$$

Where,

Oi = observed mean of i^{th} generation

Ei = expected mean of i^{th} generation

Wi = weight of i^{th} generation

n = number of generation means available

p = number of parameters estimated

The standard error of each parameter (Pi) was calculated as,

$$S.E.(P_i) = \sqrt{C_{ii}}$$

Where,

C_{ii} is the i^{th} diagonal elements of inverse matrix, J^{-1}

Following goodness-of-fit tests, when the simple additive-dominance model failed to account for variation in generation means, Hayman's (1958) six-parameter model was applied, utilizing the formulas outlined below.

Gene effects		*Equation*
Mean (m)	=	$\bar{F}_2$
Additive (d)	=	$\overline{BC_1P_1} - \overline{BC_1P_2}$
Dominance (h)	=	$2\overline{BC_1P_1} + 2\overline{BC_1P_2} - 4\bar{F}_2 + \bar{F}_1 - ½\bar{P}_1 - ½\bar{P}_2$
Additive × additive (i)	=	$2\overline{BC_1P_1} + 2\overline{BC_1P_2} - 4\bar{F}_2$
Additive × dominance (j)	=	$2\overline{BC_1P_1} - \bar{P}_1 - 2\overline{BC_1P_2} - \bar{P}_2$
Dominance × dominance (l)	=	$\bar{P}_1 + \bar{P}_2 + 2\bar{F}_1 + 4\bar{F}_2 - 4\overline{BC_1P_1} - 4\overline{BC_1P_2}$

Where,

$\bar{P}_1$, $\bar{P}_2$, $\bar{F}_1$, $\bar{F}_2$, $\overline{BC_1P_1}$ and $\overline{BC_1P_2}$ are the means of $\bar{P}_1$, $\bar{P}_2$, $\bar{F}_1$, $\bar{F}_2$, $\overline{BC_1P_1}$ and $\overline{BC_1P_2}$ generations, respectively.

The variances and standard errors for each parameter estimate were calculated as follows.

Gene effects	*Equation*
$V_{(m)}$	$= V_{\bar{F}2}$
$V_{(d)}$	$= V_{\overline{BC1P1}} + V_{\overline{BC1P2}}$
$V_{(h)}$	$= 4V_{\overline{BC1P1}} + 4V_{\overline{BC1P2}} + 16V_{\bar{F}2} + V_{\bar{F}1} + ¼V_{\bar{P}1} + ¼V_{\bar{P}2}$
$V_{(i)}$	$= 4V_{\overline{BC1P1}} + 4V_{\overline{BC1P2}} + 16V_{\bar{F}2}$
$V_{(j)}$	$= V_{\overline{BC1P1}} + ¼V_{\bar{P}1} + V_{\overline{BC1P2}} + ¼V_{\bar{P}2}$
$V_{(l)}$	$= V_{\bar{P}1} + V_{\bar{P}2} + 4V_{\bar{F}1} + 16V_{\bar{F}2} + 16V_{\overline{BC1P1}} + 16V_{\overline{BC1P2}}$

Where,

$V_{\bar{P}1}$, $V_{\bar{P}2}$, $V_{\bar{F}1}$, $V_{\bar{F}2}$,

$V_{\bar{B}1}$ and $V_{\bar{B}2}$ are the variances of the means of P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 generations, respectively.

$$S.E._{(m)} = \sqrt{V_m} \qquad S.E._{(i)} = \sqrt{V_i}$$

$$S.E._{(d)} = \sqrt{V_d} \qquad S.E._{(j)} = \sqrt{V_j}$$

$$S.E._{(h)} = \sqrt{V_h} \qquad S.E._{(l)} = \sqrt{V_l}$$

The test of significance of each parameter was done by usual t-test.

$$\text{t for m} = \frac{\text{m}}{\text{S.E. of m}} \qquad \text{t for (i)} = \frac{\text{(i)}}{\text{S.E.of(i)}}$$

$$\text{t for (d)} = \frac{\text{(d)}}{\text{S.E.of(d)}} \qquad \text{t for (j)} = \frac{\text{(j)}}{\text{S.E.of(j)}}$$

$$\text{t for (h)} = \frac{\text{(h)}}{\text{S.E.of(h)}} \qquad \text{t for (l)} = \frac{\text{(l)}}{\text{S.E.of(l)}}$$

Computation of Genetic Variances

Phenotypic, genetic and environmental variances were analysed methodically, with the assumption that epistasis was absent, as described by Kearsey and Pooni (1998). The phenotypic variance was regarded as equivalent to the total variance within the F_2 generation. The variance components were determined according to the following four formulas:

Environmental variance, $V_E = \frac{V_{P_1} + V_{P_2} + 2V_{F_1}}{4}$

Where, V_{P1} = variance within parent P_1, V_{P2} = variance within parent P_2 and V_{F1} = variance within F_1 generation.

Genetic variance, $V_G = V_P - V_E$

Where, V_P = phenotypic variance = variance within the F_2 generation (V_{F2}) and V_E= environmental variance.

Additive variance, $V_A = 2V_{F2} - (V_{BC1P1} + V_{BC1P2})$

Where, V_{F2} = variance within F_2 generation, V_{BCP1} = variance within backcross with parent P_1, V_{BC1P2} = variance within backcross with parent P_2.

Dominance variance, $V_D = V_{BC1P1} + V_{BC1P2} - V_{F2} - V_E$

Where, V_{F2} = variance within F_2 generation, V_{BCP1} = variance within backcross with parent P_1, V_{BC1P2} = variance within backcross with parent P_2 and V_E= environmental variance.

Heritability estimation

The heritability in broad-sense (H_b^2) was worked out as the quotient of genotypic variance (V_G) to phenotypic variance (V_P), while the heritability in narrow-sense (H_n^2) was computed as the ratio of additive variance (V_A) to phenotypic variance (V_P) (Akhshi *et al.,* 2014). The heritability estimates less than 30 per cent were considered low, 31–60 per cent were considered moderate and more than 60 per cent were considered high (Robinson *et al.,* 1949).

The broad-sense heritability was estimated as follows:

Broad-sense heritability, $H_b^2 = \frac{V_A + V_D}{V_A + V_D + V_E}$

The narrow sense heritability was estimated as follows:

Narrow-sense heritability, $H_b^2 = \frac{V_A}{V_A + V_D + V_E}$

The dominance (governing) ratio used to evaluate the prominence of dominance and additive gene effects was calculated according to Kearsey and Pooni (1998).

Dominance ratio, $DR = \sqrt{\frac{4V_D}{2V_A}}$

Where: DR = dominance ratio, V_D = dominance variance, and V_A = additive variance.

Results and Discussion

The disease severity means of generational progenies and parents across the four crosses (C1: CL0732 × CL0699; C2: CL0732 × CL0695; C3: CL0732 × CL0033; C4: CL0732 × CL0413) are summarized in Table 3. Parent P_1(CL 0732) consistently exhibited the lowest mean severity (0.20 ± 0.13) indicating high resistance, while parents P_2's displayed significantly higher severity, with means ranging from 5.80 ± 0.64 (CL 0695), 7.20 ± 0.20 (CL 0699) and 8.60 ± 0.16 (CL 0033 and CL 0413), reflecting high susceptibility. The large difference of GSB severity means of parents used in this study indicates that they were divergent for the studied character, which is a requirement for the success of a generation mean analysis study (Mather & Jinks 1982 and Walker & White, 2001).

The BC_1P_1 progeny showed moderate severity (1.18 ± 0.22 to 1.54 ± 0.24), closer to P_1, whereas BC_1P_2 had elevated means (2.62 ± 0.28 to 5.01 ± 0.33), trending toward P_2. The F_1 generation exhibited

Table 3

Estimates of means among six generations with their standard error for Gummy stem blight resistance in watermelon

Generations/ populations	C1 (CL0732 × CL 0699)		C2 (CL 0732 × CL 0695)		C3 (CL 0732 × CL 0033)		C4 (CL 0732 × CL 0413)	
	Sample size	Mean	Sample size	Mean	Sample size	Mean	Sample size	Mean
P_1	10	0.20 ± 0.13	10	0.20 ± 0.13	10	0.20 ± 0.13	10	0.20 ± 0.13
P_2	10	7.20 ± 0.20	10	5.80 ± 0.64	10	8.60 ± 0.16	10	8.60 ± 0.16
F_1	10	0.90 ± 0.31	10	1.40 ± 0.42	10	0.7 ± 0.21	10	0.80 ± 0.29
F_2	219	2.56 ± 0.21	177	3.31 ± 0.26	240	3.57 ± 0.24	248	3.03 ± 0.21
BC_1P_1	100	1.46 ± 0.27	100	1.54 ± 0.24	100	1.22 ± 0.26	100	1.18 ± 0.22
BC_1P_2	100	2.63 ± 0.19	100	2.62 ± 0.28	100	5.01 ± 0.33	100	3.11 ± 0.29

intermediate values (0.70 ± 0.21 to 1.40 ± 0.42), lower than the mid-parent mean in all crosses, suggesting incomplete dominance or quantitative nature of disease resistance. In contrast, the F_2 populations displayed increased severity (2.56 ± 0.21 to 3.57 ± 0.24), though still below the mid-parent values, indicating potential transgressive segregation or epistatic interactions. Notably, C3 exhibited the highest severity in F_2 (3.57 ± 0.24) and BC_1P_2 (5.01 ± 0.33), highlighting cross-specific variability. These patterns underscore the influence of genetic background on disease expression and the complexity. The distribution of disease severity across the generations highlights genetic variability, with F_2 and BC_1P_2 generations consistently showing higher severity than F_1 and BC_1P_1, indicating partial dominance of GSB resistance.

The variance data across generations and crosses (Table 4) provides critical insights into the genetic architecture and stability of disease severity traits. The low variances in non-segregating generations (P_1, P_2, F_1: 0.18-1.82) reflect stability and genetic uniformity. Notably, F_2 populations displayed markedly higher variances (10.15-13.66), reflecting extensive genetic segregation, with C3 (13.66) showing the greatest variance, indicative of complex genetic interactions or epistasis. Backcross

Table 4

Estimates of variance among six generations for Gummy stem blight resistance in watermelon

Generations/ populations	C1 (CL0732 × CL 0699)		C2 (CL 0732 × CL 0695)		C3 (CL 0732 × CL 0033)		C4 (CL 0732 × CL 0413)	
	Sample size	Variance	Sample size	Variance	Sample size	Variance	Sample size	Variance
P_1	10	0.18	10	0.18	10	0.18	10	0.18
P_2	10	0.4	10	4.18	10	0.26	10	0.26
F_1	10	0.99	10	1.82	10	0.45	10	0.84
F_2	219	10.15	177	11.93	240	13.66	248	11.55
BC_1P_1	100	7.6	100	6.19	100	6.8	100	5.26
BC_1P_2	100	3.78	100	8.05	100	11.02	100	8.40

populations also varied: BC_1P_1 variances (5.26-7.6) were lower than BC_1P_2 (3.78-11.02), with C3-BC_1P_2 again exhibiting the highest variance (11.02). The significant variances within segregating generations revealed a high level of heredity variation for GSB disease resistance that existed among the progenies derived from the crosses (Kearsey and Pooni 1998). The elevated variances in F_2 populations suggest a promising genetic pool, likely harbouring alleles for both susceptibility and resistance, which could be exploited to isolate stable, high-performing progenies in advanced breeding cycles.

Narrow-sense heritability values were 53 per cent in C3, 58 per cent in C2, 68 per cent in C4 and 72 per cent in C1, reflecting a moderate to high level of additive genetic variation (Fig. 1). This pattern suggests the presence of incomplete dominance, with both additive and non-additive effects contributing to resistance. Notably, the higher narrow-sense heritability in C1 and C4 indicates a stronger influence of additive effects, making these crosses promising candidates for selective breeding programs aimed at enhancing GSB resistance. The selection for a trait with a high narrow sense heritability is easy, while the selection of a moderately heritable character is more complicated and with a lower probability of success, as breeders would rely on transgressive segregation to register the progress (Ajay *et al.,* 2012) Moreover, the dominance ratio was below unity in all crosses (Fig. 2), further confirming the incomplete dominance observed across the populations.

The adequacy of the Additive-Dominance (A-D) model was evaluated using the joint scaling test (Cavalli, 1952), a statistical framework that assesses the goodness-of-fit between observed generation means (e.g., parents, F_1, F_2, backcrosses) and those predicted by the model. This test assumes that genetic effects - additive (*d*) and dominance (*h*) are sufficient to explain phenotypic variation, with deviations indicating the presence of epistasis, gene-environment interactions or other non-Mendelian phenomena. In the current study, the A-D model was uniformly rejected across all four crosses (C1-C4) based on significant χ^2 values (e.g., 54.12 for C1, 40.33 for C4; Table 5), which far exceeded critical thresholds ($p < 0.001$). These results unambiguously highlight the inadequacy of the A-D model in explaining the

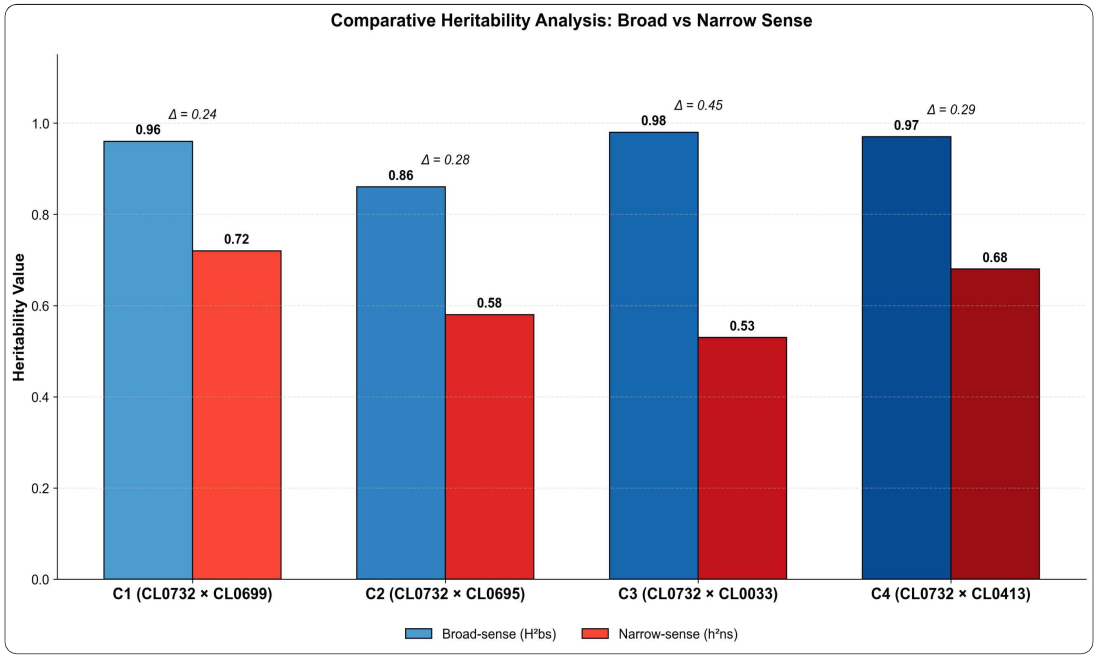

Fig. 1 : Comparison of broad and narrow sense heritability for GSB disease resistance across watermelon crosses

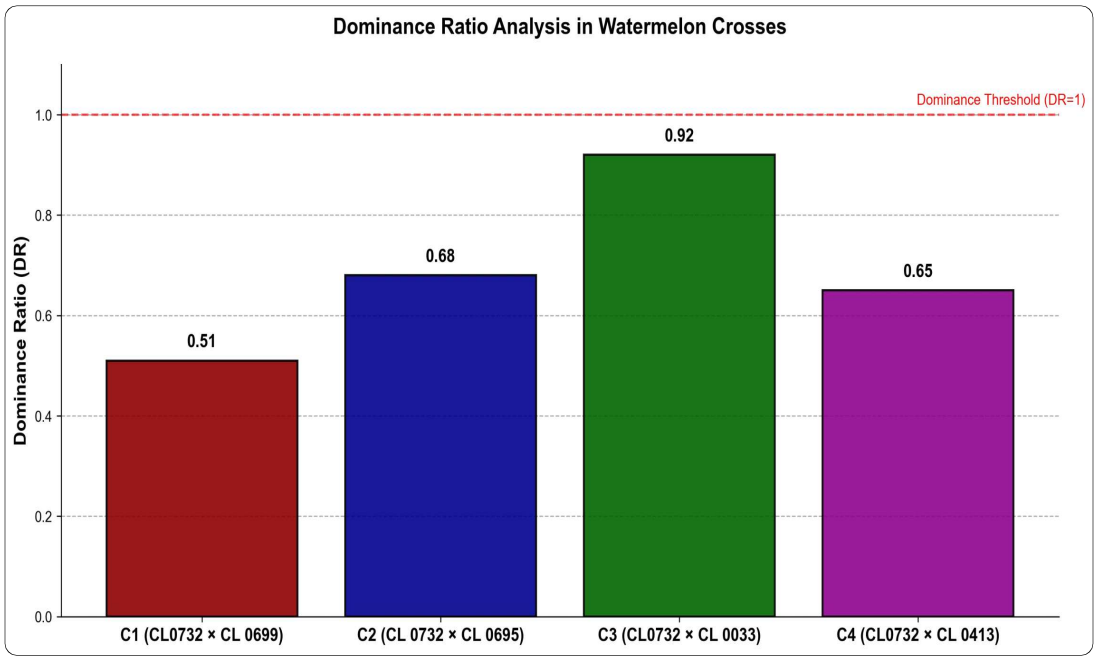

Fig. 2 : Estimates of dominance ratio for all the four crosses for GSB disease resistance across watermelon crosses

TABLE 5

Estimates of components of generation means and test for adequacy of additive-dominance model in the inheritance of resistance to gummy stem blight in watermelon

	C1 (CL0732 × CL 0699)		C2 (CL 0732 × CL 0695)		C3 (CL 0732 × CL 0033)		C4 (CL 0732 × CL 0413)	
		t-Calculated		t-Calculated		t-Calculated		t-Calculated
m	3.55 ± 0.12 **	31.23	2.61 ± 0.24 **	10.97	4.50 ± 0.10 **	43.35	4.38 ± 0.10 **	42.92
d	3.19 ± 0.11 **	28.62	2.30 ± 0.23 **	9.94	4.18 ± 0.10 **	40.31	4.04 ± 0.10 **	39.88
h	-2.90 ± 0.27 **	-10.66	-0.48 ± 0.45	-1.06	-3.34 ± 0.22 **	-15.57	-3.50 ± 0.25 **	-13.87
$\chi 2$	54.12		30.33	18.78	40.33			
Adequacy of Additive Dominance (A-D) model	Inadequate		Inadequate		Inadequate		Inadequate	

inheritance patterns GSB resistance in watermelon strongly implicating epistatic interactions as a major driver of phenotypic variation. The rejection of the A-D model underscores the necessity of incorporating epistatic terms (e.g., additive × additive, dominance × dominance and additive × dominance) into genetic models to capture the full complexity of trait architecture to account for epistasis to avoid under estimating genetic potential or misinterpreting trait heritability. These findings align with those of Gusmini *et al.* (2017), who reported non-Mendelian segregation ratios in crosses developed with a resistant genotype for GSB, indicating that the inheritance of GSB resistance involves complex polygenic and epistatic interactions.

Table 6

Estimates of components of generation means based on perfect fit solution for reaction to gummy stem blight in watermelon

	C1 (CL0732 × CL 0699)	C2 (CL 0732 × CL 0695)	C3 (CL 0732 × CL 0033)	C4 (CL 0732 × CL 0413)
m	2.57 ± 0.21 **	3.32 ± 0.26 **	3.57 ± 0.24 **	3.04 ± 0.22 **
d	-1.17 ± 0.34 **	-1.08 ± 0.38 **	-3.79 ± 0.42 **	-1.93 ± 0.37 **
h	-4.86 ± 1.14 **	-6.52 ± 1.39 **	-5.52 ± 1.29 *	-7.16 ± 1.17 **
i	-2.08 ± 1.09 *	-4.92 ± 1.28 **	-1.82 ± 1.27	-3.56 ± 1.14 **
j	4.66 ± 0.72 **	3.44 ± 1.00 **	0.82 ± 0.87	4.54 ± 0.76 **
l	3.10 ± 1.73	5.40 ± 2.12 *	-0.43 ± 1.99	5.38 ± 1.82 **
Type of epistasis	DEDD	DEDD	CEDD	DEDD

The Six-Parameter Model, which extends the classical Additive-Dominance (A-D) framework by incorporating epistatic interactions (non-allelic gene interactions), provides a more nuanced understanding of the genetic architecture underlying GSB disease resistance in the four crosses (C1-C4). This model accounts for additive (*m*, *d*), dominance (*h*) and three types of epistatic effects: additive × additive (*i*), additive × dominance (*j*) and dominance × dominance (*l*). By integrating these parameters, the model captures the complexity of trait inheritance beyond linear genetic effects, offering insights into how inter-locus interactions shape phenotypic outcomes. The data presented in Table 6 reveal striking cross-specific patterns in these parameters, underscoring the pervasive role of epistasis in modulating disease resistance. The digenic interaction model as per Hayman (1958) was significant and the additive effect [d], dominance effect [h], additive × additive epistasis [i] and dominance × dominance epistasis had significant values for crosses like C1, C2 and C3 indicating the significant influence in control of resistance to the GSB disease. The parameters specifying additive [d], dominance [h] and additive × additive [i] gene effects were negative and this implies the tendency towards the reducer parent as reported by Gaoh *et al.* (2020), thus towards disease resistance. The significance of additive [d], additive × additive [i] and additive × dominance [j] gene effects indicates that the resistance could be fixed and exploited in advanced breeding lines (Carlborg and Haley, 2004).

Epistasis in genes controlling quantitative traits is classified as duplicate or complementary based on the signs of [h] and [l]. Complementary epistasis arises when both are positive (dominant increasing alleles) or both negative (dominant decreasing alleles). Duplicate epistasis occurs with opposite signs: positive [h] and negative [l] (increasing alleles) or negative [h] and positive [l] (decreasing alleles) (Kearsey and Pooni 1996). In this study, GSB resistance genes in three crosses (C1, C2, C4) exhibited duplicate epistasis between dominant decreasing alleles (DEDD), while C3 showed complementary epistasis between dominant decreasing alleles (CEDD). If epistatic effects are significant estimates of additive [a] and dominance [d] effects and their variances (σ^2_A, σ^2_D) cannot be precisely calculated. However, Epistasis plays a major role in influencing additive genetic variance [σ^2_A], even if the variance attributed to epistasis (σ^2_I) is minimal (Rasmusson and Philip, 1997 and Bernardo, 2010).

To generate unbiased estimates of [a] and [d] gene effects, it is necessary to assume that in a given homozygous parental genotype, alleles at all loci affecting the target trait have either rising or

decreasing effects and exhibit unidirectional dominance. However, in practical, each genotype may contain a mix of increasing and decreasing effect alleles with variable degrees and directions of dominance. In extreme circumstances, increasing and decreasing alleles are equally distributed. As a result, additive gene effects [a] are frequently under estimated, with the extent of under estimating determined by allele dispersion. Similarly, ambidirectional dominance reduces dominance gene effects [d]. Variances compensate for the disadvantages associated with internal cancellation of positive and negative effects generated by gene dispersion and ambi directional dominance. However, any level of dominance and all types of epistasis contribute to σ^2_A, σ^2_D and σ^2_I (Bernardo, 2014). Hence, it is difficult to infer predominant modes of action of genes even from second degree statistics. The joint application of both first and second-degree statistics provide more comprehensive and dependable information about genetic control of quantitative traits (Kearsey and Pooni, 1996).

The additive genetic variance [σ^2_A] is unaffected by gene dispersion and remains orthogonal to the additive genetic effect [a], implying no correlation between them. Thus, diverse combinations of [a] and [σ^2_A] are possible. For example, when [a] is small or negligible (e.g., due to dispersion of increasing/ decreasing alleles between parents), opposing effects cancel out, yet [ó²$_A$] remains substantial, reflecting latent additive potential from dispersed loci. Conversely, large [a] paired with large [σ^2_A] indicates strong additive effects with minimal cancellation, typical of crosses dominated by unidirectional alleles. Alternatively, large [a] alongside nonsignificant [σ^2_A] occurs when individual gene effects are minor (<1.0), cumulatively contributing to directional additive effects but insufficient to generate notable variance (Kearsey and Pooni 1996). Similarly, the relationship between dominance effect [d] and dominance variance [σ^2_D] follows this pattern, except [σ^2_D] is influenced by ambi directional dominance rather than gene dispersion.

Significance of both [a] and higher [σ^2_A] in all the four crosses suggested the substantial contribution of [a] gene effects in the inheritance of GSB disease resistance (Table 7). Further, significant negative [d] and significant [σ^2_D] indicated the directional dominance for decreasing alleles in all four crosses. Our results indicating the role of dispersed genes with predominantly additive effects and variance. This study augurs well with those reported by Hill *et al.* (2008). The predominance of additive effects and variance in this study for the GSB resistance draw adequate support from theoretical expectations of greater σ^2_A than σ^2_D (Moll & Stubber, 1974; Hallauer, 1985; Dudley, 1997; Bernardo, 2010 and Bernardo, 2014). This is because, loci that exhibit dominance as well as epistasis also contribute to σ^2_A indicating that any segregating locus with either no dominance or partial dominance or complete dominance or overdominance contribute to σ^2_A (Bernardo, 2010). Selection for additive gene effects to exploit latent additive variance, enabling durable resistance improvement through accumulation of dispersed favourable alleles.

TABLE 7

Estimates of additive genetic effect and variance (σ^2_A) and dominant genetic effect and variance (σ^2_D) for response to gummy stem blight in watermelon

Cross combination	[a]	σ^2_A	[d]	σ^2_D
C1 (CL0732 × CL 0699)	-1.17 ± 0.34 **	17.87	-4.86 ± 1.14 **	4.65
C2 (CL 0732 × CL 0695)	-1.08 ± 0.38 **	19.25	-6.52 ± 1.39 **	8.96
C3 (CL 0732 × CL 0033)	-2.99 ± 0.46 **	19.16	-3.66 ± 1.68 *	16.12
C4 (CL 0732 × CL 0413)	-1.93 ± 0.37 **	18.96	-7.16 ± 1.17 **	8.09

The joint scaling tests showed that the additive-dominance model did not adequately describe the genetics of gummy stem blight disease resistance in the watermelon crosses used in this study, which means epistatic interactions were involved. The additive, dominance and additive × additive effects were negative and this showed inclination towards resistant parent, therefore desirable. However, the dominance × dominance effect had significant positive values in crosses like C2 and C4 and it was undesirable since it was toward the susceptible parent. The digenic interactions, namely additive × additive and additive × dominance was more prevalent than the dominance × dominance effects. The significance of additive, additive × additive and additive × dominance gene effects, along with moderate to high narrow-sense heritability with high additive variance. These findings strongly support the feasibility of initiating selection in early segregating generations to fix resistance efficiently. The breeding materials developed through this generation mean analysis, particularly from crosses C1 and C4 with high additive variance and heritability, offer promising parental lines and segregants for advancing resistant cultivars. The presence of favourable additive and epistatic gene interactions in these populations provides a robust genetic foundation for durable resistance. Moving forward, these selected lines will be further evaluated and integrated into advanced breeding programs to develop high-yielding watermelon cultivars with stable resistance to GSB, thereby contributing to sustainable cucurbit production and reduced reliance on chemical control.

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