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Integrated assessment of photosynthesis, water use efficiency and yield in cotton under differential irrigation regimes

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Abstract
Water scarcity poses a significant constraint to cotton productivity in semi-arid regions. This study evaluated the physiological responses and yield performance of two cotton genotypes (*Gossypium hirsutum* L.), GSHV-97/162 (V_1) and LRA-5166 (V_2), under four irrigation regimes: 100% (M_1), 75% (M_2), 50% (M_3) of field capacity (FC) and rainfed (M_4). The experiment was conducted using a split-plot design with stage-specific drought stress imposition. Key physiological parameters photosynthetic rate, stomatal conductance, transpiration, water use efficiency (WUE), light absorption and leaf water potential were measured at 60, 90 and 120 days after sowing (DAS). Results indicated a substantial decline in seed cotton yield from 1603.05 kg ha^{-1} in M1 to 1405.88 kg ha^{-1} in M4. Photosynthetic rate at 90 DAS dropped from 28.7 µmol CO_2 m^{-2} s^{-1} (M_1) to 24.1 µmol CO_2 m^{-2} s^{-1} (M_4), while transpiration decreased from 6.12 to 3.95 mmol H_2O m^{-2} s^{-1}. WUE peaked under moderate stress (M_2) at 0.385 µmol CO_2 mmol^{-1} H_2O, but declined under severe stress (M_4). Light absorption at 120 DAS reduced from 42.7% (M_1) to 26.4% (M_4) and leaf water potential declined from -18.44 bars to -23.08 bars. Genotype V_1 consistently outperformed V_2 under stress, with higher photosynthetic activity (26.60 vs. 23.92 µmol CO_2 m^{-2} s^{-1}), more bolls (17.55 vs. 9.55 per plant) and greater boll weight (4.15 vs. 2.83 g) under full irrigation. Heatmap visualization revealed strong trait interdependence and confirmed the detrimental impact of moisture deficit on gas exchange, water balance and yield. The study highlights the value of integrating physiological and agronomic traits to identify drought-resilient genotypes and optimize irrigation strategies for sustainable cotton production.

Keywords: Cotton genotypes, deficit irrigation, photosynthesis, water use efficiency, yield components, light absorption, leaf water potential, physiological response, heatmap analysis

Introduction
Cotton (*Gossypium hirsutum* L.) is one of the most widely cultivated fiber crops globally, serving as a vital component of the textile industry and providing significant income to farmers in tropical and subtropical regions (Khan *et al*., 2019) [6]. In India, cotton occupies nearly 12 million hectares, with a substantial proportion grown in semi-arid zones where rainfall is erratic and irrigation resources are limited. Despite its moderate drought tolerance, cotton's growth, reproductive development and final yield are highly dependent on adequate water availability during critical phenological stages, particularly flowering and boll formation (Pettigrew, 2004; Sarwar *et al*., 2015) [9, 12].

The increasing frequency of drought events, declining groundwater tables and climate variability are exacerbating water scarcity in many cotton-growing regions (Abdelraheem *et al*., 2019) [1]. These challenges underscore the need for strategies that enhance water productivity while maintaining yield stability. Deficit irrigation, when carefully timed, has shown potential in improving water use efficiency (WUE) in cotton without causing severe yield losses (Reddy *et al*., 2015; Zhang *et al*., 2010) [10, 14]. However, crop response to water deficit is highly genotype-dependent and stage-specific, making it essential to dissect physiological responses across genotypes and growth stages.

Physiological traits such as photosynthetic rate, stomatal conductance, transpiration and leaf water potential offer valuable insights into plant water relations and have been proposed as functional indicators of drought tolerance (Flexas *et al*., 2004; Chaves *et al*., 2009) [4, 3]. Additionally, traits like light interception and WUE are important determinants of crop performance under water-limited environments (Gaju *et al*., 2011) [5]. Integrating these physiological indicators with yield traits can aid in identifying resilient genotypes and optimizing irrigation schedules.

Despite these advances, comprehensive field studies evaluating cotton responses to moisture deficit using integrated physiological and agronomic parameters remain limited. Therefore, the present study aimed to (i) assess the effects of irrigation levels and stress imposition at different growth stages on physiological traits and yield in two cotton genotypes, (ii) identify physiological markers associated with water use efficiency and drought tolerance and (iii) explore genotype-specific responses to water deficit in a semi-arid agroecological context.

Material and Methods

Experimental Site and Climate

The field experiment was conducted during the *kharif* season at the Agriculture Research Station, Hebballi Farm, UAS, Dharwad. The site falls under a semi-arid agroclimatic zone with an average annual rainfall of approximately 659.06 mm. The soil at the site was classified as clayey, neutral pH and good drainage.

Experimental Design and Treatments

The study was laid out in a split-plot design with three replications. The main plot factor comprised four irrigation levels, based on field capacity (FC): M_1 - 100% FC (Well-irrigated control), M_2 - 75% FC, M_3 - 50% FC and M_4 - Rainfed (No irrigation after establishment). The sub-plot factor included two cotton genotypes: V_1 - GSHV-97/162 and V_2 - LRA-5166.

Water was applied via surface irrigation. Soil moisture content was monitored using the gravimetric method to maintain field capacity thresholds in M_1-M_3. In each main plot, three irrigation withdrawal stages were imposed as sub-subplot treatments to induce growth-stage-specific water stress: S_1 - No irrigation from 10 to 40 days after sowing (vegetative stage), S_2 - No irrigation from 50 to 80 DAS (flowering stage) and S_3 - No irrigation from 85 to 120 DAS (boll development stage)

Crop Management

Standard agronomic practices for cotton cultivation in the region were followed. Seeds were sown at a spacing of 90 cm × 60 cm. Fertilizer was applied at the recommended rate of 120:60:60 kg N: P_2O_5:K_2O ha^{-1}. All plots received uniform weed and pest management.

Physiological Observations

Physiological traits were recorded at 60, 90 and 120 DAS. Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}) and transpiration rate (mmol H_2O m^{-2} s^{-1}) were measured using a portable Infrared Gas Analyzer (IRGA, LI-6400, LI-COR Inc., USA). Water use efficiency (WUE) was computed as the ratio of photosynthesis to transpiration. Leaf water potential (bars) was measured using a pressure chamber (Scholander-type) at 90 and 120 DAS. Percent light absorption was measured at 90 and 120 DAS using a line quantum sensor (Model LI-191R, LI-COR Inc.), with values calculated from light intercepted by the canopy. Yield and Yield Components viz., Seed cotton yield (kg ha^{-1}) from the net plot area, Number of bolls per plant, based on five randomly selected plants per plot and Mean boll weight (g), based on 20 bolls sampled at random were recorded at harvesting.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) following the split-plot design structure. Treatment means were compared using the least significant difference (LSD) test at 5% significance level. Homogeneity of variance was verified prior to analysis. A correlation matrix and heatmap were constructed using Python 3.11 (libraries: pandas, seaborn, matplotlib) to visualize the interdependence among yield and physiological traits across irrigation treatments.

Results and Discussion

1. Effect of Irrigation Levels on Yield Performance

The influence of irrigation levels on cotton yield was profound and statistically significant (Table 7). Under full irrigation (M_1), genotype V_1 (GSHV-97/162) achieved the highest average yield (1602.33 kg/ha), whereas under rainfed conditions (M_4), the yield declined sharply to 1406.00 kg/ha, reflecting a ~12.3% yield penalty. A similar but slightly more pronounced decline was observed for genotype V_2 (LRA-5166), from 1603.77 kg/ha (M_1) to 1405.77 kg/ha (M_4). These yield reductions were directly associated with decreasing soil moisture levels and the corresponding physiological impairments, consistent with earlier studies that emphasize the vulnerability of cotton to moisture stress, particularly during critical reproductive stages (Pettigrew, 2004; Sarwar *et al*., 2015) [9, 12].

2. Photosynthesis and Gas Exchange Dynamics

Photosynthetic rate (Pn) was highly responsive to irrigation and varied across developmental stages (Table 1). At 90 DAS, cotton plants under M1 maintained high photosynthetic rates in both genotypes (V_1: 26.60 µmol/m²/s; V_2: 30.83 µmol/m²/s). These values dropped significantly under water-deficit conditions (M_4), especially in V_2, which decreased to 23.92 µmol/m²/s. Reduced photosynthetic activity under water stress is attributed to stomatal limitations, decline in chlorophyll content and impaired mesophyll conductance, corroborated by earlier work on drought responses in cotton (Flexas *et al*., 2004; Abdelraheem *et al*., 2019) [4, 1].

This decline in photosynthesis coincided with a reduction in stomatal conductance (gs), as shown in Table 2. Under M_1, V_2 maintained a gs of 0.052 µmol/m²/s at 90 DAS, which dropped to 0.049 under M_4. This suggests partial stomatal closure to conserve water, but at the cost of limiting CO_2 influx. Transpiration rates followed a parallel trend (Table 3), with M_1 conditions supporting high evapotranspiration (6.74 µmol CO_2/m²/s in V_2 at 90 DAS), while M_4 saw a reduction to 4.02 µmol CO_2/m²/s. These reductions in Pn, gs and E under drought stress demonstrate the complex interplay between gas exchange parameters, which collectively affect dry matter production and yield (Chaves *et al*., 2009) [3].

3. Water Use Efficiency (WUE)

Water use efficiency, defined as the ratio of carbon gained to water lost, improved modestly under moderate stress (M_2), but declined under severe stress (M_4), especially at 120 DAS (Table 4). Genotype V1 maintained higher WUE than V_2 across most stages and treatments. At 90 DAS, WUE reached 0.41 µmol CO_2/mmol H_2O in V_1 under M_2, but fell to 0.34 under M_4. These results align with prior findings that moderate stress may enhance WUE by optimizing stomatal function, but excessive water deficit suppresses both photosynthesis and transpiration, thus compromising efficiency (Zhang *et al*., 2010; Liu *et al*., 2013) [14, 7].

4. Light Absorption and Canopy Behavior
A marked reduction in percent light absorption was observed under reduced irrigation (Table 5). At 120 DAS, V_2 absorbed up to 43.20% of incident light under M_1, but only 25.20% under M_4. Reduced light interception under drought conditions may result from a decrease in leaf area index, chlorophyll degradation and premature senescence (Gaju *et al*., 2011) [5]. Genotype V_1 maintained better light absorption under stress, suggesting superior canopy retention and possibly deeper rooting, which may have facilitated better water uptake.

5. Water Potential and Plant Water Status
Water potential measurements (Table 6) confirmed significant stress under limited irrigation. At 120 DAS, V_2 recorded a water potential of −23.08 bars under M_4, indicating severe dehydration. In contrast, M_1 conditions maintained relatively higher water potential values (−18.44 bars in V_2), reflecting adequate soil moisture availability. These values are consistent with the physiological thresholds identified by Turner *et al*. (2001) [13], who noted that cotton's critical water potential threshold for stomatal closure is around −20 bars.

6. Yield Components and Their Contribution
Yield decline under drought was also linked to reductions in boll number and boll weight (Table 7). Under M_1, V_1 produced 17.55 bolls per plant and an average boll weight of 4.15 g. These values dropped significantly under M_4 to 9.55 bolls and 2.83 g boll weight, respectively. The sensitivity of reproductive structures to drought—particularly flower shedding and reduced boll filling—has been documented in numerous cotton studies (Saeed *et al*., 2014; Nawaz *et al*., 2020) [11, 8].

7. Genotypic Response and Stability
Across all measured parameters, V_1 (GSHV-97/162) outperformed V_2 (LRA-5166) under limited water availability. V_1 exhibited better retention of photosynthesis, higher WUE, more stable light absorption and less negative water potential values. These traits contribute to better drought resilience and are desirable in breeding programs for semi-arid regions. Similar genotypic stability under stress conditions has been reported by Ali *et al*. (2015) [2], who found that genotypes with higher WUE and leaf retention maintain yield under drought.

Table 1: Effect of irrigation levels on Rate of photosynthesis (μ mol/m^2/sec) at different growth stages in cotton hybrids

Treatments		Days after sowing					
		60		90		120	
		V_1	V_2	V_1	V_2	V_1	V_2
M_1	S_1	21.46	22.56	28.06	31.56	14.46	14.93
	S_2	21.83	22.33	22.86	34.36	14.93	15.06
	S_3	22.13	20.80	28.86	26.56	14.43	14.70
Mean		21.81	21.90	26.60	30.83	14.61	14.90
M_2	S_1	22.06	20.20	23.90	22.93	13.26	12.40
	S_2	20.33	20.66	24.50	20.56	13.80	12.66
	S_3	19.90	20.36	22.06	21.93	13.16	12.73
Mean		20.76	20.41	23.48	21.81	13.41	12.60
M_3	S_1	21.33	18.00	20.53	21.73	12.33	13.57
	S_2	19.90	17.93	20.93	23.03	12.16	13.30
	S_3	18.31	16.23	20.83	23.13	12.21	14.70
Mean		19.84	17.38	20.76	22.63	12.23	13.85
M_4	S_1	18.86	14.33	24.73	21.96	14.50	14.20
	S_2	24.10	19.96	24.23	24.36	14.23	13.53
	S_3	20.26	15.00	23.73	25.43	14.56	10.23
Mean		21.07	16.43	24.23	23.92	14.43	12.65
Mean	S_1	20.93	18.77	24.03	24.55	13.64	13.77
	S_2	21.54	20.22	23.13	25.58	13.78	13.64
	S_3	20.15	18.10	23.87	24.26	13.59	13.09
Grand mean		20.87	19.03	23.77	24.80	13.67	13.50
For comparing		SEm ±	CD @ 5%	SEm ±	CD @ 5%	SEm ±	CD @ 5%
Moisture regimes (M)		2.623	NS	1.010	3.497	1.044	NS
Genotypes (G)		1.149	NS	0.338	NS	0.365	NS
M X G		2.299	NS	0.676	2.206	0.730	NS
Stages (S)		0.476	1.370	0.409	NS	0.316	NS
M X S		0.951	2.741	0.818	NS	0.632	NS
G X S		0.673	NS	0.578	NS	0.447	NS
M X G X S		1.345	NS	1.156	3.331	0.894	NS

V_1- GSHV-97/162, V_2-LRA-5166, M_1- Irrigated (100% Field capacity), M_2- Irrigated (75% Field capacity), M_3- Irrigated (50% Field capacity), M_4- Rainfed, S_1- Vegetative stage (No irrigation between 10-40 DAS), S_2- Flowering stage (No irrigation between 50-80 DAS), S_3- Boll development stage (No irrigation between 85-120 DAS)

Table 2: Effect of irrigation levels on Stomatal conductance (μ mol/m^2/sec) at different growth stages in cotton hybrids

Treatments		Days after sowing					
		60		90		120	
		V_1	V_2	V_1	V_2	V_1	V_2
M_1	S_1	0.049	0.049	0.051	0.047	0.092	0.124
	S_2	0.052	0.049	0.052	0.050	0.098	0.125
	S_3	0.049	0.043	0.052	0.050	0.096	0.131
Mean			0.047	0.052	0.049	0.095	0.127
M_2	S_1	0.058	0.059	0.050	0.058	0.074	0.092
	S_2	0.059	0.064	0.051	0.059	0.069	0.089
	S_3	0.057	0.063	0.052	0.057	0.060	0.086
Mean			0.062	0.051	0.058	0.068	0.089
M_3	S_1	0.049	0.055	0.056	0.049	0.060	0.071
	S_2	0.049	0.049	0.053	0.049	0.057	0.064
	S_3	0.050	0.049	0.051	0.050	0.055	0.064
Mean			0.051	0.053	0.049	0.058	0.067
M_4	S_1	0.058	0.065	0.052	0.058	0.038	0.043
	S_2	0.060	0.055	0.051	0.060	0.032	0.043
	S_3	0.059	0.056	0.049	0.058	0.034	0.044
Mean			0.058	0.051	0.059	0.035	0.043
Mean	S_1	0.054	0.057	0.052	0.053	0.066	0.086
	S_2	0.055	0.054	0.052	0.055	0.064	0.080
	S_3	0.054	0.053	0.051	0.054	0.061	0.081
Grand mean		0.054	0.055	0.052	0.054	0.064	0.081
For comparing		SEm ±	CD @ 5%	SEm ±	CD @ 5%	SEm ±	CD @ 5%
Moisture regimes (M)		0.002	0.008	0.002	NS	0.004	0.013
Genotypes (G)		0.001	NS	0.001	NS	0.003	0.010
M X G		0.003	NS	0.002	0.007	0.006	NS
Stages (S)		0.001	NS	0.001	NS	0.001	NS
M X S		0.001	NS	0.001	NS	0.002	0.006
G X S		0.001	0.003	0.001	NS	0.001	NS
M X G X S		0.002	NS	0.001	NS	0.003	NS

V_1- GSHV-97/162, V_2-LRA-5166, M_1- Irrigated (100% Field capacity), M_2- Irrigated (75% Field capacity), M_3- Irrigated (50% Field capacity), M_4- Rainfed, S_1- Vegetative stage (No irrigation between 10-40 DAS), S_2- Flowering stage (No irrigation between 50-80 DAS), S_3- Boll development stage (No irrigation between 85-120 DAS)

Table 3: Effect of irrigation levels on rate of transpiration (μ mol CO_2/m^2/sec) at different growth stages in cotton hybrids

Treatments		Days after sowing					
		60		90		120	
		V_1	V_2	V_1	V_2	V_1	V_2
M_1	S_1	1.23	1.32	5.55	6.67	2.55	2.48
	S_2	1.19	1.19	5.49	6.57	2.62	2.46
	S_3	1.40	1.18	5.45	6.98	2.54	2.56
Mean			1.23	5.50	6.74	2.57	2.50
M_2	S_1	1.16	1.13	5.25	5.98	2.47	2.24
	S_2	1.11	1.12	5.09	5.91	2.46	2.36
	S_3	1.11	1.12	5.33	5.98	2.44	2.39
Mean			1.12	5.22	5.96	2.45	2.33
M_3	S_1	1.08	1.07	4.17	4.84	2.29	2.22
	S_2	1.07	1.08	3.97	4.70	2.21	2.16
	S_3	1.05	0.74	4.12	4.63	2.21	2.00
Mean			0.96	4.09	4.72	2.23	2.12
M_4	S_1	1.01	1.00	4.28	3.87	1.99	1.90
	S_2	1.00	0.943	3.08	4.06	2.09	1.57
	S_3	1.04	0.981	3.98	4.14	2.13	1.24
Mean			0.97	3.78	4.02	2.07	1.57
Mean	S_1	1.12	1.13	4.81	5.34	2.32	2.21
	S_2	1.09	1.08	4.41	5.31	2.34	2.14
	S_3	1.15	1.01	4.72	5.43	2.33	2.05
Grand mean		1.12	1.07	4.65	5.36	2.33	2.13
For comparing		SEm ±	CD @ 5%	SEm ±	CD @ 5%	SEm ±	CD @ 5%
Moisture regimes (M)		0.045	0.155	0.428	1.481	0.044	0.153
Genotypes (G)		0.035	NS	0.140	0.458	0.054	0.176
M X G		0.069	NS	0.281	NS	0.108	NS
Stages (S)		0.032	NS	0.059	0.170	0.026	NS
M X S		0.064	NS	0.118	NS	0.053	NS
G X S		0.046	NS	0.084	NS	0.037	NS
M X G X S		0.091	NS	0.167	0.482	0.075	0.216

V_1- GSHV-97/162, V_2-LRA-5166, M_1- Irrigated (100% Field capacity), M_2- Irrigated (75% Field capacity), M_3- Irrigated (50% Field capacity), M_4- Rainfed, S_1- Vegetative stage (No irrigation between 10-40 DAS), S_2- Flowering stage (No irrigation between 50-80 DAS), S_3- Boll development stage (No irrigation between 85-120 DAS)

Table 4: Effect of irrigation levels on water use efficiency (µ mol CO_2/ m mol H_2O) at different growth stages in cotton hybrids

Treatments		Days after sowing			
		90		120	
		V_1	V_2	V_1	V_2
M_1	S_1	0.36	0.36	0.16	0.13
	S_2	0.35	0.37	0.13	0.13
	S_3	0.38	0.28	0.12	0.12
Mean			0.34	0.14	0.12
M_2	S_1	0.41	0.37	0.15	0.14
	S_2	0.41	0.35	0.15	0.16
	S_3	0.40	0.38	0.16	0.15
Mean			0.36	0.15	0.15
M_3	S_1	0.40	0.28	0.17	0.18
	S_2	0.37	0.32	0.18	0.20
	S_3	0.36	0.34	0.18	0.18
Mean			0.31	0.18	0.19
M_4	S_1	0.35	0.40	0.19	0.21
	S_2	0.36	0.41	0.21	0.20
	S_3	0.36	0.22	0.22	0.20
Mean			0.34	0.21	0.20
Mean	S_1	0.38	0.35	0.17	0.16
	S_2	0.37	0.36	0.17	0.17
	S_3	0.37	0.31	0.17	0.16
Grand mean		0.38	0.34	0.17	0.17
For comparing		SEm ±	CD @ 5%	SEm ±	CD @ 5%
Moisture regimes (M)		0.022	NS	0.004	0.013
Genotypes (G)		0.016	NS	0.002	NS
M X G		0.031	NS	0.005	NS
Stages (S)		0.012	NS	0.003	NS
M X S		0.024	NS	0.005	0.014
G X S		0.017	NS	0.004	NS
M X G X S		0.034	NS	0.007	NS

V_1- GSHV-97/162, V_2-LRA-5166, M_1- Irrigated (100% Field capacity), M_2- Irrigated (75% Field capacity), M_3- Irrigated (50% Field capacity), M_4- Rainfed, S_1- Vegetative stage (No irrigation between 10-40 DAS), S_2- Flowering stage (No irrigation between 50-80 DAS), S_3- Boll development stage (No irrigation between 85-120 DAS)

Table 5: Effect of irrigation levels on percent light absorption at different growth stages in cotton hybrids

Treatments		Days after sowing			
		90		120	
		V_1	V_2	V_1	V_2
M_1	S_1	19.00	20.00	44.53	44.85
	S_2	20.00	19.00	41.38	43.28
	S_3	21.33	19.66	40.37	41.48
Mean			19.55	42.22	43.20
M_2	S_1	17.33	17.00	40.36	37.25
	S_2	18.66	17.00	38.80	36.68
	S_3	17.66	16.66	37.92	37.14
Mean			16.88	39.02	37.02
M_3	S_1	15.33	15.00	35.92	31.99
	S_2	14.00	15.00	33.82	30.83
	S_3	15.00	15.00	30.00	30.90
Mean			15.00	33.25	31.24
M_4	S_1	12.66	11.66	29.59	25.78
	S_2	12.00	12.00	27.78	25.43
	S_3	11.66	13.33	25.32	24.41
Mean			12.33	27.56	25.20
Mean	S_1	16.08	15.91	37.60	34.97
	S_2	16.16	15.75	35.44	34.05
	S_3	16.41	16.16	33.49	33.48
Grand mean		16.22	15.94	35.51	34.17
For comparing		SEm ±	CD @ 5%	SEm ±	CD @ 5%
Moisture regimes (M)		0.318	1.100	0.538	1.862
Genotypes (G)		0.223	NS	0.320	1.043
M X G		0.446	NS	0.640	NS
Stages (S)		0.202	NS	0.298	0.858
M X S		0.404	NS	0.595	NS
G X S		0.286	NS	0.421	1.213
M X G X S		0.571	NS	0.842	2.426

V_1- GSHV-97/162, V_2-LRA-5166, M_1- Irrigated (100% Field capacity), M_2- Irrigated (75% Field capacity), M_3- Irrigated (50% Field capacity), M_4- Rainfed, S_1- Vegetative stage (No irrigation between 10-40 DAS), S_2- Flowering stage (No irrigation between 50-80 DAS), S_3- Boll development stage (No irrigation between 85-120 DAS)

Table 6: Effect of irrigation levels on water potential (- bars) at different growth stages in cotton hybrids

Treatments		Days after sowing			
		90		120	
		V_1	V_2	V_1	V_2
M_1	S_1	13.66	13.33	22.66	18.00
	S_2	14.66	13.00	20.50	18.16
	S_3	13.33	13.00	18.50	19.16
Mean			13.11	20.55	18.44
M_2	S_1	18.00	14.00	20.00	19.83
	S_2	17.33	16.00	20.16	19.66
	S_3	15.00	17.66	20.00	19.33
Mean			15.88	20.05	19.61
M_3	S_1	18.33	18.00	20.33	22.50
	S_2	18.00	18.66	21.16	23.00
	S_3	18.33	18.00	22.00	21.50
Mean			18.22	21.16	22.33
M_4	S_1	22.00	19.41	21.66	23.36
	S_2	21.50	20.50	22.33	23.06
	S_3	21.66	21.00	23.83	22.83
Mean			20.30	22.61	23.08
Mean	S_1	18.00	16.18	21.16	20.92
	S_2	17.87	17.04	21.04	20.97
	S_3	17.08	17.41	21.08	20.70
Grand mean		17.65	16.88	21.09	20.86
For comparing		SEm ±	CD @ 5%	SEm ±	CD @ 5%
Moisture regimes (M)		0.333	1.153	0.539	1.865
Genotypes (G)		0.173	0.564	0.189	NS
M X G		0.346	NS	0.378	1.233
Stages (S)		0.294	NS	0.285	NS
M X S		0.588	NS	0.570	NS
G X S		0.416	1.198	0.403	NS
M X G X S		0.832	NS	0.807	2.324

V_1- GSHV-97/162, V_2-LRA-5166, M_1- Irrigated (100% Field capacity), M_2- Irrigated (75% Field capacity), M_3- Irrigated (50% Field capacity), M_4- Rainfed, S_1- Vegetative stage (No irrigation between 10-40 DAS), S_2- Flowering stage (No irrigation between 50-80 DAS), S_3- Boll development stage (No irrigation between 85-120 DAS)

Table 7: Effect of irrigation levels on yield and yield components at different growth stages in cotton hybrids

Treatments		Yield (Kg/ha)		Boll weight (g/boll)		Number of bolls/plants	
		V_1	V_2	V_1	V_2	V_1	V_2
M_1	S_1	1613.00	1612.00	4.33	4.10	20.00	17.66
	S_2	1605.00	1610.66	4.36	4.00	16.00	16.33
	S_3	1589.00	1588.66	3.76	3.63	16.66	15.66
Mean			1603.77	4.15	3.91	17.55	16.55
M_2	S_1	1528.00	1521.00	3.63	3.36	15.33	15.00
	S_2	1515.00	1511.62	3.76	3.36	15.00	13.33
	S_3	1506.66	1487.66	3.23	3.16	14.00	13.33
Mean			1506.77	3.54	3.30	14.77	13.88
M_3	S_1	1487.66	1466.00	3.33	3.33	13.66	12.00
	S_2	1450.33	1441.33	3.00	3.20	11.66	11.33
	S_3	1422.00	1423.33	3.00	3.00	11.33	12.66
Mean			1443.55	3.11	3.17	12.22	12.00
M_4	S_1	1418.33	1418.33	3.03	2.80	11.00	8.33
	S_2	1412.00	1410.33	2.83	2.56	8.66	6.33
	S_3	1387.66	1388.67	2.63	2.50	9.00	7.00
Mean			1405.77	2.83	2.62	9.55	7.22
Mean	S_1	1511.75	1504.33	3.58	3.40	15.00	13.25
	S_2	1495.58	1493.05	3.49	3.28	12.83	11.83
	S_3	1476.33	1472.08	3.15	3.07	12.75	12.16
Grand mean		1494.56	1489.97	3.41	3.25	13.52	12.41
For comparing		SEm ±	CD @ 5%	SEm ±	CD @ 5%	SEm ±	CD @ 5%
Moisture regimes (M)		0.544	1.884	0.051	0.175	0.638	2.209
Genotypes (G)		0.707	2.307	0.020	0.064	0.247	0.805
M X G		1.414	4.613	0.039	0.128	0.494	NS
Stages (S)		0.935	2.695	0.037	0.106	0.314	0.905
M X S		1.871	5.390	0.074	NS	0.628	NS
G X S		1.323	NS	0.052	NS	0.444	NS
M X G X S		2.646	7.622	0.104	NS	0.888	NS

V_1- GSHV-97/162, V_2-LRA-5166, M_1- Irrigated (100% Field capacity), M_2- Irrigated (75% Field capacity), M_3- Irrigated (50% Field capacity), M_4- Rainfed, S_1- Vegetative stage (No irrigation between 10-40 DAS), S_2- Flowering stage (No irrigation between 50-80 DAS), S_3- Boll development stage (No irrigation between 85-120 DAS)

8. Integrated Analysis of Physiological and Agronomic Traits Using Heatmap Visualization

A heatmap was used to visualize the integrated response of cotton to four irrigation regimes (M_1-M_4), based on key physiological traits and yield (Fig.1). The analysis showed a clear decline in yield, photosynthetic rate, transpiration, light absorption and water potential from full irrigation (M_1) to rainfed (M_4) conditions. Seed cotton yield dropped from 1603.05 kg/ha (M_1) to 1405.88 kg/ha (M_4), closely tracking reductions in photosynthesis (from 28.7 to 24.1 μmol m^{-2} s^{-1}) and light absorption (from 42.7% to 26.4%).

Water use efficiency (WUE) was highest under moderate stress (M_2), indicating partial physiological compensation. Stomatal conductance showed minor variation, suggesting a balance between water conservation and carbon assimilation. Water potential became more negative with increasing stress, confirming drought severity.

The heatmap highlighted strong trait interdependence and showed that drought stress impairs photosynthetic efficiency, water transport and ultimately yield. Genotype V_1 generally outperformed V_2 under stress, showing better physiological stability. This visual analysis supports the use of integrated trait-based approaches to guide irrigation management and genotype selection for drought resilience.

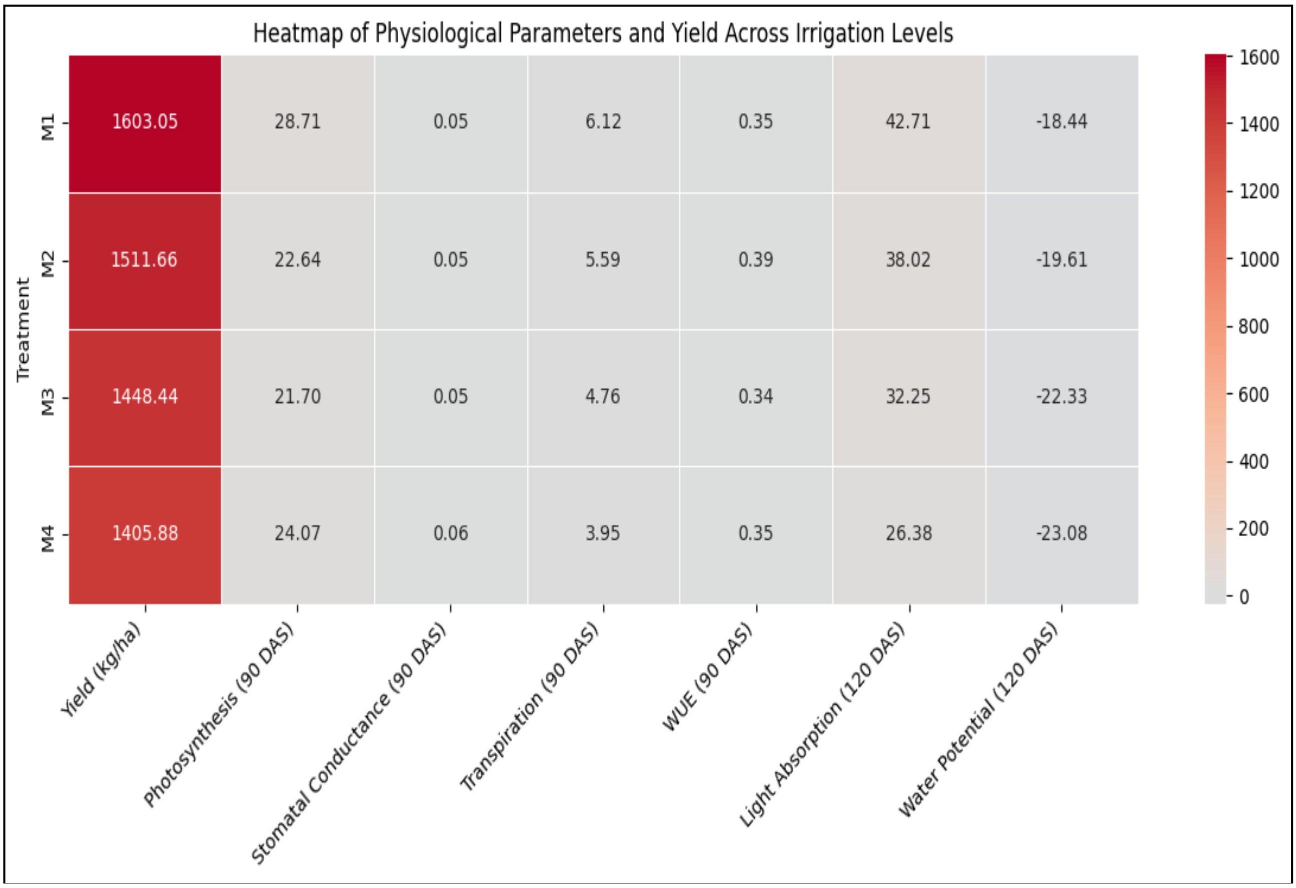

Fig 1: Heatmap visualizing the variation in physiological traits and yield performance of cotton averaged across two genotypes (V_1: GSHV-97/162 and V_2: LRA-5166) under four irrigation treatments: M_1 (100% Field Capacity), M_2 (75% FC), M_3 (50% FC), and M_4 (Rainfed). The measured variables include seed cotton yield (kg/ha), photosynthetic rate at 90 DAS (μmol m^{-2} s^{-1}), stomatal conductance (μmol m^{-2} s^{-1}), transpiration rate (μmol CO_2 m^{-2} s^{-1}), water use efficiency (WUE, μmol CO_2 $mmol^{-1}$ H_2O), percent light absorption at 120 DAS, and leaf water potential (bars)

Conclusion

The present study demonstrated that deficit irrigation significantly affects the physiological performance and yield of cotton. Among the treatments, moderate water stress (75% field capacity) optimized water use efficiency without causing substantial yield loss, while severe stress (rainfed) led to marked declines in photosynthesis, transpiration, light absorption, and boll development. Genotype GSHV-97/162 (V1) exhibited superior drought tolerance compared to LRA-5166 (V2), maintaining higher gas exchange rates, WUE, and yield under stress. The integrated use of physiological indicators and heatmap analysis proved effective in identifying trait interdependencies and genotype resilience. These findings underscore the importance of genotype selection and growth-stage-specific irrigation management for sustaining cotton productivity in water-limited environments.

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