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Quantifying the effects of drought stress on cucumber genotypes differing in membrane integrity

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Abstract

Cucumber yield is profoundly influenced by soil moisture, with drought representing a pivotal factor. This study evaluated four cucumber lines (CBECS-37, CBECS-38, CBECS-19, and CBECS-7) within a split-plot experimental design comprising four replications. Irrigation occurred once every seven days, spanning from sowing to the flowering stage. Drought stress was imposed at two critical stages: from flower bud initiation to harvesting (withheld irrigation for 25 days) and from flowering to harvesting (withheld irrigation for 15 days). Morphological and physiological parameters, including plant height, primary branch count, days to first male and female flower appearance, total soluble solids (TSS), relative water content (RWC), chlorophyll content, leaf electrolyte leakage, and malondialdehyde, were assessed 15 days after drought stress. Results indicated greater membrane damage during the flower bud initiation to the harvesting stage (404.5%) compared to the flowering to the harvesting stage (304.6%). Thus, drought stress during flower bud initiation to harvesting was more critical. CBECS-7 demonstrated the highest tolerance to drought conditions, displaying superior outcomes in primary branches, plant height (20.6%), chlorophyll a (16.7%), chlorophyll b (53.4%), total chlorophyll (26.7%), and RWC (6.7%). CBECS-7 exhibited increased chlorophyll content, enhanced photosynthetic activity, robust vegetative growth, and prolific flower and fruit production. These findings establish CBECS-7 as a drought-tolerant line during flower bud initiation to harvesting. In conclusion, this study underscores the critical nature of the flower bud initiation to the harvesting stage and identifies CBECS-7 as a drought-tolerant cucumber line.

Key words: Cucumber, drought stress, lines, tolerance, TSS, plant height, chlorophyll

Introduction

Cucumber (*Cucumis sativus* L.) is a monoecious annual crop, ranking as the second most cultivated vegetable globally after watermelon. In Asia, it stands as the fourth most significant vegetable crop, offering vital vitamins and minerals, notably vitamin A. Cucumber is renowned for its cooling properties, preventing digestive issues and aiding in jaundice control (Nandkarni and Prakash, 1927). This low-calorie vegetable (15 calories/100g) is 95% water, an ideal hydrator, rich in antioxidants, potassium, vitamin K (Pandey *et al.,* 2020), vitamins 'B' and 'C,' plus minerals like calcium, phosphorus, iron, and potassium. Its antioxidants combat harmful free radicals, preventing diseases (Pandey *et al.,* 2020). Nutritionally, cucumber offers carbohydrates (3%), protein (1%), total fat (0.5%), and fiber (1%) per 100g (Nwofia *et al.,* 2015).

India cultivates cucumbers across 0.122 million ha, yielding 1.71 MT (Indiastat, 2022), with increased yield as the primary target (Devi *et al.,* 2022). However, abiotic stresses, especially drought challenge cucumber production, affecting plant growth and fruit quality (Chmielewska *et al.,* 2016; Aujla *et al.,* 2007). Cucumber's global importance, nutritional value, and resilience underscore its significance in agriculture.

The cucumber is a relatively shallow-rooted crop and it is susceptible to drought. Typically, cucumber requires much water, particularly at the fruiting stage (Swiader *et al.,* 2002). Reduced plant growth and yield under drought stress are associated with phytohormones and reactive oxygen species (ROS) signaling changes, plant hydraulic status, and osmotic adjustment (Khan *et al.,* 2015). To mitigate the damage from ROS, plants evolve enzymatic antioxidants like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), as well as non-enzymatic antioxidants like ascorbic acid and glutathione (Xu *et al.,* 2008).

Wahb-Allah *et al.* (2011) studied the impact of irrigation regime on tomato growth and yield and reported that tomato growth parameters and yield decreased significantly at a lower soil moisture level than its fully irrigated conditions. In muskmelon, RWC was found to be significantly associated with yield (Barzegar *et al.,* 2017). Similarly, Rehman *et al.* (2023) showed that chlorophyll content was significantly decreased in melon genotypes under drought stress conditions, and the highest decrease was observed in sensitive genotypes. Ansari *et al.* (2018) found in muskmelon that drought stress altered the activity of antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX).

Due to its osmotic effect, drought has been widely studied in many crops, but cucumber has not been extensively investigated in relation to drought despite seedlings being more susceptible to water changes than mature plants.

This research aims to assess the impact of different irrigation regimes, specific cucumber lines, and their interplay on the growth and physiological aspects of cucumber genotypes.

Materials and methods

The field experiment was carried out during the summer of 2023 at the Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The details of the line are given in Table 1.

Table 1. List of genotypes and their place of collection

S.No	Lines	Place of collection
1.	CBECS-37	Namakkal, Tamil Nadu, India
2.	CBECS-38	Vadalur, Tamil Nadu, India
3.	CBECS-19	Namanasamuthiram, Tamil Nadu, India
4.	CBECS-7	Pirattiyur, Tamil Nadu, India

Crop husbandry: The main field was ploughed thrice with 5 tyre, 3 tires, and cultivator. Pits of 45 cm diameter and 30 cm depth were formed at a spacing of 1.5 × 1.5m. Three seeds per pit were sown. The cultural and management practices were followed as per the package of practices recommended by the crop production guide.

Drought stress imposition: The experiment was conducted in a split-plot design with four replications. The main plots were irrigation regimes (T_1: irrigated once in seven days; T_2: withholding irrigation for 25 days from flower initiation to harvest stage, and T_3: withholding irrigation for 15 days from flowering stage to harvest stage) and subplots were lines (CBECS-37, CBECS-38, CBECS-19, and CBECS-7). The crop was irrigated once in seven days from the sowing to the flowering stage. After that, drought stress was imposed by withholding water. Drought stress during flower initiation to the harvest stage was imposed by withholding irrigation water for 25 days from flower bud initiation. Similarly, drought stress was imposed during the flowering to harvesting stage for 15 days.

Morphological traits: The morphological traits like plant height and number of primary branches were recorded at maturity. However, the days to the first male and female flower appearance were recorded at two days intervals. The physiological traits namely relative water content (RWC), chlorophyll content, leaf electrolyte leakage and malondialdehyde (MDA) contents, were recorded in the third leaf from the top on 15th day after drought stress. TSS (°Brix) was recorded in fruits at harvest.

Total l (°Brix): A hand refractometer was used for assessing TSS.

Relative water content: The procedure given by Barrs and Weatherley (1962) was used to estimate the RWC:

$$\text{RWC} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}}$$

Leaf electrolyte leakage: According to Liu *et al.* (2011) methodology, electrical conductivity was used to assess the stability of the cell membrane. The following formula was used for calculation:

$$\text{Electrolyte leakage (\%)} = \frac{\text{Initial electrical conductivity}}{\text{Final electrical conductivity}} \times 100$$

Chlorophyll content: The method given by Lichtenthaler and Wellburn (1983) was used to extract pigments from leaf tissue, including chlorophyll *a*, chlorophyll *b* and total chlorophyll content.

Malondialdehyde (MDA): The lipid peroxidation was determined by measuring the breakdown product MDA as suggested by Heath and Packer (1968). Using 0.1 % TCA, 0.5 g of leaf sample was extracted and centrifuged at 13,000 rpm for 20 min. After centrifugation, the supernatant was taken from 0.5 mL and added to 0.5 % TBA in 20 % TCA, which was later kept in a hot water bath for 30 mins at 95 ºC, then placed in a water bath to cool. The absorbance was calculated using the extinction value of 155 mM^{-1} cm^{-1} and estimated at 523 and 600 nm.

Statistical analysis: Data were assessed using R- program (Version 4.3.1) using the Agricolae package 1.2 -8. The Analysis of Variance was performed to determine the significance of the variance. Least significance difference (LSD) at 0.05 significance level was used to compare the difference between treatment means.

Result and discussion

Plant height: There was no significant (P<0.05) differences among the lines; however, the effect of irrigation regime and interaction of irrigation regimes and lines for plant height was significant (Table 2). Among the irrigation regime, drought stress during flower initiation to maturity decreased the plant height (96.9 %) over irrigated control. However, drought stress during flowering to maturity decreased plant height by (96.2 %). Among the lines, CBECS-7 had the highest plant height than other lines and the percentage increased over CBECS-37 (89.7 %), CBECS-38 (92.5 %), CBECS-19 (80.8 %). In interaction, the line CBECS-7 exposed to drought stress from flowering to harvest had the highest plant height (99.6 %) and CBECS–19 had the lowest plant height (99.5 %) during flowering to maturity. Drought stress has been shown to negatively impact the plant height of melon (Kusvuran, 2012) and wild barley genotypes have also been found to cause a 31 % decrease in the plant height. It might be due to the morpho-anatomical changes associated with tolerant genotypes under drought stress, which might helped the plants to complete their physiological and metabolic activities and eventually affect the growth rate.

Table 2. Plant height and number of primary branches of cucumber genotypes under different irrigation levels

Genotypes (G)	Plant height (cm)			Primary branches (plant^{-1})		
	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)
CBECS-37	182.50	175.25	174.25	3.50	2.50	2.50
CBECS-38	191.75	182.50	179.25	3.00	2.25	2.50
CBECS-19	161.00	155.00	161.25	3.25	2.50	2.25
CBECS-7	199.00	195.50	198.25	3.25	2.50	3.00
Mean	183.56	177.06	178.25	3.25	2.44	2.56
CD (0.05)						
T		NS			0.59**	
G		5.35**			NS	
T x G		NS			NS	

NS=Not Significant, **= Significance at P<0.05

Number of primary branches: There was no significant (P<0.05) differences among the lines and interaction of irrigation regimes and lines; however, the effect of irrigation regime was significant (P<0.05) (Table 2). Among the irrigation regime, drought stress during flower initiation to maturity decreased the primary branches (95.9 %) over irrigated control. However, drought stress during flowering to maturity increased primary branches by (96.2 %). Among the lines, CBECS-38 had the highest primary branches than other lines and the percentage increased over CBECS-37 (100 %), CBECS-19 (100 %), CBECS-7 (97.2 %). In interaction, the line CBECS-38 exposed to drought stress from flowering to harvest had the highest primary branches (109.3 %) and CBECS–19 had the lowest primary branches (100 %) during flowering to maturity. Parveen *et al.* (2019) observed that there was a maximum reduction in the number of branches in Kashi Amrit (14.33).

Days to first male and female flower appearance: Among the irrigation regimes, drought stress during flower initiation to maturity stage and flowering to maturity stage there was no significant difference in days to first male and female flower appearance over irrigated control. Lesser number of days to first male flower appearance was recorded in CBECS - 19 under both drought conditions. Among the lines, CBECS-37 and CBECS - 7 taken lesser number of days to first female flower appearance under drought stress condition. In interaction, there was no significant difference between the lines and irrigation regime. Early flowering had been affected by drought stress, which resulted in flower abscission and limited fertilisation (Lamin-Samu *et al.,* 2021) and drought stress increased the dropping of flowers and young fruits during flowering and fruit set stages, which has a significant negative impact on the final crop.

Table 3. Days to first male and female flower appearance under control and drought stress condition

Genotypes (G)	Days to first male flower		Days to first female flower			
	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)
CBECS-37	35.83	34.50	35.00	40.75	46.00	43.00
CBECS-38	35.00	35.17	34.67	40.25	47.50	41.00
CBECS-19	32.42	31.58	31.67	45.00	50.00	42.50
CBECS-7	34.92	32.75	32.75	41.00	47.25	40.50
Mean	34.54	36.17	33.83	41.75	47.69	41.75
CD (0.05)						
T	NS				2.28**	
G	0.76**				1.88**	
T at G	NS				NS	

NS=Not Significant, **= Significance at P<0.05

Total soluble solids (TSS): There was a significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for TSS (Table 4). Among the irrigation regimes, drought stress during flower initiation to maturity fruit set was hindered due to flower dropping. However, drought stress during flowering to maturity increased TSS by (115.5 %). Among the lines, CBECS-7 had the highest TSS than other lines and the percentage increased over CBECS-37 (84.2 %), CBECS-38 (83.4 %), CBECS-19 (95.4 %). In interaction, the line CBECS-7 exposed to drought stress from flowering to harvest had the highest TSS (112 %) over the control and CBECS-38 had the lowest TSS (84 %) over the control during flowering to maturity. Fruit TSS was increased during stress condition in tomato (Nora *et al.,* 2012). This phenomenon results from a rise in phloem sap concentration and a decrease in its flux, with the phloem flux being what causes the growth in tomato size (Guichard *et al.,* 2001). Meanwhile, a decrease in its flow combined with an increase in the concentration of sugar leads to smaller fruits with a higher dry matter content.

Relative water content: There was a significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for RWC (Table 4). Among the irrigation regimes, drought stress during flower initiation to maturity decreased the RWC (91 %) over irrigated control. However, drought stress during flowering to maturity decreased the RWC (80.9 %). Among the lines, CBECS-7 had the highest RWC over other lines and the percentage increased over CBECS-37 (82.3 %), CBECS-38 (85.8 %), CBECS-19 (84.3 %). In interaction, the line CBECS-7 exposed to drought stress from flowering to harvest had the highest RWC (87.08 %) over the control and CBECS-37 had the lowest RWC (77.6 %) over control during flowering to maturity. Patane *et al.* (2016) observed that in okra and tomato, the RWC was reduced, respectively with imposing drought tolerance. These findings suggest that these drought-resistant cucumber lines have a significant adaptation mechanism because they can maintain low leaf transpiration rates and a higher RWC, which leads to an osmotic adjustment by proline accumulation.

Table 4. TSS and RWC of cucumber genotypes under different irrigation levels

Genotypes (G)	TSS (°Brix)			RWC (%)		
	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)
CBECS-37	3.78	-	4.50	90.08	77.91	66.08
CBECS-38	3.75	-	4.45	87.08	72.88	56.28
CBECS-19	4.40	-	4.98	85.69	72.70	55.62
CBECS-7	4.63	-	5.20	88.95	73.92	63.33
Mean	4.14	-	4.78	87.95	74.35	60.33
CD (0.05)						
T	0.176**			4.30**		
G	0.120**			8.15**		
T at G	0.251**			NS		

NS=Not Significant, **= Significance at P<0.05 '- '= fruit was not developed.

Leaf electrolyte leakage (EL): There was significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for EL (Fig. 1). Among the irrigation regimes, drought stress during flower initiation to maturity had increased EL by (304.6 %) over irrigated control. However, drought stress during flowering to maturity increased EL by (404.5 %). Among the lines, CBECS-7 had the highest EL over other lines and the percentage increased over CBECS-37 (82.9 %), CBECS-38 (66.4 %), CBECS-19 (72.7 %). In interaction, the line CBECS-7 exposed to drought stress from flowering to harvest stage had the highest EL (384 %) and CBECS-38 had the lowest EL (352 %) during flowering to maturity over the control. A decrease in membrane integrity results in enhanced ion leakage, which is indicated by an increase in electrical conductivity. Plants that are subjected to stresses like drought have this trait by nature (Korkmaz *et al.,* 2007).

Chlorophyll content: There was significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for chlorophyll a, chlorophyll b and total chlorophyll content (Table 5). Among the irrigation regimes, drought stress during flower initiation to maturity decreased the chlorophyll a content (76.4 %) over irrigated control. However, drought stress during flowering to maturity decreased chlorophyll a content by (71.5 %). Among the lines, CBECS-7 had the highest chlorophyll

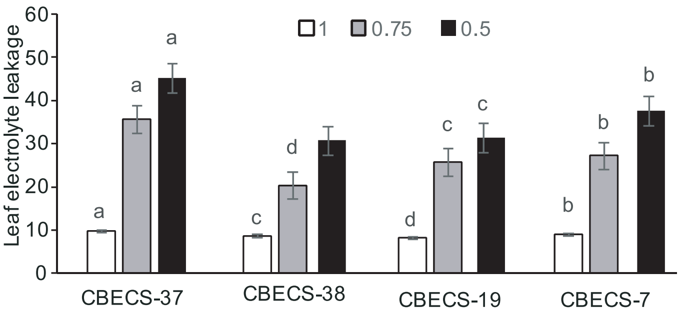

Fig. 1. Leaf electrolyte leakage of cucumber genotypes under different irrigation levels. □ irrigated once in seven days; ▒ withholding irrigation for 25 days from flower initiation to harvest stage, and ■ withholding irrigation for 15 days from flowering stage to harvest stage

a content then other lines and the percentage increased over CBECS-37 (94.7 %), CBECS-38 (85.7 %), CBECS-19 (98.8 %). Among the genotype interaction, the line CBECS-7 had the highest chlorophyll a content (74.7 %) then other treatment during flowering to harvest and CBECS-38 had the lowest chlorophyll a content (59.1 %) drought stress during flowering to maturity. Among the irrigation regimes, drought stress during flower initiation to maturity decreased the chlorophyll b content (90.5 %) over irrigated control. However, drought stress during flowering to maturity decreased chlorophyll b content (77.8 %). Among the lines, CBECS-7 had the highest chlorophyll b content then other lines and the percentage increased over CBECS-37 (91.3 %), CBECS-38 (90.8 %), CBECS-19 (40.6 %). Among the genotype interaction, the line CBECS- 7 had the highest chlorophyll b content (90.4 %) then other treatment during flowering to harvest and the line CBECS-19 had the lowest chlorophyll b content (47.7 %), drought stress during flowering to maturity. Among the irrigation regimes, drought stress during flower initiation to maturity decreased the total chlorophyll content (85.6 %) over irrigated control. However, drought stress during flowering to maturity decreased total chlorophyll content (82.1 %). Among the lines, CBECS-7 had the highest total chlorophyll content over other lines and the percentage increased over CBECS-37 (83 %), CBECS-38 (75.8 %), CBECS-19 (74 %). Among the genotype interaction, the line CBECS- 7 had the highest total chlorophyll content (92.7 %) than other treatments during flower initiation to maturity and the line CBECS-38 had the lowest total chlorophyll content (76.5 %), drought stress during flowering to maturity. This decreases may be due to oxidative stress, decreased chlorophyll synthesis and increased chlorophyll breakdown (Kumar and Singh, 1996). Asharaf and Mahmood (1990) reported drought stress would lower chlorophyll b concentrations more than chlorophyll a and under water stress conditions, plants' total chlorophyll content was reduced.

Malondialdehyde (MDA): There was significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for MDA (Fig. 2). Among the irrigation regimes, drought stress during flower initiation to maturity had increased the MDA (109.8 %) over irrigated control. However, drought stress during flowering to maturity increased MDA by 120.7 %. Among the lines, CBECS-37 had the highest MDA over other lines and the percentage increased over CBECS-38 (85.8 %), CBECS-19 (85.9 %), CBECS-7 (89.9 %). Among the genotype interaction, line CBECS- 37 had the highest MDA (141.6 %) than other treatments during flowering to maturity and line CBECS-19 had the lowest MDA (114.9 %), drought stress during flower initiation to maturity. In this study, MDA was increased under drought stress. Numerous investigations showed that there was a link between electrolyte leakage and MDA. MDA, a secondary oxidative product, is formed when the accumulated ROS degrades the polyunsaturated lipids in the cell membranes (Ayala *et al.,* 2014).

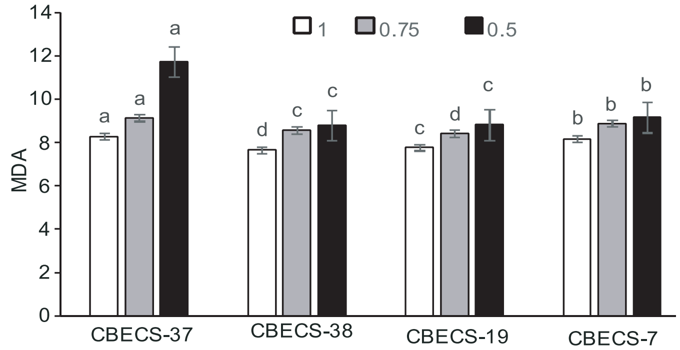

Fig. 2. Malondialdehyde activity of cucumber genotypes under different irrigation levels. □ irrigated once in seven days; ▒ withholding irrigation for 25 days from flower initiation to harvest stage, and ■ withholding irrigation for 15 days from flowering stage to harvest stage.

Overall, based on this study, it could be stated that advances in biotechnology, such as genetic engineering and gene editing techniques like CRISPR-Cas9, offer the potential to enhance drought tolerance in crops. The CRISPR/Cas9 system is a cutting-edge technology that enhances crop improvement by producing high-yielding, high-quality resistant plants to biotic and abiotic stresses. However, challenges include selecting genes for mutations, genome sequencing, and delivering CRISPR-Cas agents into plant cells. Creating a universal and effective genetic transformation and regeneration system for vegetable crops remains challenging due to the need for precise alterations and genome sequencing. Identifying and modifying specific genes responsible for drought response is possible, thereby creating more resilient and water-efficient plant varieties. A multidisciplinary approach involving genetics, biochemistry, biotechnology, physiology, plant breeding, and crop science will be appropriate to evolve superior drought-resistant genotypes. Breeding programs focused on developing climate-resilient crop varieties could lead to the cultivation of drought-tolerant

Table 5. Chlorophyll a, Chlorophyll b, Total chlorophyll of cucumber genotypes under different irrigation levels

Genotypes (G)	Chlorophyll a (mg g^{-1})			Chlorophyll b (mg g^{-1})			Total chlorophyll (mg g^{-1})		
	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)
G_1 - CBECS-37	1.07	0.92	0.86	0.61	0.54	0.49	1.86	1.46	1.36
G_2 - CBECS-38	1.15	0.75	0.68	0.60	0.55	0.47	1.65	1.37	1.27
G_3 - CBECS-19	1.19	0.92	0.86	0.30	0.28	0.14	1.54	1.35	1.30
G_4 - CBECS-7	1.19	0.93	0.89	0.63	0.60	0.57	1.97	1.84	1.80
Mean	1.15	0.88	0.82	0.54	0.49	0.42	1.76	1.51	1.43
CD (0.05									
T	0.01**			0.01**			0.01**		
G	0.01**			0.01**			0.01**		
T at G	0.02**			0.02**			0.02**		

**= Significance at P<0.05

plants that can thrive under changing environmental conditions. Drought tolerance is a complex trait influenced by multiple genes and environmental factors. Developing plants with high drought tolerance involves a deep understanding of the genetic and physiological mechanisms underlying this trait.

The results of the present study indicate that the drought exerted different effects on cucumber lines under different levels of drought conditions. It may be concluded that CBECS-7 shows maximum tolerance to imposed drought conditions with better results in plant height, TSS, chlorophyll a, chlorophyll b, total chlorophyll, leaf electrolyte leakage, RWC followed by CBECS-37 in MDA. Among the different genotypes evaluated, CBECS-7 was the best genotype for the drought conditions, as it showed maximum chlorophyll content, which increases photosynthetic activity and results in good vegetative growth with the maximum number of flowers and fruits.

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