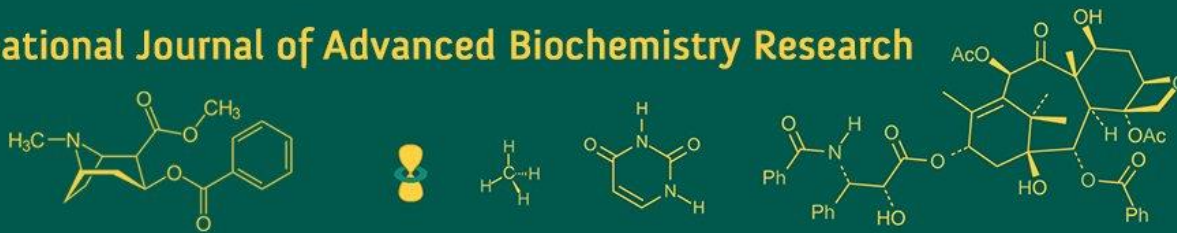


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Physiological and biochemical changes during *in vitro* germination under salinity stress in green gram (*Vigna radiata* (L.) R. Wilczek)

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Abstract

In this study, ten genotypes of green gram (V₁: GJM 1701, V₂: GJM 1714, V₃: GJM 1837, V₄: GJM 1901, V₅: GM 4, V₆: GM 6, V₇: GM 7, V₈: GAM 5, V₉: K 851, V₁₀: Meha) investigated the salt tolerance and salt susceptible under various saline water treatments (T₀ to T₄ = DW to 8 EC). Different genotypes displayed significant variation in physiological and biochemical characteristics. The experimental results revealed that with increase in salinity levels, greater reduction was observed for physiological and biochemical parameters. All physiological and biochemical parameters were found reduced in all the genotypes studied with more reduction at higher salinity (8 EC) level rather than control (Distilled water) level. In physiological parameters, the salt-tolerant variety (GM 6) showed the highest GP% (97.33%) and STI (76.91%), while GJM 1701 showed the lowest GP% (88%) and STI (64.03%) as compared to other varieties. Higher saline water stress causes a decrease in a number of biochemical parameters, including total chlorophyll content, chlorophyll a, and chlorophyll b. However, the salt-susceptible variety GJM 1701 has the lowest total chlorophyll content and the salt-tolerant variety GM 6 has the highest levels of these biochemical parameters. However, salinity causes an increase in lipid peroxidation, especially in the susceptible (GJM 1701) variety. In summary, it was concluded that the GM-6 cultivar could tolerate saline irrigation water. GM 6 cultivar exhibits superior tolerance criteria and performs better at varying salt levels. In terms of salinity tolerance, the following is the order of green gram varieties: GM 6 > GM 7 > Meha > GAM 5 > K 851 > GM 4 > GJM 1901 > GJM 1714 > GJM 1701.

Keywords: Green gram, genotype, salinity, stress, chlorophyll

Introduction

A major traditional legume crop grown in India is mungbean. Mungbean, also known as green gram [*Vigna radiata* (L.) R. Wilczek], is a warm-season, short-duration leguminous pulses crop. The main areas of cultivation for this crop are Australia, East Africa, and Asia. (Pratap *et al*, 2021) ^[1] Approximately 7.3 million ha are cultivated with mungbean around the world, producing an average yield of 721 kg/ha. (Nair and Schreinemachers, 2020) ^[2] Providing nutritious and healthful food to the poor people is a challenge for developing nations. Due to the high nutritional value mungbean used as functional food.

Food security is a major problem in developing countries because of the increasing population and soil degradation. In India, around 23 million ha of land are affected by salinity. Approximately 75% of salt affected soil in the country exist in the state of Gujarat (2.23 million ha). (Kumar and Sharma, 2020) ^[8].

Salinity stress is one of the most atrocious environmental factors restricting the productivity of mungbean in arid and semiarid regions Saline zones in Gujarat, particularly in Ghed and Bhal coastal regions, pose a significant threat to food crops in Saurashtra and Gujarat. 78% of the Saurashtra-Kutch region is covered by the Arabian Sea, causing reduced plant efficiency and lower dry matter yield per seed.

Seed germination is a critical stage of plant life; it is highly impacted by salinity. Salinity altered the growth as well as metabolism activity of plants. [(Munns, 2009) ^[3] (Ma, 2020) ^[4]] Legumes are highly sensitive to salt stress. According to Prakash (2017) ^[5], salinity decreases and delays the germination of green gram. Higher salt levels significantly decreased the overall percentage of seed germination, while lower salinity levels

delayed germination. Measurement of salt tolerance index (STI) of salt tolerance and salt susceptible genotype has revealed the different seedling dry weight. Salt tolerance genotypes has higher dry weight of seedling as compare to susceptible genotypes. (Masuda *et al.*, 2021) [9].

To use the quick screening method to determine the difference between salt tolerance and salt-susceptible genotypes, various physiological and biological characteristics are used. [(Merah *et al.*, 2023) [7] (Trivedi *et al.*, 2021) [6]]. In an attempt to provide quick screening techniques for salt tolerance, several researchers have attempted to assign genotype differences between salt-tolerant and sensitive plants based on biochemical characteristics such as chlorophyll content (El-Shaieny, 2015) [10], free proline (Misra and Gupta, 2005) [11], glycine betaine (Misra and Gupta, 2005) [11], and lipid peroxidation (Yasar *et al.*, 2008) [12].

The main objective of this particular research was to distinguish between the salt-susceptible genotype and the salt-tolerant genotypes. We selected the 10 genotypes from the pulse research station, J.A.U., Junagadh 362001.

Materials and Methods

The research was conducted at the Department of Biochemistry, Junagadh Agriculture University, Junagadh, Gujarat, India, during the 2022-23 period. The study utilized seeds from ten different green gram genotypes sourced from the Pulse Research Station, J.A.U., Junagadh. These genotypes included: GJM 1701, GJM 1714, GJM 1837, GJM 1901, GM 4, GM 6, GM 7, GAM 5, K 851, and Meha. Salinity treatments were administered at five levels: Distilled water (<2EC), 2 EC, 4 EC, 6 EC, and 8 EC. Ten seeds of each genotype were sown in petri plates under laboratory conditions with different salinity level. The experimental design employed a completely randomized block design (RBD), with three replications per treatment.

Determination of physiological parameters

Disease free healthy ten seeds of green gram genotypes sown in petridish with various saline water treatments. These seeds were distributed evenly among the petri dishes. The petri dishes were then positioned in lab condition at 28-32 °C, optimal for green gram germination.

Germination percentage

Germination percentage was recorded under control and saline condition at 6 days after sowing. The germination percentage (%) was subsequently calculated following the formula outlined by I.S.T.A. (1976).

$$\text{Germination Percentage (GP\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seed sown}}$$

Relative water content: Relative water content was measured by fresh weight, dry weight and turgid weight of green gram leaves at 15 DAS. RWC were estimated as per formula and expressed as percent relative water content.

$$\text{RWC (\%)} = \frac{[\text{Fresh weight (g)} - \text{Dry weight (g)}] \times 100}{\text{Turgid Weight (g)} - \text{Dry Weight (g)}}$$

Salt tolerance index (STI): Root length and shoot length was measured at 15 DAS and salt tolerance index (STI) was calculated using formula given by Rahman *et al.* (2008) [16].

*It was calculated using the data obtained on plant root length and shoot length in various saline water treatments and control, expressed as percentage.

$$\text{STI} = \frac{\text{Growth in particular treatments}}{\text{Growth in control}} \times 100$$

Where, Growth = Root length + Shoot length

Determination of biochemical parameters

Estimation Chlorophyll content

The chlorophyll a, chlorophyll b and total chlorophyll content were determined from green gram leaves at 15 DAS using the method.

Estimation of free proline content

Free proline content was measured from green gram seedling at 15 DAS using the method.

Estimation of lipid peroxidation

The level of lipid peroxidation was determined from green gram seedling at 15 DAS as 2-thiobarbituric acid (TBA) reactive metabolites chiefly malondialdehyde (MDA) accumulation as described.

*Lipid peroxidation was determined from green gram seedling at 15 DAS by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid reaction as described by (Panda and Khan, 2009).

Estimation of glycine betaine

Glycine betaine was measured from green gram seedling at 15 DAS using the method.

Results and Discussion

The present study was conducted to evaluate and compare the performance of ten green gram genotypes under different saline water treatments. Salinity show the genotypic difference in mung bran.

Physiological parameters

Effect of salinity on seed germination

Salinity had significant difference on germination percentage (GP%), increasing water salinity level decreased seed germination and absence of salinity almost 100% germination was observed in all genotypes. The significantly higher (97.33%) mean value of germination percentage was recorded for the genotype GM 6 (V₆) and it remained statistically at par with genotype GM 7 (V₇) with 95.53% germination. The significantly lower germination percentage (88.00%) was found in GJM 1701 (V₁) (Table 1). The results of decreasing germination percentage under salt stress align with those of Misra and Dwivedi (2004) [13], as well as with the study by Riddhi *et al.* (2019) [14].

Effect of salinity on Relative Water Content (RWC)

The relative water content of a leaf indicates its maximal hydration capacity, or full turgidity. It gives an indication of the level of stress expressed. In present study, RWC was examined from the leaves of various green gram genotypes at 15 DAS. The significantly higher mean value (89.49%) of RWC was recorded for the genotype GM 6 (V₆) and it remained statistically at par with genotype GM 7 (V₇) with 88.59%. The significantly lower (79.32%) RWC was found in GJM 1701 (V₁). Mean effect of treatments on RWC was

significantly varied between 93.54% and 76.85%. Treatment T₀ irrigated with distilled water showed highest (93.54%) mean value of RWC, while the T₄ irrigated with 8 EC showed lowest (76.85%) mean value of RWC after 15 DAS (Table 2). A significant reduction in RWC under salinity supported by Riddhi *et al.* (2019)^[14].

Effect of salinity on salt tolerance index (STI)

The salt tolerance index was recorded based on root and shoot length of green gram seedlings at 15 days after sowing (DAS) and showed statistically significant differences. The significantly higher (76.91%) mean value of salt tolerance index was recorded for the genotype GM 6 (V₆). The significantly lower (64.03%) salt tolerance index was found in GJM 1701 (V₁). Mean value of all the genotypes showed the 100% STI in T₀ (Distilled water) and STI were decrease with increase in salinity (T₁ to T₄). Salinity imposes osmotic stress on plants and osmotic stress can restrict cell expansion in both roots and shoots, leading to reduced growth rates and shorter root and shoot lengths. Here, salt tolerance index depends on root length and shoot length, STI decreases with increasing salinity level (Prakash, 2017)^[5]. Pantola *et al.* (2017)^[15] found that as salt stress increased, the salt tolerance index decreased. At a salinity stress level of 4 dsm⁻¹, both the selected leguminous crops exhibited their lowest salt tolerance.

Biochemical parameters

Effect of salinity on chlorophyll a, b and total chlorophyll content

Chlorophyll content was measured from leaves of green gram under salinity at 15 DAS. Chlorophyll a, b and total chlorophyll content was significantly decrease with increase in salinity (T₀ to T₄).

The genotype GM 6 (V₆) exhibited significantly higher chlorophyll A, chlorophyll B, and total chlorophyll content compared to GJM 1701 (V₁), which displayed significantly lower values across all three parameters. Specifically, GM 6 (V₆) had higher mean value of chlorophyll A content of 6.91 mg.100⁻¹. g⁻¹ of fr. wt., chlorophyll B content of 6.05 mg.100⁻¹. g⁻¹ of fr. wt. and total chlorophyll content of 12.96 mg.100⁻¹. g⁻¹ of fr. wt. Conversely, GJM 1701 (V₁) showed lower mean values for chlorophyll A (5.11 mg.100⁻¹. g⁻¹ of fr. wt.), chlorophyll B (4.25 mg.100⁻¹. g⁻¹ of fr. wt.), and total chlorophyll (9.36 mg.100⁻¹. g⁻¹ of fr. wt.).

Treatment T₀, irrigated with distilled water, consistently exhibited the highest chlorophyll content across all parameters measured, whereas Treatment T₄, which received irrigation with 8 EC saline water, consistently exhibited the lowest chlorophyll content compared to Treatment T₀. Specifically, Treatment T₀ showed the highest levels of chlorophyll A (8.11 mg.100⁻¹. g⁻¹ of fr. wt.) and chlorophyll B (7.26 mg.100⁻¹. g⁻¹ of fr. wt.), as well as the highest total chlorophyll content (15.38 mg.100⁻¹. g⁻¹ of fr. wt.). In contrast, Treatment T₄ displayed the lowest levels of chlorophyll A (4.15 mg.100⁻¹. g⁻¹ of fr. wt.), chlorophyll B (3.27 mg.100⁻¹. g⁻¹ of fr. wt.), and total chlorophyll content (7.43 mg.100⁻¹. g⁻¹ of fr. wt.). These trends were observed after 15 days after sowing (DAS).

Reduction in chlorophyll content might be due to higher expansion of biomass leading to reduction in chlorophyllase enzymatic activities. Similar result reported by Kumar *et al.* (2015)^[17]

Effect of salinity on free proline content

Various genotypes of green gram seedling exhibited significant difference in free proline content at 15 DAS. The significantly higher mean value (0.268 mg. g⁻¹ of fr. wt.) of free proline content was recorded for the genotype GM 6 (V₆) and lower (0.158 mg. g⁻¹ of fr. wt.) free proline content was found in GJM 1701 (V₁). Mean effect of treatments on free proline content was significantly varied between 0.095 and 0.392 (mg. g⁻¹ of fr. wt.), and treatment T₀ irrigated with tap water showed lowest (0.095 mg. g⁻¹ of fr. wt.) mean value of free proline content, while the T₄ irrigated with 8 EC showed highest (0.392 mg. g⁻¹ of fr. wt.) mean value of free proline content after 15 DAS. The interaction effect of V X T was found to be significant for free proline content in green gram seedling. The significantly lower (0.079 mg. g⁻¹ of fr. wt.) free proline content was found in genotype GJM 1701 at distilled water irrigation (V₁T₀), and significantly higher (0.563 mg. g⁻¹ of fr. wt.) in genotype (V₆) compared to other genotype under the influence of salinity at all level (< 2 EC to 8 EC = T₀ to T₄). The result agreement with Solanki *et al.* (2018)^[19] and Riddhi *et al.* (2019)^[14].

Effect of salinity on glycine betaine

The data on glycine betaine analyzed from seedling of various green gram genotype at 15 DAS. Mean data of genotypes were found significant for glycine betaine. Among the genotype, higher mean value (0.168 mg. g⁻¹ of fr. wt.) of glycine betaine content was recorded for the genotype GM 6 (V₆). The significantly lower (0.202 mg. g⁻¹ of fr. wt.) glycine betaine content was found in GJM 1701 (V₁). Among the different saline water treatment, treatment T₀ irrigated with distilled water showed lowest (0.129 mg. g⁻¹ of fr. wt.) mean value of glycine betaine content, while the treatment T₄ irrigated with 8 EC saline water showed highest (0.228 mg. g⁻¹ of fr. wt.) mean value of glycine betaine content after 15 DAS. Glycine betaine possesses the potential to significantly contribute to effective protection against salt, drought, and extreme temperature stress. (Dikilitas *et al.*, 2020; Ashraf *et al.*, 2007)^[20, 21].

Effect of salinity on lipid peroxidation

The data of lipid peroxidation was analyzed from different green gram genotype seedlings under saline water treatment at 15 DAS. In our study, lipid peroxidation was increase with respect to saline water treatments (T₁ to T₄) when compared to control suggested that salinity cause the oxidative stress. The significantly higher mean value (0.347 μmol. g⁻¹ of fr. wt.) of lipid peroxidation was recorded for the genotype GJM 1701 (V₁). The significantly lower (0.225 μmol. g⁻¹ of fr. wt.) lipid peroxidation was found in GM 6 (V₆). Mean effect of treatments on lipid peroxidation was significantly varied between 0.238 and 0.384 μmol. g⁻¹ of fr. wt. (table), and treatment T₄ irrigated with 8 EC saline water showed higher (0.384 μmol. g⁻¹ of fr. wt.) mean value of lipid peroxidation, while the treatment T₀ irrigated with distilled water showed lowest (0.238 μmol. g⁻¹ of fr. wt.) mean value of lipid peroxidation after 15 DAS.

The interaction effect of genotype and treatment was found to be significant for lipid peroxidation in green gram seedling. The significantly higher (0.417 μmol. g⁻¹ of fr. wt.) lipid peroxidation was found in genotype GJM 1701 at distilled water irrigation (V₁T₀). The lipid peroxidation was found significantly lower (0.195 μmol. g⁻¹ of fr. wt.) in

genotype (V₁) compared to other genotype under the influence of salinity at all level (<2 EC to 8 EC =T₀ to T₄). The result agreement with Rasool *et al.* (2013) and Yasar *et al.* (2008) [12]. Lipid peroxidation, a pivotal process in cellular damage under salinity stress, significantly impacts green gram seedlings. Elevated levels of salt induce reactive

oxygen species (ROS) accumulation, triggering lipid peroxidation and compromising membrane integrity. This oxidative stress disrupts normal physiological functions, impeding growth and development Sachdev *et al.* (2021) [22].

Table 1: Effect of salinity stress on germination percentage of green gram genotypes at 6 DAS

Genotypes* (V)	Germination percentage (%)					Mean V
	T ₀ (DW)	T ₁ (2 EC)	T ₂ (4 EC)	T ₃ (6 EC)	T ₄ (8 EC)	
V ₁ (GJM 1701)	100.00	93.33	90.00	83.33	73.33	88.00
V ₂ (GJM 1714)	100.00	97.00	92.33	85.00	77.67	90.40
V ₃ (GJM 1837)	100.00	96.67	90.00	84.00	75.00	89.13
V ₄ (GJM 1901)	100.00	97.67	93.33	87.33	78.00	91.27
V ₅ (GM 4)	100.00	97.67	93.33	88.67	78.00	91.53
V ₆ (GM 6)	100.00	100.00	100.00	99.97	86.67	97.33
V ₇ (GM 7)	100.00	100.00	96.67	95.33	85.67	95.53
V ₈ (GAM 5)	100.00	99.33	93.33	90.00	83.00	93.13
V ₉ (K 851)	100.00	98.67	93.33	88.00	81.00	92.20
V ₁₀ (Meha)	100.00	93.33	96.67	91.67	84.33	93.20
Mean T	100.00	97.37	93.90	89.33	80.27	
	S.Em.±	C.D. at 5%			C.V.%	3.16%
V	0.75	2.12				
T	0.53	1.50				
V X T	1.68	4.73				

Table 2: Effect of salinity on Relative Water Content (RWC) in green gram leaves at 15 DAS

Genotypes* (V)	Relative Water Content (RWC)					Mean (V)
	T ₀ (DW)	T ₁ (2 EC)	T ₂ (4 EC)	T ₃ (6 EC)	T ₄ (8 EC)	
V ₁ (GJM 1701)	91.60	81.90	77.57	74.28	71.27	79.32
V ₂ (GJM 1714)	92.90	84.37	79.49	76.78	74.55	81.62
V ₃ (GJM 1837)	91.92	83.77	78.80	75.78	70.21	80.10
V ₄ (GJM 1901)	92.98	84.99	82.03	78.91	77.26	83.23
V ₅ (GM 4)	92.99	86.46	83.16	79.90	77.91	84.09
V ₆ (GM 6)	96.03	95.54	91.38	82.90	81.59	89.49
V ₇ (GM 7)	95.23	95.01	90.14	82.48	80.09	88.59
V ₈ (GAM 5)	94.07	86.70	83.13	80.79	78.20	84.58
V ₉ (K 851)	93.09	85.78	84.12	82.80	79.14	84.99
V ₁₀ (Meha)	94.56	93.58	87.23	82.11	78.28	87.15
Mean (T)	93.54	87.81	83.70	79.67	76.85	
	S.Em.±	C.D. at 5%			C.V.%	2.12%
V	0.46	1.30				
T	0.33	0.92				
V X T	1.03	2.90				

Table 3: Effect of salinity on salt tolerance index (STI) in green gram seedling at 15 DAS

Genotypes* (V)	Salt Tolerance Index					Mean (V)
	T ₀ (DW)	T ₁ (2 EC)	T ₂ (4 EC)	T ₃ (6 EC)	T ₄ (8 EC)	
V ₁ (GJM 1701)	100.00	76.00	61.79	47.79	34.57	64.03
V ₂ (GJM 1714)	100.00	72.39	61.26	51.00	41.39	65.21
V ₃ (GJM 1837)	100.00	70.00	60.22	51.28	42.75	64.85
V ₄ (GJM 1901)	100.00	72.95	61.35	51.92	42.90	65.82
V ₅ (GM 4)	100.00	77.83	66.37	55.90	46.32	69.28
V ₆ (GM 6)	100.00	86.80	75.81	65.88	56.07	76.91
V ₇ (GM 7)	100.00	86.01	74.76	64.61	55.10	76.10
V ₈ (GAM 5)	100.00	85.35	74.18	63.91	52.88	75.26
V ₉ (K 851)	100.00	84.67	71.96	59.59	48.36	72.92
V ₁₀ (Meha)	100.00	85.37	74.16	63.91	53.91	75.47
Mean	100.00	79.74	68.19	57.58	47.42	
	S.Em.±	C.D. at 5%			C.V.%	2.33%
V	0.01	0.01				
T	0.01	0.01				
V X T	0.01	0.03				

Table 4: Effect of salinity on chlorophyll A content (mg.100⁻¹.g⁻¹ of fr. wt.) in green gram leaves at 15 DAS

Genotypes* (V)	Chlorophyll A content (mg.100 ⁻¹ .g ⁻¹ of fr. wt.)					
	T ₀	T ₁	T ₂	T ₃	T ₄	Mean (V)
	(DW)	(2 EC)	(4 EC)	(6 EC)	(8 EC)	
V ₁ (GJM 1701)	7.03	6.07	5.00	3.93	3.53	5.11
V ₂ (GJM 1714)	7.70	6.50	5.27	4.37	4.03	5.57
V ₃ (GJM 1837)	7.50	6.27	5.07	4.17	3.73	5.35
V ₄ (GJM 1901)	8.00	6.73	5.47	4.63	3.83	5.73
V ₅ (GM 4)	7.87	6.93	5.67	4.87	3.90	5.85
V ₆ (GM 6)	9.47	7.57	6.50	6.03	5.00	6.91
V ₇ (GM 7)	9.03	7.27	6.40	5.77	4.77	6.65
V ₈ (GAM 5)	8.07	6.93	6.03	5.07	4.23	6.07
V ₉ (K 851)	8.00	6.77	5.93	5.00	4.03	5.95
V ₁₀ (Meha)	8.47	7.00	6.30	5.50	4.47	6.35
Mean T	8.11	6.80	5.76	4.93	4.15	
	S.Em.±	C.D. at 5%		C.V.%	2.3%	
V	0.04	0.10				
T	0.03	0.07				
V X T	0.08	0.22				

Table 5: Effect of salinity on chlorophyll B content (mg.100⁻¹. G⁻¹ of fr. Wt.) in green gram leaves at 15 DAS

Genotypes* (V)	Chlorophyll B (mg.100 ⁻¹ . G ⁻¹ of fr. Wt.)					
	T ₀	T ₁	T ₂	T ₃	T ₄	Mean (V)
	(DW)	(2 EC)	(4 EC)	(6 EC)	(8 EC)	
V ₁ (GJM 1701)	6.17	5.20	4.13	3.07	2.67	4.25
V ₂ (GJM 1714)	6.83	5.60	4.40	3.50	3.17	4.70
V ₃ (GJM 1837)	6.63	5.40	4.20	3.30	2.87	4.48
V ₄ (GJM 1901)	7.13	5.87	4.60	3.77	2.97	4.87
V ₅ (GM 4)	7.03	6.07	4.80	4.00	2.90	4.96
V ₆ (GM 6)	8.60	6.70	5.63	5.17	4.13	6.05
V ₇ (GM 7)	8.30	6.40	5.53	4.90	3.90	5.81
V ₈ (GAM 5)	7.20	6.07	5.17	4.20	3.37	5.20
V ₉ (K 851)	7.13	5.90	5.07	4.13	3.17	5.08
V ₁₀ (Meha)	7.60	6.13	5.43	4.63	3.60	5.48
Mean T	7.26	5.93	4.90	4.07	3.27	
	S.Em. ±	C.D. at 5%		C.V.%	3.76%	
V	0.05	0.14				
T	0.04	0.10				
V X T	0.11	0.31				

Table 6: Effect of salinity on total chlorophyll content (mg.100⁻¹. g⁻¹ of fr. wt.) in green gram leaves at 15 DAS

Genotypes* (V)	Total Chlorophyll content (mg.100 ⁻¹ .g ⁻¹)					
	T ₀	T ₁	T ₂	T ₃	T ₄	Mean (V)
	(DW)	(2 EC)	(4 EC)	(6 EC)	(8 EC)	
V ₁ (GJM 1701)	13.20	11.27	9.13	7.00	6.20	9.36
V ₂ (GJM 1714)	14.53	12.10	9.67	7.87	7.20	10.27
V ₃ (GJM 1837)	14.13	11.67	9.27	7.47	6.60	9.83
V ₄ (GJM 1901)	15.13	12.60	10.07	8.40	6.80	10.60
V ₅ (GM 4)	14.90	13.00	10.47	8.87	6.80	10.81
V ₆ (GM 6)	18.07	14.27	12.13	11.20	9.13	12.96
V ₇ (GM 7)	17.33	13.67	11.93	10.67	8.67	12.45
V ₈ (GAM 5)	15.27	13.00	11.20	9.27	7.60	11.27
V ₉ (K 851)	15.13	12.67	11.00	9.13	7.20	11.03
V ₁₀ (Meha)	16.07	13.13	11.73	10.13	8.07	11.83
Mean T	15.38	12.74	10.66	9.00	7.43	
	S. Em. ±	C.D. at 5%		C.V.%	2.7%	
V	0.08	0.22				
T	0.05	0.15				
V X T	0.17	0.48				

Table 7: Effect of salinity on free proline (mg. g⁻¹ of fr. wt.) in green gram seedling at 15 DAS.

Genotypes* (V)	Free proline (mg. g ⁻¹ of fr. wt.)					Mean
	T ₀ (DW)	T ₁ (2 EC)	T ₂ (4 EC)	T ₃ (6 EC)	T ₄ (8 EC)	
V ₁ (GJM 1701)	0.079	0.095	0.122	0.176	0.320	0.158
V ₂ (GJM 1714)	0.084	0.098	0.128	0.181	0.337	0.166
V ₃ (GJM 1837)	0.082	0.102	0.132	0.185	0.342	0.169
V ₄ (GJM 1901)	0.087	0.105	0.135	0.191	0.349	0.174
V ₅ (GM 4)	0.098	0.112	0.140	0.200	0.365	0.183
V ₆ (GM 6)	0.111	0.141	0.203	0.323	0.563	0.268
V ₇ (GM 7)	0.107	0.132	0.177	0.276	0.472	0.233
V ₈ (GAM 5)	0.102	0.121	0.156	0.229	0.385	0.199
V ₉ (K 851)	0.100	0.115	0.147	0.208	0.380	0.190
V ₁₀ (Meha)	0.104	0.124	0.161	0.243	0.411	0.209
Mean T	0.095	0.114	0.150	0.221	0.392	0.195
	S.Em.±	C.D. at 5%			C.V.%	1.78%
V	0.001	0.003				
T	0.001	0.002				
V X T	0.002	0.006				

Table 8: Effect of salinity on glycine betaine (mg. g⁻¹ of fr. wt.) in green gram seedling at 15 DAS.

Genotypes* (V)	Glycine betaine (mg. g ⁻¹ of fr. wt.)					Mean V
	T ₀ (DW)	T ₁ (2 EC)	T ₂ (4 EC)	T ₃ (6 EC)	T ₄ (8 EC)	
V ₁ (GJM 1701)	0.118	0.136	0.172	0.191	0.221	0.168
V ₂ (GJM 1714)	0.122	0.137	0.173	0.197	0.215	0.169
V ₃ (GJM 1837)	0.122	0.137	0.173	0.197	0.215	0.168
V ₄ (GJM 1901)	0.131	0.143	0.179	0.211	0.223	0.177
V ₅ (GM 4)	0.123	0.141	0.180	0.210	0.220	0.175
V ₆ (GM 6)	0.145	0.171	0.193	0.227	0.276	0.202
V ₇ (GM 7)	0.135	0.151	0.170	0.220	0.240	0.183
V ₈ (GAM 5)	0.129	0.148	0.186	0.213	0.222	0.180
V ₉ (K 851)	0.131	0.143	0.175	0.202	0.217	0.174
V ₁₀ (Meha)	0.132	0.147	0.183	0.216	0.234	0.182
Mean T	0.129	0.145	0.178	0.208	0.228	
	S.Em.±	C.D. at 5%			C.V.%	3.56%
V	0.002	0.005				
T	0.001	0.003				
V X T	0.004	0.010				

Table 9: Effect of salinity on lipid peroxidation (μmol. g⁻¹ of fr. wt.) in green gram seedling at 15 DAS

Genotypes* (V)	Lipid peroxidation (μmol. g ⁻¹ of fr. wt.)					Mean V
	T ₀ (DW)	T ₁ (2 EC)	T ₂ (4 EC)	T ₃ (6 EC)	T ₄ (8 EC)	
V ₁ (GJM 1701)	0.262	0.300	0.364	0.391	0.417	0.347
V ₂ (GJM 1714)	0.237	0.266	0.328	0.368	0.398	0.319
V ₃ (GJM 1837)	0.261	0.288	0.366	0.386	0.416	0.343
V ₄ (GJM 1901)	0.232	0.276	0.337	0.370	0.396	0.322
V ₅ (GM 4)	0.249	0.261	0.328	0.387	0.401	0.325
V ₆ (GM 6)	0.195	0.204	0.224	0.236	0.266	0.225
V ₇ (GM 7)	0.226	0.241	0.283	0.313	0.369	0.286
V ₈ (GAM 5)	0.266	0.292	0.331	0.353	0.386	0.325
V ₉ (K 851)	0.226	0.273	0.332	0.361	0.397	0.318
V ₁₀ (Meha)	0.231	0.268	0.331	0.358	0.390	0.316
Mean T	0.238	0.267	0.322	0.352	0.384	
	S.Em.±	C.D. at 5%			C.V.%	3.29%
V	0.003	0.008				
T	0.002	0.006				
V X T	0.006	0.017				

Conclusion

In conclusion, the varying salinity levels tested, ranging from <2 EC to 8 EC, exerted significant stress on physiological descriptors such as germination percentage, relative water content and salt tolerance index. Optimal physiological performance was observed at salinity levels below 2 EC, with a notable decrease in these descriptors as

salinity increased, reaching a reduction of 19.73% in germination percentage, 16.69% in relative water content, and 52.58% in salt tolerance index compared to the control. Additionally, higher saline water stress led to a decline in various biochemical parameters, including chlorophyll A, chlorophyll B, and total chlorophyll content, alongside alterations in proline, glycine betaine, and lipid peroxidation

levels. Among the green gram genotypes studied, GM 6 exhibited significantly higher values across physiological and biochemical parameters under saline conditions. However, lipid peroxidation enhances due to salinity particularly in susceptible (GJM 1701) genotype. It can be concluded that the genotype GM 6 was found to be tolerant under saline irrigation water. This genotype GM 6 performs better under different salinity level and demonstrated better tolerance criteria. The order of green gram varieties with respect to salinity tolerance are GM 6 > GM 7 > Meha > GAM 5 > K 851 > GM 4 > GJM 1901 > GJM 1714 > GJM 1701.

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