

Effect of salinity tolerance in yield performance of guinea grass (*Megathyrsus maximus*) genotypes

K. ANUSREE¹*, EDNA ANTONY¹, K. SRIDHAR² AND M. B. DODDAMANI³

¹Department of Crop physiology, ³Department of Environmental Science, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad - 580 005, India

²Department of Plant Physiology, ICAR-IGFRI-SRRS, Dharwad

*E-mail: anusreethamban97@gmail.com

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Abstract: Excess salts in soil negatively impact the growth and development of plants. These types of land under the salinity impact could be changed for pasture production or fodder cultivation to improve animal husbandry when cropping is impossible due to higher salinity levels. Guinea grass performs well in cut-and-carry systems and can be used to make hay and silage. The research study was conducted in MARS, UAS Dharwad to evaluate the physiological mechanisms of salinity tolerance in Guinea grass (*Megathyrsus maximus* Jacq.), its recovery from salinity stress and tolerance contributes to its yield performance. The genotypes were studied in artificially made saline soil. Artificial saline soil was prepared by a combination of salts of NaCl, Na₂SO₄, MgCl₂ and CaSO₄ in the salt ratio: 13:7:1:2, respectively. Varieties BG-1, BG-4, DGG-1, and CO-1 were planted in control and artificially created in 12 ECe soils. After salinity was imposed, three harvests were taken. DGG 1 recorded the highest fresh biomass yield in this study, followed by BG-4, where CO-1 and BG 1 recorded the lowest biomass yield. Leaf succulence was highest in DGG1 and lowest in BG 4. More leaf succulence could ameliorate the ionic and osmotic stress effects of high salinity treatment and provide long-term storage to facilitate improved reproductive capacity under salinity stress conditions. BG4 recorded the lowest tissue tolerance because most of the salts are excreted through the micro hairs of leaves in this genotype. DGG1 has the highest leaf-to-stem ratio, which results in a higher number of leaves and leaf area. Membrane stability was increased at 45 days in the subsequent harvest in all genotypes. The increased tissue tolerance combined with increased membrane stability and more number of leaves in DGG-1 facilitated the recovery and increased biomass in DGG 1.

Key words: Leaf to stem ratio, Membrane stability, Tissue tolerance

Introduction

India has approximately 6.73 mha of salt-affected soils, with saline and sodic soil contributing 40% to 60%, respectively. Soil salinity reduces crop yield and quality. Salt-tolerant forages, particularly grasses that can grow well in saline conditions, have the potential to be valuable alternative forage resources and could play an essential role in sustaining livestock production. India's current area under fodder crop production is approximately 8.6 million hectares (Singh *et al.*, 2022). The country lacks 35.6% green fodder, 10.95% dry fodder and 44% concentrated feed materials (Anon, 2015). The only way to meet livestock fodder needs is to increase productivity per unit of land area and integrate fodder crops into existing cropping systems.

Guinea grass [*Megathyrsus maximus* (Jacq)] is an African native introduced to almost all tropical countries as a source of animal fodder. It is highly valued as an excellent fodder due to its high productivity, palatability and persistence. It is a perennial bunch of grass that grows to a height of 0.5 to 4.5m. The stem is erect or ascending, glabrous, hairy, and stout to slender. The leaves range from 10 to 100cm long and 3.5cm wide. The panicle is loose and heavily branched, with the lowermost branches forming a distinct whorl. The tiny seeds are shattered. The root system is dense, fibrous, and deep. The grass can grow in medium to highly fertile loams but prefers well-drained, light-textured soils, preferably sandy loams or loams. It cannot tolerate heavy clays or prolonged waterlogging. In 4-5 cuttings, the yield is approximately 120-150 tonnes of

green forage. Under ideal conditions, roughage contains 8-10% protein and is propagated by slips.

Salinity negatively impacts plant growth due to: (1) the low osmotic potential of the soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors. All major processes within a plant are affected by the onset and development of salt stress, including photosynthesis, protein synthesis, and energy and lipid metabolism (Parida and Das 2005).

Salt tolerant grasses can withstand increasing salt stress by utilising various mechanisms such as vacuolisation of toxic Na⁺ and Cl⁻ in mature or senescent leaves, secretion of excess salts by salt glands, accumulation of osmolytes such as proline and glycine betaine, tissue tolerance. Previously, it was discovered that the membrane stability of salt-tolerant genotypes changes after salt application. In this study, we try to understand why tolerant genotypes have higher membrane stability than sensitive genotypes.

Material and methods

This study explored soil salinity tolerance mechanisms in guinea grass. The experiment was conducted at MARS, University of Agriculture Sciences Dharwad, from September 2021 to August 2022 to assess the salinity tolerance in guinea grass genotypes. The total amount of rainfall received during cropping period 2021-22 was 183.6 mm. The mean maximum temperature (31.0°C) was recorded during February and mean

minimum temperature was recorded during January (13.2°C). The genotypes were studied in artificially made saline soil.

Preparation of saline soil

Artificial saline soils were prepared based on the USDA Agriculture handbook no 60 (Dheeravathu *et al.*, 2018). Containers which can hold 30 kg of soil were used for each treatment. The containers were without drainage holes to ensure the retention of salts in the soils and not be washed away due to irrigation. The soil salinity created based on a percentage of salt ratio was allowed to be uniformly distributed in the soil in the container for more than 45 days by subjecting it to repeated cycles of irrigation and evaporation. The desired treatments are 0 and 12 ECe. Salts of NaCl, Na₂SO₄, MgCl₂ and CaSO₄ in the salt ratio: of 13:7:1:2, respectively, are used to prepare saline soils.

Experimental design and details

An experiment was carried out in MARS, UAS Dharwad during from September 2021 to August 2022. Four guinea grass genotypes BG1, BG4, CO1, DGG1 were planted in control (0ECe) and artificially created saline soil (12ECe). The plants were grown in FRBD design with 5 replications

Morphological observation

Number of leaves at harvest

The number of leaves was counted manually

Fresh leaf weight (g plant⁻¹ harvest⁻¹)

The plants were harvested by leaving 10 cm from the ground. Leaves and stems were separated, and the weight of the leaf were weighed separately.

Leaf-to-stem ratio

The leaf-to-stem ratio was calculated as the ratio of leaf fresh weight and stem fresh weight obtained in each harvest.

Total fresh and dry biomass (g plant⁻¹ harvest⁻¹)

The fresh and dry weight of leaves and stem of each harvest were added to get the total fresh and total dry biomass, respectively and expressed as g plant⁻¹ harvest⁻¹.

2. Physiological parameters

Leaf succulence

Leaf succulence on a dry weight basis was measured using the equation:

$$\text{Succulence (g H}_2\text{O g}^{-1} \text{DW}) = \frac{\text{FW} - \text{DW}}{\text{DW}}$$

Where FW is the fresh weight; DW is the dry weight.

Membrane stability index (%)

Electrolytes tend to leak through injured cell membranes of leaves. This electrolyte leakage was measured using the conductivity meter. The membrane injury index (%) was measured according to Sullivan and Ross (1979). This observation was recorded at 30 and 45 days after each harvest, first before salinity application and later at 30 and 45 days at each harvest after salinity application. Twenty leaf discs (0.5g) were taken in a 20 ml beaker with 10 ml of distilled water. The beaker was covered and kept in a 40°C water bath for one hour. Then the water was changed, and 10 ml of distilled water was added. The beaker was exposed to 100°C for 30 min in a water bath. Using a conductivity meter, the EC was recorded earlier at 40°C (C₁) and after 100°C (C₂) exposure. The membrane stability index was calculated using C₁ the formula,

$$\text{Membrane stability index (MSI)} = 1 - \frac{C_1}{C_2} \times 100$$

Ex situ Tissue tolerance by leaf clip assay method

Ex situ tissue-tolerance assay (Chakraborty *et al.*, 2020) was performed to measure the pigment retention capacity of guinea grass genotypes under similar Na⁺ load in the mesophyll tissues. For this, leaves were collected from ~25-day-old plants, and the leaves were cut into pieces (~10 cm) and placed in Petri dishes containing saline water (EC 12 dS m⁻¹ or (0.5 M) for 14 days. The chlorophyll and tissue ion contents Na⁺ were estimated by spectrophotometer and flame photometry, respectively, from freshwater and saline water-dipped samples. From the collected data, daily chlorophyll degradation rate and tissue tolerance (LC50 score represents a sodium concentration where half (50%) of the chlorophyll pigments were destroyed) were calculated for each genotype.

Results and discussion

Effect of salinity on morphological characteristics

When the leaf parameters were analysed, it was evident from the Table 1 number of leaves per tiller was found to be maximum in DGG1 in all three harvests, 4.11, 5.77, and 8.66 at the 1st, 2nd & 3rd harvests, respectively. DGG1 significantly

Table 1. Effect of salinity on the number of leaves in guinea grass genotypes at different harvests

Genotypes	1 st harvest			2 nd harvest			3 rd harvest		
	Control	Salinity	Mean	Control	Salinity	Mean	Control	Salinity	Mean
BG1	4.53	2.73	3.63 ^b	6.60	3.53	5.06 ^{bc}	6.46	3.86	5.16 ^c
BG4	5.73	3.73	4.73 ^a	7.26	4.40	5.83 ^b	8.26	5.53	6.89 ^b
CO1	3.66	3.33	3.49 ^b	5.58	5.44	5.51 ^c	7.50	6.06	6.78 ^b
DGG1	5.13	4.11	4.62 ^a	8.46	5.77	7.11 ^a	9.93	8.66	9.29 ^a
Mean	4.76 ^a	3.47 ^b		6.97 ^a	4.78 ^b		8.03 ^a	6.02 ^b	
	S.Em±	C. D. at 5%		S.Em±	C.D. at 5%		S.Em±	C.D. at 5%	
Genotype	0.25	0.72		0.31	0.92		0.44	1.42	
Salinity	0.17	0.53		0.22	0.65		0.34	1.00	
Genotype x Salinity	0.35	NS		0.44	NS		0.69	NS	

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Table 2. Effect of salinity on leaf fresh weight (g plant⁻¹) in guinea grass genotypes at different harvest

Genotypes	1 st harvest			2 nd harvest			3 rd harvest		
	Control	Salinity	Mean	Control	Salinity	Mean	Control	Salinity	Mean
BG1	18.00 ^b	15.00 ^b	16.50	23.80	28.40	26.10 ^b	37.40	18.40	27.90 ^b
BG4	38.00 ^a	13.00 ^b	25.50	15.60	23.80	19.70 ^b	34.40	34.80	34.60 ^b
CO1	38.10 ^a	11.00 ^b	24.55	21.50	22.00	21.75 ^b	45.10	34.80	39.95 ^b
DGG1	47.00 ^a	9.33 ^b	28.16	66.40	67.70 ^a	150.80	230.66	190.73 ^a	
Mean	35.27 ^a	12.08 ^b		31.82	35.80		66.92	70.96	
	S. Em±	C.D. at 5%		S.Em±	C.D. at 5%		S.Em±	C.D. at 5%	
Genotype	3.49	NS		3.51	10.17		28.49	82.55	
Salinity	2.46	7.15		2.48	NS		20.15	NS	
Genotype x Salinity	4.93	14.30		4.96	NS		40.30	NS	

Table 3. Effect of salinity on the leaf-to-stem ratio in dry weight basis in guinea grass genotypes

Genotypes	1 st harvest			2 nd harvest			3 rd harvest		
	Control	Salinity	Mean	Control	Salinity	Mean	Control	Salinity	Mean
BG1	0.57	0.52	0.54 ^b	0.70	0.69	0.69 ^b	3.45	6.48	4.96 ^b
BG4	1.80	0.60	1.20 ^b	0.47	0.68	0.57 ^b	4.40	7.73	6.06 ^{ab}
CO1	0.65	0.52	0.58 ^b	0.89	0.41	0.65 ^b	3.69	2.71	3.20 ^b
DGG1	1.47	1.80	1.63 ^a	12.77	13.93	13.35 ^a	12.74	8.58	10.66 ^a
Mean	1.12	0.86		3.70	3.92		6.07	6.37	
	S.Em±	C.D. at 5%		S. Em±	C.D. at 5%		S. Em±	C. D. at 5%	
Genotype	0.10	0.30		1.08	3.15		1.23	3.57	
Salinity	0.07	NS		0.76	NS		0.87	NS	
Genotype x Salinity	0.14	NS		1.53	NS		1.88	NS	

1st harvest – 60 days after salt application, 2nd harvest-45 days after 1st harvest 3rd harvest-45 days after 2nd harvest

differed with BG1, BG4, and CO1 genotypes under salinity. From the Table 2 leaf fresh weight was maximum for DGG1 in both saline and under control conditions 230.66 g and 150.80 g, respectively, in 3rd harvest. The highest leaf fresh weight was seen in DGG1(190.73 g), and the lowest leaf fresh weight was seen in BG1 (16.50 g), irrespective of the treatment levels. In the second and third harvests, DGG1 significantly differed from all other genotypes CO1, BG4, and BG1. It was understood from Table 3 that the leaf-to-stem ratio was found to be more in control compared with salinity. The three harvest DGG1 showed maximum leaf-to-stem ratio, which significantly differed from all other genotypes CO1, BG4 and BG1.

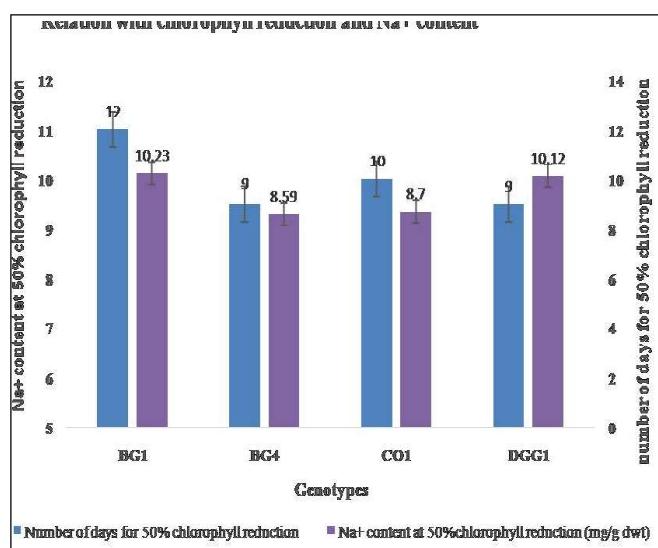


Fig 1. Relation with Na⁺ content and chlorophyll reduction

It was observed that at salinity and control, fresh leaf weight (0.99**; Table 2, Fig 2) and the number of leaves (0.92; Table 1, Fig 2) were positively correlated with fresh biomass. This observation hints that under salinity, the components contributing to yield are the number of green leaves and fresh leaf weight. Leaf to stem ratio (Table 3) was also positively correlated with fresh biomass, indicating that guinea grass yield depends on the leaf biomass. This finding suggests that genotypic traits like the number of leaves (Table 1) and fresh leaf weight (Table 2) define the biomass of guinea grass under salinity. It also implies that an increased number of leaves and

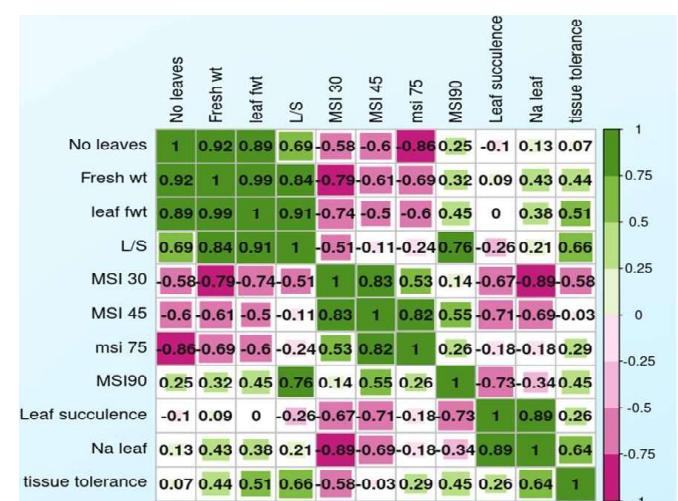


Fig 2. Pearson's correlation coefficients of morphological, physiological parameters calculated for four guinea grass genotypes; n=4; at 5% P=0.95 FW=fresh weight, MSI= membrane stability index at 12 ECe

Table 4. Effect of salinity on total fresh biomass in guinea grass genotypes (g plant⁻¹)

Genotypes	Control	Salinity	Mean
BG1	196.40	141.60	169.00 ^b
BG4	238.80	152.20	195.50 ^b
CO1	295.30	178.20	207.25 ^b
DGG1	448.20	365.66	406.93 ^a
Mean	294.67 ^a	209.41 ^b	
	S.Em±	C.D. at 5%	
Genotype	42.15	122.12	
Salinity	34.60	100.24	
Genotype x Salinity	69.21	NS	

fresh leaf weight can be considered an essential trait for selecting genotypes for salinity tolerance.

Effect of salinity on physiological characteristics of guinea grass genotypes

The membrane stability index is a critical physiological parameter detecting cellular membrane injury and electrolyte leaks. The data concerning membrane stability is presented in Table 5. In the data after salinity application, the highest stability under control conditions at 30 days was BG1 (76.40) and the least was for CO1 (56.10). Under salinity highest was for BG4 (65.80), and the least was for DGG1 (38.00). BG4 was significant with other genotypes under salinity. At 45 days after salinity application, DGG1(62.00) showed the highest and CO1(52.30) showed the lowest membrane stability under control. BG4(55.20) showed the highest, and CO1 showed the lowest membrane stability under salinity. CO1 significantly differed from other genotypes, where BG1 & BG4 were on par with each other.

In DGG1, the membrane stability steadily increased as the tillers regenerated after the second harvest. This observation on membrane stability indicated that the regenerated tillers remember the salinity stress and prepared the forthcoming generations with improved membrane stability, implying a transcriptional memory influencing the salinity response in plants. Under stress, ROS and O² increase electrolyte leakage and affect membrane stability (Hu *et al.*, 2016). During stress,

Table 6. Effect of salinity on leaf succulence(g H₂O g⁻¹ dw) and Na⁺ in leaf (mg/g) of guinea grass genotypes

Genotypes	Leaf succulence			Na ⁺ in leaf (mg/g)		
	Control	Salinity	mean	Control	Salinity	mean
BG1	1.51 ^{bc}	1.96 ^{ab}	1.738 ^{ab}	0.39 ^c	0.64 ^a	0.515 ^a
BG4	2.3 ^a	1.56 ^{bc}	1.930 ^a	0.22 ^d	0.37 ^c	0.295 ^c
CO 1	1.61 ^c	2.12 ^a	1.754 ^{ab}	0.17 ^e	0.37 ^c	0.270 ^c
DGG 1	1.56 ^{bc}	1.89 ^c	1.450 ^b	0.26 ^d	0.49 ^b	0.375 ^b
Mean	1.69	1.74		0.26 ^b	0.46 ^a	
	S.Em±	C.D at 5%		S.Em±	C.D at 5%	
Genotypes	0.112	0.325		0.011	0.033	
Salinity levels	0.079	NS		0.008	0.023	
Genotype x	0.159	0.459		0.016	0.047	
Salinity levels						

sugar accumulation was more and acted as a sugar-sensing signal for the upregulation of the gene responsible for transcriptional memory (Hu *et al.*, 2016). This trainable gene expression is responsible for transcriptional memory and metabolome response of turf grasses under stress (Hu *et al.*, 2016).

Tissue tolerance contributes towards salinity tolerance in guinea grass genotypes

Regulation of Na⁺ in the leaves is most important in salinity tolerance as leaves are the central hub of all activities in a plant. Protection of the leaves from toxic levels of Na⁺ is essential for the plant's survival.

Several mechanisms of Na⁺ ion regulation exist in plants, including recirculating the Na⁺ ion back to the stem and roots and tolerating the high concentration of Na⁺ by partitioning it into the central vacuole. Leaf tissue tolerance is the inherent capacity of the leaves to tolerate Na⁺ ion concentration without damaging the photosynthetic machinery. To check the tolerance capacity at the leaf level, we experimented to find tissue tolerance by leaf clip assay (Chakraborty *et al.*, 2020). From the experiment, tissue tolerance was high in BG1(10.23), which was followed by DGG1(10.12) and was minimum in BG4(8.59) (Fig.1). Where BG1 had high leaf succulence and Na⁺ followed by DGG1, as more Na⁺ is compartmentalised into the vacuole, resulting in

Table 5. Effect of salinity on membrane stability (%) in guinea grass genotypes

Genotype	1 st harvest						2 nd harvest					
	30 days			45 days			30 days			45 days		
	Control	Salinity	mean		Control	Salinity	mean		Control	Salinity	mean	
BG 1	76.4 ^a	52.60 ^c	64.50 ^{ab}	56.20	54.00	55.10 ^a	86.30	72.20	79.25 ^a	58.80	54.00	56.40 ^a
BG 4	72.20 ^{ab}	65.80 ^{abc}	69.00 ^a	58.75	55.20	56.97 ^a	80.12	56.00	68.06 ^{ab}	64.20	59.00	61.60 ^a
CO 1	56.10 ^{bc}	48.00 ^c	52.05 ^{bc}	52.30	46.80	49.55 ^{ab}	48.13	43.60	45.86 ^{bc}	32.70	32.00	32.35 ^b
DGG 1	62.40 ^{abc}	38.00 ^d	50.20 ^c	62.00	47.66	54.83 ^b	82.40	40.60	61.50 ^c	45.80	60.00	52.90 ^b
Mean	66.78 ^a	47.35 ^b		57.31 ^a	46.20 ^b		74.23 ^a	53.1 ^b		49.17	51.25	
	S.Em±	C.D. at 5%		S.Em±	C.D. at 5%		S.Em±	C.D. at 5%		S.Em±	C.D. at 5%	
Genotype levels	3.16	9.18		4.53	13.12		3.77	10.92		4.46	12.90	
Salinity Level	4.48	12.98		3.20	9.27		5.33	15.45		3.15	NS	
Genotype x Salinity	6.33	18.36		7.24	NS		7.54	NS		6.30	NS	

Before Salinity application membrane stability was found to be between 70-90%. The observation was recorded at 30, 45 days of 1st harvest and 2nd harvest after Salinity application in 3rd leaf of guinea grass genotypes

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Table 7. Effect of salinity on recovery biomass (g plant⁻¹) in guinea grass genotypes

Genotypes	Recovery biomass					
	Fourth harvest			Fifth harvest		
	Control	Salinity	Mean	Control	Salinity	Mean
BG1	408.00 ^b	482.00 ^b	445.00 ^b	214.00 ^d	322.00 ^{bc}	68.00 ^c
BG4	440.00 ^b	496.00 ^b	468.00 ^b	287.00 ^{cd}	383.00 ^{bc}	35.00 ^{bc}
CO1	480.00 ^b	420.00 ^b	450.00 ^b	342.50 ^{bc}	381.00 ^{bc}	61.75 ^c
DGG1	788.00 ^a	493.33 ^b	640.66 ^a	799.00 ^a	410.00 ^b	04.50 ^a
Mean	529.00 ^a	472.82 ^b		410.62	374.00	
	S.Em \pm	C.D. at 5%		S.Em \pm	C.D. at 5%	
Genotype 23.57	78.81		22.08	73.84		
levels						
Salinity	16.66	55.72		15.61	NS	
levels						
Genotype 33.32	111.45		31.22	104.43		
x Salinity levels						

higher succulence and tissue tolerance. BG1 took 12 days for loss in 50% chlorophyll, whereas it could retain 10.23 mg/g of

Na⁺ until it lost its 50% chlorophyll. DGG1 also exhibited high tissue tolerance (Fig 1). However, genotype BG1 recorded higher tissue tolerance and minimum biomass under salinity.

Conclusion

The fresh biomass in DGG1 and BG4 was higher indicating the relative tolerance to salinity stress, whereas BG1 and CO1 found to be sensitive with respect to biomass accumulation. In salt tolerant varieties the physiological traits like tissue tolerance, membrane stability was higher compare to the sensitive varieties. The above-mentioned physiological traits were higher in BG4 compared to DGG1 which was evident with respect to fresh biomass accumulation. In varieties like BG1 and DGG1 tissue tolerance act as salinity tolerance strategy also. In the present study tolerant genotypes under salinity exhibit favourable characteristics such as increased leaf number, leaf fresh weight. The findings of the study concludes that the genotypes DGG1 and BG4 are recommended for saline soils >12ECe because of its high biomass recovery (Table 7).

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