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Effect of Electrolyte Leakage and Proline Content on Growth and Yield of Small-Fruited Bitter Gourd, *Momordica charantia* L. var. *muricata* under Sodic Soil Condition

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ABSTRACT: Fifty genotypes of small-fruited bitter gourd were evaluated for yield under sodic soil. The seeds were sown in sodic soil with pH of 9.1 and EC of 0.12 dS/m. The lowest electrolyte leakage was recorded in MCM 10 (29.85%) followed by MCM 5 (31.43%) and MCM 2 (32.18%). The higher accumulation of proline was recorded in MCM 41 (0.610 mg/g) followed by MCM 14 (0.547 mg/g), MCM 1 (0.545 mg/g) and MCM 16 (0.543 mg/g). The germination percentage ranged from 51.33 to 100. The number of days for germination was significantly lower (5.79) in MCM 3, followed by MCM 50 (5.95). The number of days taken for first female flower opening ranged from 41.52 to 59.27. The number of days for first female flower opening was significantly lower (41.52) in MCM 25, followed by MCM 7(22.78) and MCM 24 (44.94). The significantly longer fruits (5.97 cm) were recorded in MCM 45, followed by MCM 41 (5.23). The individual fruit weight was significantly higher (9.94 g) in MCM 45, followed by MCM 39 (8.88) and MCM 41 (8.82). The number of fruits per plant ranged from 29.43 to 48.38. The number of fruits/plant was significantly higher in MCM 12 (48.38) followed by MCM 1 (48.33). The higher yield per plant was recorded significantly higher in MCM 45 (399.4 g) followed by MCM 41 (324.3g) and MCM 1(309.3 g). The high yielding small-fruited bitter gourd genotypes with sodicity tolerance can be successfully used in breeding programmes for developing new varieties and hybrids.

Keywords: Small-fruited bitter gourd, yield, sodic soil, proline, electrolyte leakage.

INTRODUCTION

The bitter gourd is an important vegetable of cucurbitaceae family. The small-fruited bitter gourd *Momordica charantia* var. *muricata* with small, tuberculate fruits is considered as progenitor of commercially grown large-fruited bitter gourd (Walters and Decker-Walters, 1988). It is an herbaceous vine with wide variations in leaves, flowers and fruits (Neelavathi *et al*., 2015). The immature fruits contain charantin, momordicin, polypeptide P, vicine, insulin-like peptides and other steroidal glycosides. Bitter gourd fruits are known to possess antihyperglycemic (Ali *et al*., 1993; Virdi *et al*., 2003), antilipidemic (Fernandes *et al*., 2007), antiviral, antiulcerogenic, antitumorigenic and anti-inflammatory properties. Bitter gourd fruits can regulate uptake of glucose in diabetic rats (Ahmed *et al*., 2004). The yield of bitter gourd is influenced by genotypes, nutrients, irrigation water, soil pH and soil properties.

Salt affected soil is a problem in certain pockets of Tamil Nadu. Out of 4.7 lakh hectares salt affected soils in Tamil Nadu, 3.0 lakh hectares in inland and 1.7 lakh hectares in coastal areas. Out of the 3 lakhs inland salt affected soil, 2.00 lakh was due to alkalinity and 1.00 lakh hectares due to salinity. Salinity is a constraint limiting plant growth and productivity of vegetable crops. Salinity inhibits water uptake by plants, causes ionic imbalance leading to ionic toxicity and osmotic stress which affects the growth and fruit yield of horticultural crops. Sodicity is due to the presence of sodium salts in soil. Sodicity tolerance involves a complex of physiological responses and metabolic processes. Understanding the mechanism underlying plant response to salinity and sodicity provides new insights into the improvement of salt tolerance. Plasma membrane of the cell is a site of salt injury (Mansour and Salama 2004). Electrolyte leakage is a key physiological parameter to evaluate abiotic tolerance (Arvin and Donnelly 2008). Salt tolerant genotypes maintained lower electrolyte leakage under sodic condition compared to salt sensitive genotypes. The plasma membrane integrity is maintained in genotypes having lower electrolyte leakage.

Plants accumulate a group of metabolites particularly amino acids when exposed to biotic and abiotic stresses. Proline is an amino acid that accumulates in plants under abiotic stresses. Proline accumulation is a common physiological response in many plants in response to a wide range of biotic and abiotic stresses such as drought, salinity, low temperature, heavy metals and high acidity (Verbruggen and Hermans 2008; Hossain *et al*., 2014). Proline is an excellent osmolyte and compatible solute. It plays a role as a metal chelator, an anti-defence molecule and a signalling molecule. Proline imparts stress tolerance (Farkhondeh *et al*., 2012; Gharsallah *et al*., 2016) by maintaining cell turgor or osmotic balance and bringing concentration of reactive oxygen species within normal range. It protects folded protein structures against denaturation, stabilises cell membranes by interacting with phospholipids, functions as a hydroxyl radical scavenger, or serves as an energy and nitrogen source. The accumulation of free proline resulted in osmotic adjustment and salt tolerance in bitter gourd by facilitating water absorption, scavengers and reactive oxygen species molecules. There is a positive correlation between proline accumulation and stress tolerance (Dar *et al*., 2016; Mansour and Ali 2017). In this context, growth, yield, electrolyte leakage and proline were studied in small-fruited bitter gourd.

MATERIALS AND METHODS

The present investigation was carried out at the farm of Horticultural College and Research Institute for Women, Tamil Nadu Agricultural University, Tiruchirappalli, Tamil Nadu during 2018-20. Fifty genotypes of small-fruited bitter gourd collected from Tamil Nadu were evaluated for growth, yield, electrolyte leakage and proline content. The small-fruited bitter gourd seeds were sown in clay loam soil with three replications and Randomized Block Design (RBD). The experimental soil is sodic in nature with pH of 9.1 and EC of 0.12 dS/m and ESP of 33.62 % (Table 1). The available nitrogen, phosphorus and potash in the soil was 176 kg/ha, 24 kg/ha and 258 kg/ha, respectively. The seeds were sown in the beds at 2 x 1.5 m spacing in December 2018, June 2019 and January 2020. The vines were allowed to creep on the ground.

Table 1: Soil properties of experimental field.

Sr. No.	Soil properties	Value
1.	pH	9.1
2.	Electrical Conductivity (EC)	0.17 dS/m
3.	Exchangeable Sodium Percentage (ESP)	33.62 %
4.	Available nitrogen	176 kg/ha
5.	Available phosphorus	24 kg/ha
6.	Available potash	258 kg/ha

Electrolyte leakage. Ten discs of fresh leaf (0.5 cm diameter) were cut from the fully expanded leaves and the leaves were washed three times with deionized water to remove surface-adhered electrolytes. Leaf discs were placed in test tubes containing 5 ml of deionized water. The initial electrical conductivity of the solution (EC 1) was determined using a conductivity meter. After 30 minutes, electrical conductivity of the solution (EC 2) was determined. The leaf discs were then incubated in a water bath for 10 minutes to release all electrolytes, cooled down to 25°C and their final electrical conductivity (EC 3) was measured. The electrolyte leakage (EL) was calculated as

$$\text{Electrolyte leakage (\%)} = (\text{EC }1 - \text{EC2} / \text{EC }3) \times 100$$

Proline. Proline accumulation in leaf tissue was determined via reaction with ninhydrin. Purified proline was used to build a standard curve for proline content quantification. 0.5 gram of fresh leaf samples were homogenized in 10 ml of 3% aqueous sulfosalicylic acid and centrifuged at 3000 rpm for 1 minute. 2 ml of supernatant was reacted with 2 ml of ninhydrin acid and 2 ml of glacial acetic acid for 1 hour at 100°C in a heater. The chromophore was extracted using 2 ml of toluene, and its absorbance at 520 nm was determined by UV Spectrophotometer with toluene used as blank.

Proline content =

$$((\mu\text{g proline/mL} \times \text{mL toluene})/115.5\ \mu\text{g}/\mu\text{mole}) \times (\text{g sample}/5) = \mu\text{moles proline gram FW}^{-1}$$

Statistical analysis. The data were statistically analysed (Panse and Sukhatme 1985). Level of significance is 5 per cent.

RESULTS AND DISCUSSION

Electrolyte leakage. Electrolyte leakage has been used as an indicator of cell membrane permeability under abiotic stresses. The electrolyte leakage from plasma membranes is reported as one of the most important selection criterion for identification of salt-tolerant plants (Ashraf and Ali 2008). The electrolyte leakage was significantly influenced by bitter gourd accessions. The electrolyte leakage value varies with bitter gourd accessions. The lowest electrolyte leakage indicates decrease in membrane permeability and increased cell tolerance to salt stress. The lowest electrolyte leakage was recorded in MCM 10 (29.85%) followed by MCM 5 (31.43%) and MCM 2 (32.18%). The genotypes with higher electrolyte leakage are not desirable not only due to salt stress but also higher content of potassium (Mansour and Salama 2004). Demidchik *et al*. (2014) also stated that electrolyte leakage is mainly related to the efflux of K+ in plant cells.

Proline content. Accumulation of proline in plants is an indication of disturbed physiological condition, triggered by biotic or abiotic stress condition. Proline is a measure of stress in vegetable crops (Claussen, 2005).

Determination of free proline levels is a useful assay to monitor physiological status and to assess salt tolerance of plants. There was a significant difference in accumulation of proline in the leaves of bitter gourd (Table 2). The higher accumulation of proline was recorded in MCM 41 (0.610 mg/g) followed by MCM 14 (0.547 mg/g), MCM 1 (0.545 mg/g) and MCM 16 (0.543 mg/g). Free proline content can increase upon exposure of plants to salinity and drought (Ábrahám *et al*., 2010). In some of the bitter gourd genotypes, the accumulation of higher quantity of proline was positively correlated with yield per plant. Protective role of proline against salt stress was reported by Huang *et al*., 2009. The proline content was positively or negatively correlated with yield that could be due to genetic character of the bitter gourd genotypes. The difference in the yield is due to the role of proline in flower transition (Saxena *et al*., 2008).

Table 2: Electrolyte leakage and proline content in leaves of small fruited bitter gourd.

Sr. No.	Bitter gourd genotypes	Electrolyte leakage (%)	Proline (mg/g)
1.	MCM 1	34.29	0.545
2.	MCM 2	32.18	0.254
3.	MCM 3	37.18	0.271
4.	MCM 4	37.78	0.232
5.	MCM 5	31.43	0.255
6.	MCM 6	32.29	0.250
7.	MCM 7	34.38	0.245
8.	MCM 8	41.46	0.182
9.	MCM 9	39.82	0.466
10.	MCM 10	29.85	0.525
11.	MCM 11	38.30	0.267
12.	MCM 12	43.06	0.492
13.	MCM 13	34.85	0.531
14.	MCM 14	41.24	0.547
15.	MCM 15	37.50	0.509
16.	MCM 16	43.90	0.543
17.	MCM 17	36.59	0.512
18.	MCM 18	41.32	0.495
19.	MCM 19	43.09	0.519
20.	MCM 20	41.11	0.354
21.	MCM 21	33.94	0.515
22.	MCM 22	49.00	0.467
23.	MCM 23	41.51	0.450
24.	MCM 24	46.53	0.199
25.	MCM 25	46.55	0.255
26.	MCM 26	49.45	0.267
27.	MCM 27	43.56	0.288
28.	MCM 28	41.05	0.509
29.	MCM 29	44.00	0.244
30.	MCM 30	51.47	0.242
31.	MCM 31	54.76	0.291
32.	MCM 32	50.70	0.510
33.	MCM 33	50.00	0.531
34.	MCM 34	55.21	0.524
35.	MCM 35	51.59	0.248
36.	MCM 36	32.76	0.255
37.	MCM 37	53.13	0.485
38.	MCM 38	34.30	0.312
39.	MCM 39	35.92	0.196
40.	MCM 40	35.77	0.255
41.	MCM 41	34.11	0.610
42.	MCM 42	37.23	0.263

43.	MCM 43	36.21	0.305
44.	MCM 44	38.74	0.340
45.	MCM 45	45.90	0.498
46.	MCM 46	48.57	0.270
47.	MCM 47	42.31	0.517
48.	MCM 48	41.44	0.330
49.	MCM 49	35.63	0.479
50.	MCM 50	34.74	0.460
	Mean	40.95	0.382
	SEd	2.24	0.09
	CD(0.05)	5.12	0.21

Growth and yield characteristics. The pooled data on growth and yield parameters of small-fruited bitter gourd grown during 2018-2020 was calculated. The presence of salts was greatly influenced the germination of bitter gourd seeds. The germination percentage was ranged from 51.33 to 100. The number of days taken for germination ranged from 5.79 to 6.58. The number of days for germination was significantly lower (5.79) in MCM 3, followed by MCM 50 (5.95).

The yield parameters of small fruited bitter gourd under saline soil is presented in Table 3. The number of days taken for first male flower opening ranged from 34.28 to 49.85. The number of days for first male flower opening was significantly lower (34.28) in MCM 25. The number of days taken for first female flower opening ranged from 41.52 to 59.27. The number of days for first female flower opening was significantly lower (41.52) in MCM 25, followed by MCM 7 (22.78) and MCM 24 (44.94). The fruit length ranged from 2.35 cm to 5.97 cm. The significantly higher value (5.97 cm) for fruit length was recorded in MCM 45, followed by MCM 41 (5.23). The individual fruit weight was significantly higher (9.94 g) in MCM 45, followed by MCM 39 (8.88) and MCM 41 (8.82). The number of fruits per plant ranged from 29.43 to 48.38. The significantly higher number (48.38) of fruits/plant was recorded in MCM 12, followed by MCM 1 (48.33). The higher yield per plant was recorded significantly higher in MCM 45 (399.4 g) followed by MCM 41 (324.3g) and MCM 1(309.3 g). Number of seeds/fruit ranged from 3.66 to 12.33. A significantly higher number (12.33) of seeds/fruit was recorded in MCM 47, followed by MCM 39 (9.23). The level of salt tolerance varied with cultivars which are corrected with growth (Arvin and Donnelly 2008). The growth of New Zealand spinach varied with different soil texture and salinity (Kim *et al*., 2011) and water spinach (Yousif *et al*., 2010).

Table 3: Yield characteristics of small-fruited bitter gourd.

Bitter gourd genotypes	Days taken for first male flower opening	Days taken for first female flower opening	Individual fruit weight (g)	No. of fruits/ plant	Yield (g)/plant
MCM 1	41.43	49.33	6.40	48.33	309.3
MCM 2	48.22	59.18	3.71	33.89	125.7
MCM 3	42.57	46.17	4.81	39.24	188.7
MCM 4	43.26	56.15	5.16	35.21	181.7
MCM 5	41.49	49.33	7.69	36.90	283.8
MCM 6	41.32	49.15	3.92	30.10	118.0
MCM 7	37.81	44.78	2.83	39.66	112.2
MCM 8	47.89	58.13	4.03	31.56	127.2
MCM 9	39.14	46.34	3.17	32.44	102.8
MCM 10	41.27	49.22	3.56	46.25	164.7
MCM 11	41.87	50.55	5.10	39.27	200.3
MCM 12	42.36	49.66	4.93	48.38	238.5
MCM 13	35.71	45.19	2.99	47.11	140.9
MCM 14	38.25	48.66	5.43	45.11	244.9
MCM 15	42.68	51.57	2.91	42.34	123.2
MCM 16	45.17	52.63	4.92	45.76	225.1
MCM 17	44.33	52.74	3.90	44.12	172.1
MCM 18	39.27	48.21	4.21	39.25	165.2
MCM 19	45.38	52.54	3.87	43.33	167.7
MCM 20	35.47	45.72	4.88	38.46	187.7
MCM 21	44.28	51.54	2.89	43.28	125.1
MCM 22	45.31	52.58	2.87	37.24	106.9
MCM 23	38.24	46.76	2.95	39.26	115.8

MCM 24	35.65	44.94	2.76	29.43	81.2
MCM 25	34.28	41.52	5.23	35.13	183.7
MCM 26	42.66	50.65	5.92	39.89	236.1
MCM 27	39.90	48.22	3.76	39.27	147.7
MCM 28	38.33	45.25	3.14	43.53	136.7
MCM 29	38.17	48.90	3.59	34.26	123.0
MCM 30	42.58	51.33	3.90	37.92	147.9
MCM 31	41.57	49.33	5.48	39.33	215.5
MCM 32	43.46	50.59	2.79	44.62	124.5
MCM 33	47.20	55.32	2.86	46.75	133.7
MCM 34	34.56	44.97	4.01	45.54	182.6
MCM 35	39.14	47.65	3.67	38.23	140.3
MCM 36	39.21	47.23	5.96	38.35	228.6
MCM 37	43.82	50.65	4.89	35.37	173.0
MCM 38	39.26	47.18	2.45	39.25	96.2
MCM 39	39.53	48.21	8.88	31.26	277.6
MCM 40	44.48	51.85	2.75	35.63	98.0
MCM 41	42.33	49.49	8.82	36.77	324.3
MCM 42	43.67	50.26	4.75	37.16	176.5
MCM 43	44.11	51.17	2.01	38.24	76.9
MCM 44	41.65	49.26	2.69	39.48	106.2
MCM 45	42.66	49.15	9.94	40.18	399.4
MCM 46	43.73	50.28	2.76	39.88	110.1
MCM 47	42.55	49.55	2.52	42.33	106.7
MCM 48	49.85	59.27	3.18	39.13	124.4
MCM 49	45.24	55.33	3.33	40.25	134.0
MCM 50	49.21	57.66	3.92	40.28	157.9
Mean	41.83	50.03	4.26	39.48	167.4
SEd	0.23	1.06	0.77	1.94	5.11
CD(0.05)	0.46	2.13	1.55	3.88	11.53

CONCLUSIONS

Among 50 small-fruited bitter gourd genotypes evaluated, MCM 12 (44.38 fruits/plant) and MCM 1 (48.33 fruits/plant) recorded better yield performance. The lowest electrolyte leakage was recorded in MCM 10 (29.85 %) followed by MCM 5 (31.43 %) and MCM 2 (32.18 %). The higher accumulation of proline was recorded in MCM 41 (0.610 mg/g) followed by MCM 14 (0.547 mg/g), MCM 1 (0.545 mg/g) and MCM 16 (0.543 mg/g). The presence of wide variation in yield, electrolyte leakage and proline content in small-fruited bitter gourd offered a great scope for selecting the suitable genotypes in the breeding programmes for development of varieties/hybrids for sodic soil.

FUTURE SCOPE

Study on proline and electrolyte in response to salt stress will provide the better understanding of developing technologies and varieties for salt affected soils. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of plants to salt-stress. Addressing the problems of salinity is necessary to sustain the vegetable production in future. The improvement in understanding of the responses of vegetable crops to salinity is one of the necessary prerequisites for increased cultivation and their use for phytoremediation could contribute to solving the problem of salinization.

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Conflict of interest. The authors declare that they have no conflict of interests.

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