

EVALUATION OF SWEET PEPPER VARIETIES FOR SALINITY TOLERANCE BASED ON MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES

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ABSTRACT

Soil salinity is a big threat to the world and has become a major concern to agricultural productivity. Sweet pepper (*Capsicum annuum* L.) is a high value vegetable; due to its quality nutrition. Despite growing all over the country, its production is not so high in Bangladesh. As a popular crop and also available everywhere, some varieties of sweet pepper were put under this study to assess their salt tolerance level. A pot experiment was conducted at net house, Botanical Garden, BAU, Mymensingh during the period from November, 2020 to April, 2021. A factorial with CRD (Completely Randomized Design) was applied, where Factor A consisted of six sweet pepper varieties and Factor B included three salinity levels. In the vegetative stage, most of the parameters showed significant variation with salinity levels among varieties except chlorophyll content. In case of physio-morphological traits, all the parameters were significantly reduced with increasing salinity levels. In case of biochemical attributes, leaf proline contents were significantly increased with the higher level of salinity. Among six varieties being examined under the study, Messi had a relatively higher tolerance level than other varieties and therefore can be recommended for salt tolerance breeding studies.

Keywords: Chlorophyll, Photosynthesis, Proline, Salinity, Stomata, Transpiration

INTRODUCTION

Soil salinity is a growing concern across the world, and it is a major impediment to agricultural production, particularly in places where irrigation is required (Sagar et al., 2019). In many regions, soil salinity is becoming a major factor in

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determining crop yield and distribution (Gucci et al., 1997). Salt stress has become a major constraint to agricultural production particularly in arid and semi-arid parts of the world due to low rainfall and high evapotranspiration demand, as well as inadequate soil and water management practices (Farooq et al., 2015). Around 77 million hectares out of 1.5 billion hectares land across the world are currently affected due to high salt concentration (Sheng et al., 2008). The presence of excessive salt (Sodium chloride) in soil and irrigation water has a significant impact on crop productivity globally (Maibody and Feizi, 2005; Demiral and Turkan, 2005).

Salt-affected soils are a significant biological component in Bangladesh's environment, particularly along the coast and offshore. In the past four decades, the country's overall salt-affected land has increased by roughly 1.056 million hectares from 0.833 million hectares (SRDI, 2010). Only approximately 10% land of Bangladesh is less than 1 meter above mean sea level, and one-third of the nation is subject to tidal excursions (SRDI, 2010). Due to frequent flooding from the sea in the southern part of Bangladesh, the saline impacted region is growing at an alarming rate every year.

Plant development and metabolism are both harmed by salinity, which is a key environmental stress or Osmotic stress, particularly ion toxicity, ion imbalance, and oxidative stress all impeding plant growth (Tester and Davenport, 2003). Plant growth and yield are mostly determined by photosynthesis. Under salt stress, it is clear that stomatal and non-stomatal function are limited, resulting in lower net CO₂ absorption, transpiration, and water usage efficiency (Ashraf, 2012). This is true for a wide range of crops, including rice (Moradi and Ismail, 2007), wheat (Kanwal et al., 2011), sunflower (Noreen and Ashraf, 2008), and others. Plants have evolved a variety of physiological and biochemical strategies to maintain a reasonably constant intracellular environment under salty conditions by accumulating different solutes (Gupta and Huang, 2014 and Roy et al., 2014). Plants use osmotic adjustment to sustain water intake and cell turgor, allowing for normal physiological metabolisms (Radi et al., 2013). Proline, as a key osmo-protectant, aids in osmotic adjustment and protects enzymes from oxidative damage in a salty environment (Ashraf and Harris, 2004; Gupta and Huang, 2014).

Now-a-days sweet pepper is considered as an important vegetable crop across the world. Sweet pepper output in the world totals over 47,000 million tons (FAO STAT, 2015). It is also a good source of vitamins, carotenoids, and phenolic compounds in terms of nutrition. Seed germination, seedling growth, and crop production of sweet pepper are all affected by salinity. Sweet pepper (*Capsicum annum* L.), was formerly considered as moderately tolerant to salinity (De Pascale et al., 2003). The suppressing effects of salinity on sweet pepper plants were also reported by Supanjani and Lee, (2006). Furthermore, being a developing crop in Bangladesh, sweet pepper may play an important role in the selection of salt-tolerant cultivars. To reduce crop loss, a small number of high yielding salt tolerant cultivars have been

created. As a result, a thorough and well-organized research program is required to assess certain salt-tolerant sweet pepper cultivars using morpho-physiological and biochemical selection characteristics. In view of the above facts, six high producing sweet pepper varieties namely, BARI Mistimorich-1, Green bell, Dream, Thunder, Messi, and Maria were cultivated in pots under salt stress to investigate the morpho-physiological and biochemical responses of sweet pepper varieties under salinity stress and identify potential salt tolerant cultivar(s) for saline prone areas of Bangladesh.

MATERIALS AND METHODS

Study location and soil

The experiment was conducted at the rooftop of the net house in the Botanical Garden of Bangladesh Agricultural University (BAU), Mymensingh. The six varieties were grown in plastic pot of 10L size with well manured soil for 30 days and different levels of salinity treatments were started from 15 DAS to 18 DAS. Each pot contained 8 kg of Brahmaputra River side and well decomposed cow dung mixed soil. The experimental site was located in the AEZ 9 (Old Brahmaputra Floodplain) of Bangladesh and situated at latitude 24.75°N and Longitude of 90.50°E.

Experimental design and data collection

Seeds of BARI Mistimorich-1 was collected from BARI (Bangladesh Agricultural Research Institute) and other five varieties (Green bell, Dream, Thunder, Messi, and Maria) from local non-government seed company. The experiment was comprised of two factors. Factor A included 6 Varieties: V_1 = BARI Mistimorich-1, V_2 = Green bell, V_3 = Dream, V_4 = Thunder, V_5 = Messi and V_6 = Maria; Factor B consisted of three levels of salinity: T_1 = 0 dSm^{-1} , T_2 = 4 dSm^{-1} and T_3 = 8 dSm^{-1} . The experiment was laid out in Completely Randomized Design (CRD) with three replications. The six varieties were randomly assigned to each pot; total 54 pots were used for the experiment. Manures and fertilizers doses used in this experiment were: Cow dung @ 0.025 kg pot^{-1} , Urea@ 0.652 g pot^{-1} , Triple Super Phosphate@ 1.1 g pot^{-1} , Muriate of Potash@ 0.45 g pot^{-1} , Gypsum@ 1 g pot^{-1} . Intercultural operations were done in time to maintain healthy growth of the seedlings.

The salinity levels (0, 4 and 8 dSm^{-1}) were stimulated by applying NaCl to the soil and the EC was measured by a portable EC meter until it reached the desired point. Plants were grown in 10 L pots with 8 kg air dried soil in each pot under pot house condition. Seeds were sown in individual pot. Only one seedling pot^{-1} was allowed after 4 days of germination. The NaCl treatment started on 15 DAS with $\frac{1}{4}$ of the required amount of salt in four daily increments till the desired EC was reached on 18 DAS. After 12 days of full salinity treatment, plants were harvested. The plants under treatments were harvested on 45 days after sowing (DAS). Different morpho-physiological and biochemical parameters namely, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight relative

chlorophyll content (SPAD value), photosynthesis, transpiration, stomatal conductance, leaf proline content and chlorophyll content were measured at different salinity levels. Relative chlorophyll content was measured using a handheld chlorophyll meter (measured as the SPAD unit, Chlorophyll meter, SPAD502.15 Plus, Konica Minolta, Japan).

Leaf net photosynthesis (A), transpiration (E) and stomatal conductance (g_s) were measured on three replications per variety and salinity level on 12 days after the salinization by placing the recently matured leaf in the cuvette of a portable photosynthesis system (Lci-SD Photosynthetic system, ADC Bio Scientific LCi-SD System, Herts, UK). Physiological and biochemical analyses were performed in the physiology and ecology laboratories of Crop Botany Department, BAU, Mymensingh. Proline content of leaves was determined according to the method developed by Bates et al. (1973).

Chlorophyll content was determined from the leaf samples using the method of Coombs et al. (1985). Collected data were statistically analyzed for ANOVA in accordance with the principles of Completely Randomized Design (Gomez and Gomez, 1984). The t-test was performed to test significance among control and salinity levels. The significance of difference between the pairs of means was separated by LSD test 5% and 1% levels of probability.

RESULTS AND DISCUSSIONS

Morphological attributes

Root fresh weight (g)

The interaction effect of salt concentration with varieties showed significant influence on the root fresh weight (Table 1). At 4 dSm^{-1} salinity level, the highest root fresh wt. (1.45 g) was found in Messi followed by Maria (2.70 g), BARI Mistimorich-1 (2.16 g), and the lowest root fresh wt. (1.04 g) was recorded from Thunder followed by Green bell (1.30 g), Dream (1.82 g). The same result was obtained for control treatment and 8 dSm^{-1} treatment. Franco et al. (2001) also found the similar results in horticultural plants.

Root dry weight (g)

The interaction effect of salt concentration with varieties showed significant influence on the root dry weight (Table 1). At 4 dSm^{-1} salinity level, the highest root dry wt. (0.46 g) was found in Messi followed by Maria (0.36 g), BARI Mistimorich-1 (0.32 g), and the lowest root dry wt. (0.14 g) was recorded from Thunder followed by Green bell (0.21 g), Dream (0.24 g). The same result was obtained for control treatment and 8 dSm^{-1} treatment. The results revealed that root dry weight was decreased with the increasing salinity in each variety. Mwai, (2001) observed that higher salinity caused lower root dry weight in spider plants.

Shoot fresh weight (g)

The interaction effect of salt concentration with varieties showed significant influence on the shoot fresh weight (Table 1). At 4 dSm⁻¹ salinity level, the highest shoot fresh wt. (30.17 g) was found in Messi followed by Maria (25.54 g), BARI Mistimorich-1 (19.78 g), and the lowest shoot fresh wt. (10.11 g) was recorded from Thunder followed by Green bell (11.23 g), Dream (15.09 g). The same result was obtained for control treatment and 8 dSm⁻¹ treatment. Similarly, considerable variation in salt tolerance among sweet pepper genotypes using 40, 80 and 120 mM NaCl has been reported by Prasad and Chakravorty, (2015).

Table 1. Effects of salinity level on Root fresh wt. (g), Root dry wt. (g), Shoot fresh wt. (g), Branch dry wt. (g) and Leaf dry wt. (g) of different sweet pepper varieties

Variety X treatment	Root fresh wt. (g)	Root dry wt. (g)	Shoot fresh wt. (g)	Branch dry wt. (g)	Leaf dry wt. (g)
V ₁ T ₁	2.01ef	0.28f	18.24h	0.75f	1.05h
V ₁ T ₂	1.30hi	0.21h	11.23j	0.42h	0.73k
V ₁ T ₃	0.71j	0.13j	6.45l	0.18i	0.45m
V ₂ T ₁	1.74fg	0.21h	14.26i	0.62g	0.91i
V ₂ T ₂	1.04i	0.14ij	10.11jk	0.39h	0.58l
V ₂ T ₃	0.58j	0.05k	5.127m	0.14i	0.30n
V ₃ T ₁	3.01bc	0.40c	25.46d	1.20c	1.46d
V ₃ T ₂	2.16e	0.32e	19.78g	0.86e	1.15g
V ₃ T ₃	1.36hi	0.23gh	14.24i	0.43h	0.78j
V ₄ T ₁	2.50d	0.32e	21.13f	0.88e	1.23f
V ₄ T ₂	1.82ef	0.24g	15.09i	0.57g	0.95i
V ₄ T ₃	1.13hi	0.16i	9.247k	0.23i	0.61l
V ₅ T ₁	3.18ab	0.67a	36.26a	1.55a	1.83a
V ₅ T ₂	1.45gh	0.46b	30.17c	1.25c	1.52c
V ₅ T ₃	1.32hi	0.37d	23.24e	0.85e	1.150 g
V ₆ T ₁	3.40a	0.45b	31.39b	1.38b	1.60b
V ₆ T ₂	2.70cd	0.36d	25.55d	1.10d	1.31e
V ₆ T ₃	1.94ef	0.28f	17.87h	0.73f	0.95i
LSD _{0.05}	0.31	0.02	1.13	0.09	0.05
CV (%)	10.20	4.17	3.67	7.54	2.32

In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT).

** V₁= BARI Mistimorich-1, V₂= Green bell, V₃= Dream, V₄= Thunder, V₅= Messi and V₆= Maria; T₁= 0 dSm⁻¹, T₂= 4 dSm⁻¹ and T₃= 8 dSm⁻¹

Branch dry weight (g)

The interaction effect of salt concentration with varieties showed significant influence on the branch dry weight (Table 1). At 4 dSm⁻¹ salinity level, the highest branch dry wt. (1.25 g) was found in Messi followed by Maria (1.1 g), BARI Mistimorich-1 (0.86 g), and the lowest branch dry wt. (0.39g) was recorded from Thunder followed by Green bell (0.42 g), Dream (0.56 g). The same result was obtained for control treatment and 8 dSm⁻¹ treatment. Similar results were obtained in white seed coat bambara (Tafouo et al., 2008). Results mainly revealed that branch dry weight was decreased with the increasing salinity in each variety.

Leaf dry weight (g)

The interaction effect of salt concentration with varieties showed significant influence on the branch dry weight (Table 1). At 4 dSm⁻¹ salinity level, the highest branch dry wt. (1.52 g) was found in Messi followed by Maria (1.3 g), BARI Mistimorich-1 (1.15 g), and the lowest branch dry wt. (0.58 g) was recorded from Thunder followed by Green bell (0.73 g), Dream (0.95 g). Tafouo et al. (2008) reported that leaf dry weight was decreased with the increasing salinity in white seed coat bambara. The same result was obtained for control treatment and 8 dSm⁻¹ treatment. So, leaf dry weight was decreased with the increasing salinity in each variety.

Physiological attributes**Relative chlorophyll content (SPAD value)**

The interaction effect of salt concentration with varieties showed significant influence on the transpiration (Table 2). At 4 dSm⁻¹ salinity level, the highest relative chlorophyll content (50.08) was found in Messi followed by Maria (44.8), BARI Mistimorich-1 (41.5), and the lowest relative chlorophyll content (29.33) was recorded from Thunder followed by Green bell (34.5), Dream (37.4). The same result was obtained for control treatment and 8 dSm⁻¹ treatment. Nozulaidi et al. (2015) reported that relative chlorophyll content was decreased with the increment of salinity levels in plants.

Photosynthesis (Pn; A)

The interaction effect of salt concentration with varieties showed significant influence on the transpiration (Table 2). At 4 dSm⁻¹ salinity level, the highest photosynthesis (10.89 $\mu\text{molm}^{-2}\text{s}^{-1}$) was found in Messi followed by Maria (10.12 $\mu\text{molm}^{-2}\text{s}^{-1}$), BARI Mistimorich-1 (8.92 $\mu\text{molm}^{-2}\text{s}^{-1}$), and the lowest photosynthesis content (5.67 $\mu\text{molm}^{-2}\text{s}^{-1}$) was recorded from Thunder followed

by Green bell ($7.12 \mu\text{molm}^{-2}\text{s}^{-1}$), Dream ($8.08 \mu\text{molm}^{-2}\text{s}^{-1}$). The photosynthetic process under saline conditions reduced due to reduction in carbon uptake, lower stomatal conductance and inhibition in photochemical capacity or combination of all these (Gaballah et al., 2006 and Hussain et al., 2008). Same result was obtained for control treatment and 8 dSm^{-1} treatments.

Table 2. Effects of salinity level on SPAD value, Photosynthesis, Transpiration and Stomatal conductance of different sweet pepper varieties

Variety X treatment	SPAD value	Photosynthesis (Pn; A) ($\mu\text{molm}^{-2} \text{ s}^{-1}$)	Transpiration (E) ($\text{mmolm}^{-2} \text{ s}^{-1}$)	Stomatal conductance (g _s) ($\text{molm}^{-2} \text{ s}^{-1}$)
V ₁ T ₁	38.80f	9.51g	0.31f	3.86e
V ₁ T ₂	34.50h	7.12l	0.21i	2.78gh
V ₁ T ₃	26.81j	5.01p	0.13k	1.84j
V ₂ T ₁	33.34h	8.06j	0.26h	2.96g
V ₂ T ₂	29.33i	5.67n	0.17j	1.74j
V ₂ T ₃	22.32k	3.68q	0.10l	0.87k
V ₃ T ₁	46.46c	11.64c	0.43c	5.11c
V ₃ T ₂	41.50e	8.93h	0.32f	3.93e
V ₃ T ₃	33.56h	6.45m	0.22i	2.71h
V ₄ T ₁	41.64e	10.34e	0.35e	4.68d
V ₄ T ₂	37.40fg	8.08j	0.25h	3.54f
V ₄ T ₃	30.63i	5.37o	0.15j	2.27i
V ₅ T ₁	56.87a	13.33a	0.54a	6.32a
V ₅ T ₂	50.09b	10.89d	0.43c	5.23c
V ₅ T ₃	42.43e	8.213i	0.32f	3.87e
V ₆ T ₁	50.97b	12.60b	0.49b	5.85b
V ₆ T ₂	44.80d	10.12f	0.39d	4.75d
V ₆ T ₃	36.56g	7.41k	0.28g	3.59f
LSD _{0.05}	1.49	0.09	0.02	0.22
CV (%)	2.32	0.66	5.61	3.54

In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT).

** V₁= BARI Mistimorich-1, V₂= Green bell, V₃= Dream, V₄= Thunder, V₅= Messi and V₆= Maria; T₁= 0 dSm⁻¹, T₂= 4 dSm⁻¹ and T₃= 8 dSm⁻¹

Transpiration (E)

The interaction effect of salt concentration with varieties showed significant influence on the transpiration (Table 2). At 4 dSm⁻¹ salinity level, the maximum transpiration (0.43 mmolm⁻²s⁻¹) was found in Messi followed by Maria (0.39 mmolm⁻²s⁻¹), BARI Mistimorich-1 (0.32 mmolm⁻²s⁻¹), and the lowest transpiration Content (0.17 mmolm⁻²s⁻¹) was recorded from Thunder followed by Green bell (0.21 mmolm⁻²s⁻¹), Dream (0.25 mmolm⁻²s⁻¹). The same result was obtained for control treatment and 8 dSm⁻¹ treatment. Transpiration of plant was significantly reduced with the increasing salinity (Munns and Tester, 2008).

Stomatal conductance (g_s)

The interaction effect of salt concentration with varieties showed significant influence on the stomatal conductance (Table 2). At 4 dSm⁻¹ salinity level, the maximum stomatal conductance (5.23 molm⁻²s⁻¹) was found in Messi followed by Maria (4.75 molm⁻²s⁻¹), BARI Mistimorich-1 (3.94 molm⁻²s⁻¹), and the lowest stomatal conductance content (1.75 molm⁻²s⁻¹) was recorded from Thunder followed by Green bell (2.79 molm⁻²s⁻¹), Dream (3.54 molm⁻²s⁻¹). The same result was obtained for control treatment and 8 dSm⁻¹ treatment. A similar result was also found by Niu et al. (2012).

Biochemical attributes

Leaf proline content

The interaction effect of salt concentration with varieties showed significant influence on the leaf proline content (Fig. 1). At 4 dSm⁻¹ salinity level, the maximum leaf proline content (0.766 mg/100 g F) was found in Messi followed by Maria (0.710 mg/100g F), BARI Mistimorich-1 (0.633 mg/100g FW), and the lowest leaf proline content (0.420 mg/100g FW) was recorded from Thunder followed by Green bell (0.483 mg/100 g FW), Dream (0.590 mg/100g FW). That result was similar to Cicek and Cakirlar, (2002). The same result was obtained for control treatment and 8 dSm⁻¹ treatment.

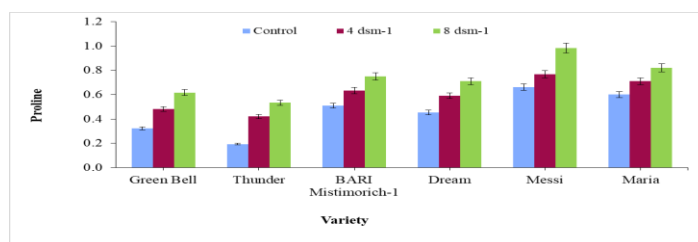


Figure 1. Effects of salinity levels on leaf proline content of different sweet pepper varieties. Vertical bars represent SEM (n=3)

Chlorophyll content

The interaction effect of salt concentration with varieties showed significant influence on chlorophyll-a content. At 4 dSm⁻¹ salinity level, the maximum Chl-a content (2.24 mg/g FW) was found in Messi followed by Maria (2.07 mg/g FW), BARI Mistimorich-1 (1.908 mg/g FW), and the lowest Chl-a content (1.45 mg/g FW) was recorded from Thunder followed by Green bell (1.61 mg/g FW), Dream (1.73 mg/g FW) (Fig. 2). The same result was obtained for control treatment and 8 dSm⁻¹ treatment. The results explored that Chl-a content was decreased with the increasing salinity in each variety.

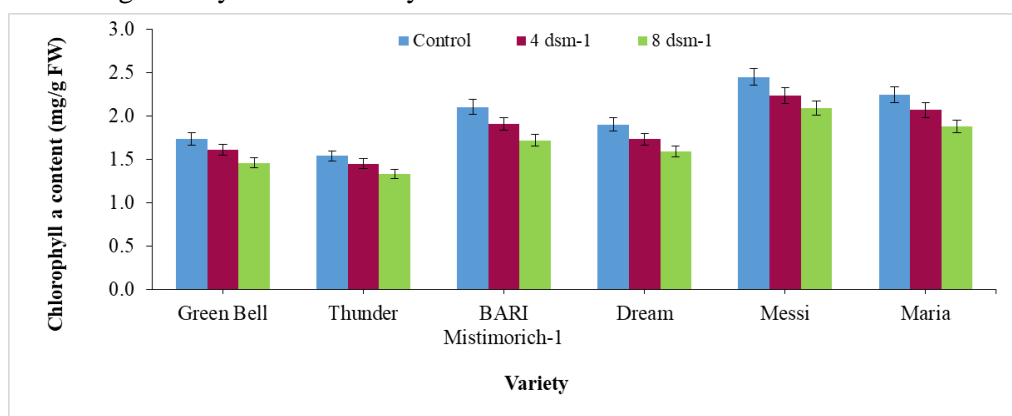


Figure 2. Effects of salinity levels on chlorophyll-a (Chl-a) content of different sweet pepper varieties. Vertical bars represent SEM (n=3)

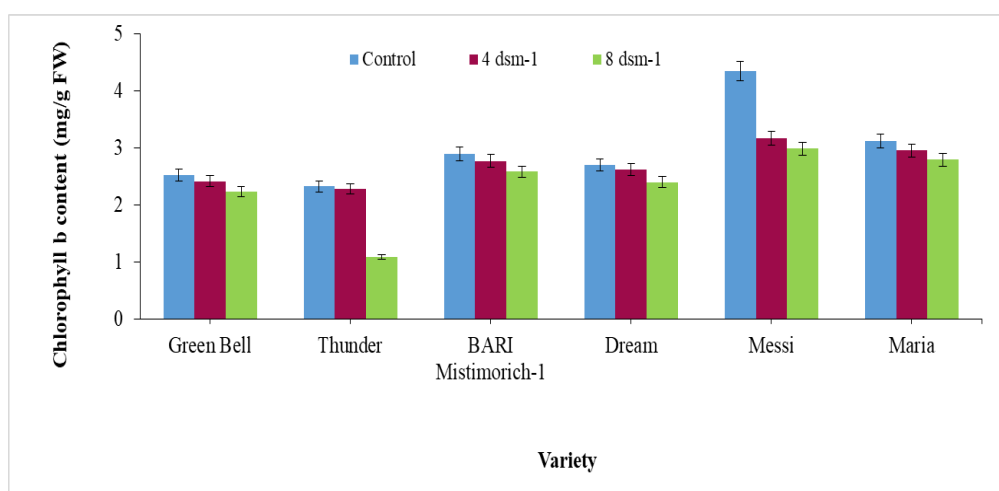


Figure 3. Effects of salinity levels on chlorophyll-b (Chl-b) content of different sweet pepper varieties. Vertical bars represent SEM (n=3)

The interaction effect of salt concentration with varieties showed significant influence on chlorophyll-b content. The maximum Chl-b content (3.16 mg/g FW) was found in Messi followed by Maria (2.95 mg/g FW), BARI Mistimorich-1 (2.77mg/g FW) and the lowest Chl-b content (2.28 mg/g FW) was recorded from Thunder followed by Green bell (2.41 mg/g FW), Dream (2.62 mg/g FW) at 4 dSm⁻¹ salinity level (Fig. 3). The same result was obtained for control treatment and 8 dSm⁻¹ treatment.

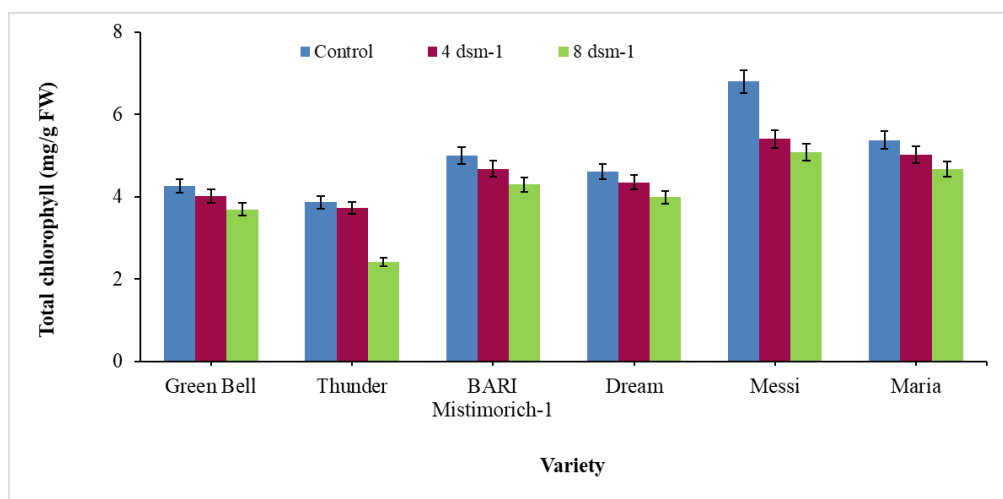


Figure 4. Effects of salinity levels on total chlorophyll content of different sweet pepper varieties. Vertical bars represent SEM (n=3)

The combined effect of salinity with the varieties showed significant influence on the total chlorophyll content (Fig. 4). At 4 dSm⁻¹ salinity level, the maximum total chlorophyll content (3.10 mg/g FW) was found in Messi followed by Maria (2.91 mg/g FW), BARI Mistimorich-1 (2.74 mg/g FW), and the lowest Chl-a content (2.28 mg/g FW) was recorded from Thunder followed by Green bell (2.41 mg/g FW), Dream (2.59 mg/g FW). Reduction in chlorophyll under salinity stress has been reported in many plant species including sweet pepper (Abdul-Jaleel et al., 2007). The same result was obtained for control treatment and 8 dSm⁻¹ treatment.

CONCLUSION

The study was done to evaluate the sweet pepper varieties based on the morpho-physiological and biochemical responses under salinity stress and to identify potential salt tolerant cultivar(s) for saline prone areas of Bangladesh. The results revealed that growth and development of sweet pepper was adversely invaded by salinity. A huge variation in vegetative stage in sweet pepper caused by salinity has been derived from the experiment. Salt tolerant varieties exhibited minimum

relative reduction of morpho-physiological properties and increased value of leaf proline content compared to sensitive ones, at vegetative stage under salt stress. And the variety, Messi was found salt tolerant while Thunder appeared as salt sensitive from overall performance based on morpho-physiological and biochemical attributes. Moreover, further study is needed to evaluate the varieties at reproductive phase in the field condition, especially in the coastal areas of Bangladesh for their adaptability to grow.

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