Evaluating physiological responses of Butterfly pea, *Clitoria ternatea* L. var. *Pleniflora* to salt stress

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Abstract. Nowadays, proportions of agriculture land had become saline. Many plants and herbs have evolved series of defences to mitigate against salinity. There were critical appraisal studies on salinity using the butterfly pea, *Clitoria ternatea* var *Pleniflora* that analysed their potential role as salt tolerant legume. However, study on the physiological responses of *Clitoria ternatea* is still lacking. Therefore, the purpose of this study was to introduce and evaluate the growth traits of *Clitoria ternatea* var *Pleniflora* with regards to the number of branches and leaves, plant’s height, fresh and dry weight of plant parts (stem, leaves and roots) during the exposure to six different doses of NaCl (0, 100, 150, 200, 250 and 300 mM). In addition, controlled growth environment was watered daily with equal NaCl-supplemented distilled water. The results from this study had revealed that all of the test growth traits: the number of branches and leaves, height of plants, fresh weight of leaves and dry weight of stem, leaves and roots were the highest under 200 mM of NaCl. In conclusion, 200 mM of NaCl can improve the overall growth of *Clitoria ternatea* var *Pleniflora*.

1. Introduction

These days, world confronts and deal with one of the predominant problems which gives to many sensitivities towards growth and future. Salinity becomes one of the limiting factors towards environment and life. Productivity and growth of plants are reduced since most of them are present in low tolerance towards soil salinity. Moreover, the plants undertake the evolution by two main mechanisms either by limiting the salt’s entrance by roots or controlling its concentration and distribution [1] which is relevant to improve their tolerance and adaptations. The notable impacts of salt stress have been faced by *Clitoria ternatea* in semi-arid and arid region where they are considered as low to medium salt tolerant [2] as well as more tolerant among some of other comparative legumes. Thus, this study was focusing on the effects of various concentrations of sodium chloride, NaCl on the physiological part of *Clitoria ternatea* growth. It might be prominent as it can shows their endurance and capabilities to survive on salt stress over time. This study also might fill the gap to certain physiological part of halophytes.
2. Methodology

2.1 Plant Materials
The stems of *Clitoria ternatea var. Pleniflora* were cut for 10cm and placed in soil about ¼ depth of the soil. Once the new leaves emerged from the stems, they were transferred into another bigger pot. After a few days, the propagation stage of *Clitoria ternatea var. Pleniflora* were observed. Then, the plants were transferred into new big pots that contain the mixture of topsoils, organic soil and sands at ratio of 2:1:1 [3]. Three hundred pots of *Clitoria ternatea var. Pleniflora* were prepared for preliminary and full-scale NaCl treatment as well as an early preparation of plants from getting any diseases. The species of *Clitoria ternatea* var. *Pleniflora* was identified by botanist in UTHM and FRIM with a voucher number of SUNR-25395 and was deposited under UTHM herbarium.

2.2 Soil Treatment
Responses of *Clitoria ternatea* var. *Pleniflora* were introduced under five different doses (100, 150, 200, 250 and 300 mM) of NaCl treatment of moderate stress as keeping a control (0 mM added NaCl). In addition, controlled growth environment was conducted by a pot experiment, and subsequently they were watered with equal daily of NaCl-supplemented distilled water of 10 mL for twice in a day. The soil treatment was conducted until 14 days of plant growth.

2.3 Measurement of Physiological

2.3.1 Plant Height
The main plant height was measured from the apex of *Clitoria ternatea* var. *Pleniflora*’s leaves to the border of the container in meters (m). The frequency of the measurement was calculated for every 2 to 3 days [4].

2.3.2 Number of Leaves
The number of visible leaves on each pot were recorded and counted and the tip of the new emerging leaves were included. The *Clitoria ternatea* var. *Pleniflora* were placed over the graph paper to avoid the counting error. The frequency of the measurement was calculated for every 2 to 3 days.

2.3.3 Number of Branches
The branches number were recorded and counted including the new emerged branches. The frequency of the measurement was calculated for every 2 to 3 days.

2.3.4 Fresh Weight
*Clitoria ternatea* var. *Pleniflora* were removed from soil and the loose soil were washed off. They were blotted gently with soft paper towel to ensure and remove any free surface moisture on the plant. After that, each plant was weighed immediately as the plant still consist the high composition of water and to ensure the production of accurate result [5, 6].

2.3.5 Dry Weight
*Clitoria ternatea* var. *Pleniflora* were removed from the soil and the loose soil were washed off. The plants were blotted gently to avoid and remove the free surface moisture. After that, they were dried in an oven for overnight at low heat of 104 ° F (40 °C). At the next day, they were let to cool in a Ziploc bag, a dry environment to keep the moisture out. Then, they were weighed on the scale that were set up to grams (g) [6].

3. Results and Discussions

3.1 Number of branches of *Clitoria ternatea* var. *Pleniflora* over 14-days of treatment.
From Figure 1, as the concentration increasing, some of the treated branches (200 mM and 250 mM) were increasing until day-12 but were lower than control. Meanwhile, more treated branches (0 mM, 100 mM and 150 mM) were increasing only until day-10 but were also lower than control. Then, only branches treated with 300 mM was increasing until day-14 and was also lower with increment than control (0 mM). The similar effect of treated branches lower than control also can be seen when, Egypt soybean (*Glycine max* L.) were treated with salt stress and it shown that, their number of
branches were lower than the control [7]. These effects can be seen with the advancement of time. This might happen due to the formation of new branches were impeded by salt stress, as well as facilitating the aging process of the old branches [7].

![Figure 1](image1.png)

**Figure 1.** Total increment of *Clitoria ternatea* var *Pleniflora*’s number of branches over 14 days of measurement.

### 3.2 Number of leaves of *Clitoria ternatea* var *Pleniflora* over 14-days of treatment.

From Figure 2, the highest total increment number of leaves of *Clitoria ternatea* var *Pleniflora* was at concentration of 200 mM NaCl (6.222) compared to control (5.111). A research was treating pistachio, *Pistacia vera* L. with salinity of 0, 50, 100 and 150 mM of NaCl up to 15 days. Based on the results, the number of leaves were higher compared to control at each concentration [6]. Likewise, this might also give the same outcome to the treated-200 mM NaCl of *Clitoria ternatea*. This might happen due to a mechanism of salt-tolerant to mitigate the toxicity of salt [8]. Thus, it might allow them to grow continuously in long period. Meanwhile, the number of leaves was the lowest at the concentration of 300 mM of NaCl. Likewise, *Populus euphratica* were treated with 0, 50, 100, 150,200, 250 and 300 mM of NaCl. In their study, *P. euphratica* shown that, leaves’ number were the lowest when treated with 300 mM NaCl. They were significantly reduced to 20 % compared with the control [9].

![Figure 2](image2.png)
Figure 2. Total increment of *Clitoria ternatea* var *Pleniflora*’s number of leaves over 14 days of measurement.

3.3 Plant height of *Clitoria ternatea* var *Pleniflora* over 14-days of treatment

From Figure 3, the plant height of *Clitoria ternatea* var *Pleniflora* was the highest when treated with 200 mM of NaCl compared to control, 0 mM of NaCl. The similar trend occurred to Noni plants, *Morinda citrifolia* L. when treated under 200 mM of NaCl [10]. Based on the result, it is shown that plant height was the tallest (44 cm ± 3.6) compared to control (32 cm ± 2.4) and under 100 mM of NaCl (36 cm ± 1.6) [10]. The similar effect of increasing shoot growth treated under 200 mM of *Clitoria ternatea* might happen since 200 mM is still tolerable and not reaching to the level of toxic towards plants [11]. This adaptation might occur due to strong correlations between salt tolerance and salt exclusion within a plant [11]. Meanwhile, *Clitoria ternatea* have the shortest in height when treated with 300 mM of NaCl at day-14. A study used Kallar grass, *Leptochloa fusca* to be treated under 0, 100, 200 and 300 mM of NaCl [12]. Based on the results, it was show that there was a progressive decrease in plant height when treated under 300 mM of NaCl. The plant height was the shortest among the four treatments [12].

![The total increment of the plant height over 14 days of measurement](image)

Figure 3. Total increment of *Clitoria ternatea* var *Pleniflora* plant height over 14 days of measurement.

3.4 Fresh weight on different part of *Clitoria ternatea* var *Pleniflora* over 14-days of treatment

Based on the Figure 4, the highest amount of stem’s fresh weight was at 300 mM of NaCl compared to control, 0 mM of NaCl. In a study, Evergreen mangrove, *Excoecaria agallocha* were treated with 0, 100, 200, 300, 400 and 500 mM of NaCl and obtain the highest stem fresh weight when treated with 300 mM at day 60 (4.75 g) and 120 (5.89 g) compared to control at day 60 (2.64 g) and 120 (4.21 g) [13].

Additionally, the leaves fresh weight reached a peak significantly at 200 mM at 13.729 g compared to control 11.493 g. This data correlates well with a study by Khedr et al., 2011 [14], where the leaf fresh weight of Mediterranean salt bush, *Atriplex halimus* under 200 mM (0.2533 g ±0.0233) of NaCl was the highest when compared to control (0.1600 g ±0.0147) at p ≤ 0.05. Meanwhile, fresh weight of leaves in this study hit to its lowest point when treated under the concentration of 300 mM of NaCl relatives to control. This is in agreement with a study by Eisa et al., 2012 [15] in which the leaves fresh weight of quinoa, *Chenopodium quinoa* was the lowest under 300 mM of NaCl treatment.

Furthermore, the highest root’s fresh weight, 1.4504 g was at 200 mM of NaCl compared to the control, 1.1076 g. Besides, fodderbeet, *Beta vulgaris* were treated under two concentrations, 0 and
200 mM of NaCl and root fresh weight of fodderbeet is higher (12.602 g) than control (9.856 g) (p<0.01) [16]. On contrary, the lowest fresh weight of root was at 100 mM, 1.0763 g compared with the control, 1.1076 g.

Figure 4. Fresh weight of each part of stem, leaves and root of *Clitoria ternatea* var *Pleniflora*.

3.5 Dry weight on different part of *Clitoria ternatea* var *Pleniflora* over 14-days of treatment.
Based on the Figure 5, at 200 mM of NaCl treatment, it obtained the highest stem dry weight compared to control. Based on a study, water spinach, *Ipomoea aquatica* were treated under 50, 100 and 200 mM of NaCl and obtained the highest stem dry weight under treatment of 200 mM of NaCl (63.5 g ±0.17) compared to control, 0 mM (3.69 g ±0.29) (p<0.05) [17]. Then, the lowest stem dry weight hit at the 100 mM of NaCl treatment.

On the other hand, leaves dry weight reached the highest at 200 mM of NaCl compared to control. Furthermore, 100 and 200 mM of NaCl were introduced to deciduous tree, *Elaeagnus angustifolia* L. Highest leaves dry weight was under 200 mM of NaCl (48.68 g ±1.28) compared to control (46.65 g ±0.64) [18]. Meanwhile, treatment under 100 mM of NaCl obtained the lowest leaves dry weight compared to control. Apart from that, mungbean, *Vigna radiata* L was treated under 10, 50 and 100 mM of NaCl against control, 0 mM of NaCl and under 100 mM of NaCl, *V. radiata* obtained the lowest leaves dry weight (0.0033 g ± 0.0013) compared to control (0.0141 g ± 0.0022) [19].

In addition, the highest root dry weight was at 200 mM compared to control. In a study by Pessarakli et al., 2005, Russian olive, *Elaeagnus angustifolia* were used and treated under 0, 100 and 200 mM of NaCl. The root dry weight treated under 200 mM of NaCl hit the highest (14.53 g ±0.55) compared to control (9.13 g ±0.32). Moreover, under 200 mM of NaCl, desert saltgrass, *Distichlis spicata* L. possessed the highest root dry weight (0.549 g) compared to control (0.441 g) [20].

Despite, at 100 mM of NaCl treatment, it possessed the lowest root dry weight compared to control. Two species of persimmons, *Diospyros kaki* and *D. virginiana* possessed the lowest root dry weight under 100 mM (0.62 g, 1.04 g) compared to control respectively (1.81 g, 1.79 g) when treated under 0, 50, 75 and 100 mM of NaCl [21].
Figure 5. Dry weight of each part of stem, leaves and root of *Clitoria ternatea* var *Pleniflora*.

4. Conclusions

*Clitoria ternatea* var *Pleniflora* can considerably portray as salt-tolerant plants as it can survive up to 250 mM of NaCl in the advancement of time. Presence of salt stress might enhance and considered as a factor for achieving the optimum growth for *Clitoria ternatea* var *Pleniflora*. Most of the growth parameters of *Clitoria ternatea* var *Pleniflora* grown at its best and hit at its highest point under 200 mM of NaCl. These results suggest that *clitoria ternatea* var *Pleniflora* showed a better performance under salinity stress and more appropriate under salinity condition. On the other hand, the information from this study may also possibly provide a stepping stone for future researchers particularly, in genetics, to produce plant breeds with better endurance towards salinity.

Acknowledgement

The authors would like to thank FRGS-RACER National Grant (Vot K 165), GPPS University Grant (Vot H631, H421 and U814) for assistance and sponsorship.

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