Tolerance Level of *Stylosanthes guianensis* Cultivar Cook against Salinity Stress

A.Z. Fauzan[1], P.D.M.H. Karti[2], I. Prihantoro[2], L. Abdullah[2]

10.18805/ag.DF-744

ABSTRACT

Background: *Stylosanthes guienensis* Cook cultivar has the potential to be cultivated in saline areas, especially in the tropics. This study aims to evaluate the tolerance level of *Stylosanthes guianesis* cultivar Cook to different levels of salinity stress.

Methods: The method used was single randomized complete block design (CRD) with 5 treatments and 20 replicates. The experimental design consists of P0 (NaCl 0 ppm), P1 (NaCl 2000 ppm), P2 (NaCl 3000 ppm), P3 (NaCl 4000 ppm) and P4 (NaCl 5000 ppm). Parameters measured include plant *via*bility, vertical height of plants, number of trifoliate leaves, number of leaflets, plant weight and leaf color. Data were analyzed using ANOVA and if the data were significantly different, further tested using Duncan with SPSS software version 25.

Result: The results showed that *Stylosanthes guianensis* Cook in NaCl treatment had a significant effect (P<0.05) on plant vertical height, number of leaflets, plant length and plant weight and NaCl treatment up to the level of 5000 ppm decreased plant *via*bility and leaf color became darker. The conclusion of the study showed that *Stylosanthes guianensis* Cook can survive in saline conditions up to 2000 ppm and at moderate stress (3000 ppm) experienced a significant decrease.

Key words: Legumes, Salinity, Stylosanthes, Tissue culture, Tolerance.

INTRODUCTION

Stylosanthes guianensis is a legume native to Central and South America and can grow in tropical to subtropical regions, including Suriname, Venezuela, Brazil and Indonesia (Palsaniya *et al.,* 2017). *Stylosanthes guianensis* as a feed source has a fairly high nutrient content with a crude protein content of 10.5%, crude fiber 25% and crude fat 3% (Schultze-Kraft *et al.,* 2023). The International Center for Tropical Agriculture reported that there are ≥500 Stylosanthes cultivars in various countries since the 1960s (Santos-Garcia *et al.,* 2012). One cultivar that is widely cultivated in Indonesia is *S. guianensis cv. Cook*. This plant has the advantage of improving soil fertility. According to Latief *et al.* (2020), *S. guianensis* has the ability to fix nitrogen from the air through a symbiotic process with Rhizobium bacteria so that it can improve the quality of tropical grazing land.

Efforts to develop and spread *S. guianensis* cv. Cook need to be done especially in marginal areas such as saline soils. Indonesia has 81,290 km of coastline (Tome *et al.,* 2024) that can be utilized as land for *S. guianensis* cultivation. Currently, *S. guianensis* has been cultivated as a forage that grows in soils that tend to be acidic with a pH ranging from 3-5 (Putra *et al.,* 2021). The Agricultural Research and Development Agency (2020) reported that 6.2% of Indonesia's total land area is saline soils ranging from 0.5 - 32 dS m^{-1}. Saline soils have low availability of P, N, Cu, Mn, Fe and Zn nutrients, high osmotic pressure, low activity of soil microorganisms and weak water and air movement (Liu *et al.,* 2017). The use of saline soils causes lethargy and death in non-halophytic plants (Wang *et al.,* 2022). The level of tolerance of plants to salinity is divided into four categories: halophytes (>4000 ppm), salt-tolerant plants (2000-4000 ppm), moderate salt-tolerant (1000-2000 ppm), salt-sensitive plants (<1000 ppm) (Moussa *et al.,* 2022).

Most cultivars of *Stylosanthes guianensis* have sensitivity to salinity as in the study (Liu *et al.,* 2022) *S. guianensis* variety FM05-2 claimed to be sensitive to 100 mM salinity. Studies on *S. guianensis* cv. Cook on saline soils has not been reported in Indonesia, so research on the growth of *S. guianensis* cv. Cook on saline soils as a basis for effective development of *S. guianensis*. This study aims to evaluate the tolerance level and obtain information on the adaptation limit of *S. guianensis* cv. Cook to different levels of salinity stress.

[1]Graduate School of Nutrition and Feed Science, Faculty of Animal Science, IPB University (Bogor Agriculture University), Bogor 16680, Indonesia.

[2]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University (Bogor Agriculture University), 16680, Bogor, Indonesia.

Corresponding Author: A.Z. Fauzan, Graduate School of Nutrition and Feed Science, Faculty of Animal Science, IPB University (Bogor Agriculture University), Bogor 16680, Indonesia.
Email: abdulzumar@apps.ipb.ac.id
ORCID: 0009-0009-1939-2693

How to cite this article: Fauzan, A.Z., Karti, P.D.M.H., Prihantoro, I. and Abdullah, L. (2026). Tolerance Level of *Stylosanthes guianensis* Cultivar Cook against Salinity Stress. *Agricultural Science Digest.* **46(1):** 31-37. doi: 10.18805/ag.DF-744.

Submitted: 11-04-2025 **Accepted:** 12-06-2025 **Online:** 02-07-2025

MATERIALS AND METHODS

Study period and location

This research was conducted from January to May 2024 at the Feed Plant Tissue Culture Laboratory, Division of Feed and Pasture Plant Science and Technology, Faculty of Animal Science, Bogor Agricultural University.

Work procedure

Seeds of *Stylosanthes guanensis* cultivar Cook were obtained from the Agrostology Field Laboratory of IPB University. Stylo seeds were given seed treatment by soaking the seeds in NaClO solution for 10 minutes, after which they were immersed in warm water at 60°C for 3 minutes, then soaked with distilled water for 24 hours. Seeds were sterilised in a laminar air flow using clorox solution and distilled water. Seeds that have been sterilised can be planted on control media as many as 20 seeds and 80 seeds in NaCl treatment. Each seed was put into one bottle measuring 10.4 × 4.9 cm with a volume of 100 ml. The time of planting seeds into the media shows the age of 0 Days After Planting (DAP). Observations of this study were carried out by observing morphological characteristics (vertical height, number of *trifoliate* leaves and leaf blade) which were carried out every three days, leaf color was observed and crown weight was weighed on the 30th day. Observations and measurements started from the first day after planting until 30 DAP.

Statistical analysis

This study was conducted using a single randomised complete block design (CRD) consisting of 5 treatments and 20 replicates. Data from the study were analysed by analysis of variance (ANOVA), using the method of Steel and Torrie (1995) if significantly different ($P<0.05$) Duncan's further test was conducted with IBM SPSS Statistic version 25. While the results of leaf colour analysis at the age of 30 days using Munshell Color Chart 1.0.11. The results of the study using the stress index formula based on Fernandez (1992):

$$IC = 1 - \frac{H\bar{c}}{H\bar{p}}$$

Keterangan :

IC = Stress Index.

$H\bar{c}$ = Average yield of all replicates per treatment under stressed conditions.

$H\bar{p}$ = Average yield of all replicates under no stress (control) condition.

RESULTS AND DISCUSSION

Viability of *Stylosanthes guianensis* under different salinity stresses

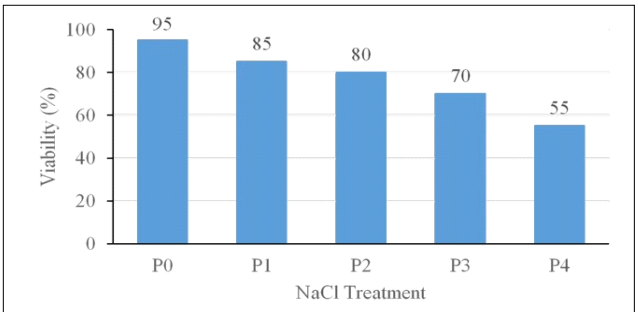
Plant viability illustrates the level of tolerance of *S. guianensis* to salinity stress, which is very important specially to evaluate the quality and growth potential of *S. guianensis*. The results of *S. guianensis* viability with different NaCl levels (30 DAT) are presented in Fig 1. Viability of *S. guianensis* cv. Cook decreased as the NaCl level increased. Media treated with NaCl at the highest level had lower growth by 40% than without NaCl. The decrease in viability occurs due to increased osmotic stress, impaired nutrient absorption, cell death and even excessive Na^+ and Cl^- ion poisoning, thus disrupting cell ionic balance and physiological processes such as respiration and photosynthesis. In accordance with the research of Veraplakorn *et al.* (2013) excess Na^+ and Cl^- ions damage the cell membrane of *S. guianensis* so that the metabolic process is disrupted, severe cell membrane damage causes plant cell death.

Growth stages can show different sensitivity to salinity stress. Tolib *et al.* (2017) reported that legumes are still tolerant in moderate salinity soils at 2500 ppm and are not resistant to high salinity. NaCl stress causes plant cell death and reduced growth (up to 40%) either by inhibiting water uptake or toxic effects of ions in the cell. According to Farooq *et al.* (2017) ion toxicity in plant tissues damages cell structure and enzyme function thereby inhibiting growth and causing plant development to be inhibited up to 70%.

Vertical height of *S. guianensis* under different salinity stresses

The results of vertical height of *S. guianensis* at different NaCl levels are presented in Table 1. Giving the highest level of NaCl had a very significant effect at 15 DAP. Media without NaCl (control) showed the best vertical height compared to *S. guianensis* with NaCl media. The decrease in vertical height of *S. guianensis* was influenced by the level of NaCl which caused differences in the expression of plant height in each treatment. This results in cell damage in *S. guianensis* inhibiting growth.

The concentration of NaCl in the media affects osmosis and causes water to move out of *S. guianensis* cells. Based on Veraplakorn *et al.* (2012) the height growth of *S. guianensis* CIAT184 began to be significantly inhibited at a salinity of 20-25 mM NaCl (about 1100-1400 ppm). According to Chen *et al.* (2023), an increase in osmotic pressure causes cell turgor pressure to decrease so that stem growth is inhibited and as a result, plants become short. Excess Na^+ and Cl^- ions in the media interfere with the activity of gibberellin hormone in *S. guianensis*.



P0 = 0 ppm, P1 = 2000 ppm, P2 = 3000 ppm, P3 = 4000 ppm, P4 = 5000 ppm.

Fig 1: Viability of *S. guianensis* with different NaCl levels (30 DAT).

Gibberellin hormone plays a role in cell elongation, excess Na^+ and Cl^- ions in gibberellin activity causes inhibited stem growth (Ramadhani and Ulpah, 2022).

Number of trifoliate leaves under different salinity stresses

Stylosanthes guianensis has trifoliate leaves or consists of three leaflets attached to one stalk. The results of the number of trifoliate leaves at different NaCl levels are presented in Fig 2. Trifoliate leaves began to form on day 12 with the highest number in media without NaCl. The provision of more NaCl caused a decrease in the number of trifoliate leaves reaching 72.7% of the media without NaCl (control). The ability of NaCl can reduce the absorption of water by the roots, the potential water in the media becomes lower than the potential water in the root cells.

Media supplemented with NaCl can increase osmotic pressure, so that plants are dehydrated and interfere with the division of meristematic cells responsible for the formation of primordial leaves of *S. guianensis*. According to Rizki *et al.* (2022) the number of *Stylosanthes* trifoliate leaves at two weeks after planting in the range of 3-6 leaves. Zhao *et al.* (2021) reported that salinity stress affects the slow formation of primordial leaves, so it is necessary to have genetic mutations that can protect plants from this stress. Salinity stress inhibits respiratory enzymes and increases free radicals, thus impairing leaf respiration of *S. guianensis*. In line with the statement of Das *et al.* (2019) salinity stress inhibits the activity of enzymes such as *pyruvate dehydrogenase* and *isocitrate dehydrogenase* that play a role in cellular respiration.

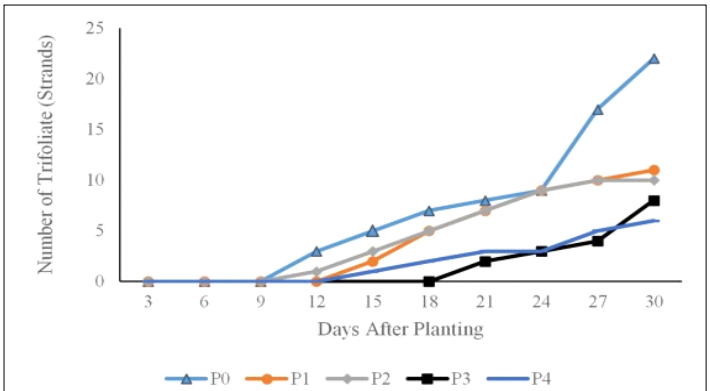
Number of leaves of *S. guianensis* under different salinity stresses

The results of the number of leaves of *S. guianensis* against different NaCl levels have been done presented in Table 2. The higher the NaCl level in the media, the fewer leaf blades produced. The decrease in the number of leaves shows a significant inhibitory effect on the number of leaves due to high NaCl levels. The impact will be increasingly visible as the level and duration of NaCl exposure increase in the media. High NaCl levels in the media interfere with nutrient absorption by means of Na^+ ion concentrations competing with K^+, Ca^{2+} and Mg^{2+} ions in *S. guianensis* cells

Table 1: Vertical height of *S. guianensis* at different NaCl levels.

Age (DAP)	NaCl level				
	P0	P1	P2	P3	P4
	mm				
3	12.77±7.90^a	5.75±3.96^b	7.23±5.13^b	4.27±4.55^b	3.53±3.98^b
6	28.42±7.46^a	15.52±7.07^b	14.12±9.72^b	13.51±7.48^b	12.03±8.77^b
9	34.04±6.89^a	22.50±8.84^b	21.20±8.94^b	21.61±9.25^b	17.10±8.25^b
12	38.21±6.26^a	26.36±7.61^b	25.57±7.89^b	24.98±9.43^b	21.24±7.26^b
15	43.04±8.35^a	32.15±5.99^b	30.33±8.51^{bc}	28.62±7.93^{bc}	25.56±5.78^c
18	45.95±11.36^a	36.55±6.30^b	33.38±9.32^{bc}	31.11±6.30^{bc}	29.14±4.91^c
21	47.98±11.31^a	38.20±6.23^b	35.13±10.02^{bc}	32.10±6.21^c	31.33±5.09^c
24	49.33±11.49^a	39.72±6.43^b	37.28±9.94^{bc}	33.35±6.02^c	32.83±5.10^c
27	50.94±11.53^a	41.04±6.05^b	38.00±9.98^{bc}	34.50±6.17^c	34.01±5.01^c
30	52.51±11.75^a	43.02±5.52^b	38.95±10.08^{bc}	36.73±6.20^c	35.15±5.34^c

P0 = 0 ppm, P1 = 2000 ppm, P2 = 3000 ppm, P3 = 4000 ppm, P4 = 5000 ppm; Different superscript letters in the same row indicate significant differences in the results of Duncan's further test ($P<0.05$).



P0 = 0 ppm, P1 = 2000 ppm, P2 = 3000 ppm, P3 = 4000 ppm, P4 = 5000 ppm;

Fig 2: Number of trifoliate leaves of *S. guianensis* with different NaCl levels (30 DAP).

and can interfere with cell metabolism. In line with the research of Feng *et al.* (2020) which states that Na+ ions have a high affinity which causes the absorption of nutrient ions to be inhibited, so that photosynthesis is disrupted and leaf growth is inhibited. High NaCl concentrations increase reactive oxygen species in plant cells, inhibit axillary branching and reduce leaf blade production (Chauhan *et al.,* 2024).

Leaf length of *S. guianensis* under different salinity stresses

The difference in leaf surface area significantly affects the rate of photosynthesis that takes place in *S. guianensis*. The results of leaf length of *S. guianensis* with different NaCl levels are presented in Table 3. Long-term exposure to NaCl and high NaCl levels can cause the growth of *S. guianensis* leaf length to be inhibited. The provision of higher levels of NaCl causes water stress, as a result the water content transported for photosynthesis is reduced.

High NaCl levels can be toxic to *S. guianensis* by damaging cells and inhibiting metabolic processes. This process disrupts the process of cell division at the leaf growth point, so that the leaves cannot grow optimally long. Research by Feng *et al.* (2020) showed that higher NaCl causes water stress, thus reducing the availability of water for photosynthesis and inhibiting the activity of photosynthetic enzymes, such as RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) which is important in CO_2 fixation. Disruption of photosynthesis causes leaf damage and leaf length growth is not optimal. In accordance with Zhao *et al.* (2021) If the plant lacks water, the photosynthetic rate will drop, so that cells will be regulated to perform limited growth.

Fresh weight of *S. guianensis* under different salinity stresses

The measurement results of *S. guianensis* weight with different NaCl levels are presented in Fig 3. The highest NaCl level (P4) showed the most significant decrease in *S. guianensis* weight (25.97%) compared to media without NaCl (control). NaCl application to *S. guianensis* causes dehydration due to NaCl ions pulling water out of the cells, reducing turgor and inhibiting growth. In accordance with Liu *et al.* (2017) high salinity causes damage to cell structures resulting in loss of cell turgor and cell death which results in the growth of *S. guianensis* being inhibited. Prolonged exposure to NaCl and high levels in tissue culture media can result in necrosis (cell death) of apical buds and young leaf tissue. Tissue death in *S. guianensis* directly reduces mass, so the weight of *S. guianensis* is reduced. Hossain *et al.* (2024) stated that the provision of higher NaCl levels in legumes [*Vigna mungo* Hepper] resulted in a decrease in fresh and dry weights due to a decrease in photosynthetic capacity and osmotic imbalance in plant tissues.

Stress index of *S. guianensis* cv. cook

Salinity stress index is an important method to measure crop tolerance to salinity stress. This method can be used to

Table 2: Number of leaves of *S. guianensis* under different salinity stresses.

Age (DAP)	NaCl level				
	P0	P1	P2	P3	P4
	Strands				
15	2.71±1.25	2.29±0.49	2.20±1.10	2.00±0.00	1.67±1.15
18	3.25±1.67	2.71±0.49	2.43±0.98	2.00±0.00	2.00±1.15
21	3.08±1.68	2.36±0.81	2.40±0.97	2.20±0.84	2.17±1.33
24	3.50±2.07	2.73±0.90	2.58±1.00	2.33±0.82	2.33±1.21
27	4.43±2.10^{a}	3.18±1.40^{ab}	2.92±1.44^{ab}	2.63±1.60^{b}	2.22±1.72^{b}
30	5.21±2.01^{a}	3.55±1.29^{b}	3.17±1.59^{b}	2.90±2.28^{b}	2.56±2.13^{b}

P0 = 0 ppm, P1 = 2000 ppm, P2 = 3000 ppm, P3 = 4000 ppm, P4 = 5000 ppm; Different superscript letters in the same row indicate significant differences in the results of Duncan's further test (P<0.05).

Table 3: Leaf length of *S. guianensis* at different NaCl levels.

Age (DAP)	NaCl level				
	P0	P1	P2	P3	P4
	mm				
15	3.97±0.60^{a}	3.40±0.47^{ab}	3.29±0.37^{ab}	3.13±0.15^{b}	2.87±0.56^{b}
18	4.40±0.66^{a}	3.74±0.50^{ab}	3.51±0.34^{b}	3.46±0.53^{b}	3.32±0.46^{b}
21	4.62±0.61^{a}	3.76±0.64^{b}	3.61±0.56^{b}	3.41±0.85^{b}	3.32±0.69^{b}
24	4.79±0.72^{a}	4.17±0.57^{ab}	3.73±0.67^{b}	3.71±0.79^{b}	3.62±0.64^{b}
27	5.16±0.63^{a}	4.70±0.42^{ab}	4.21±0.47^{bc}	3.97±0.78^{c}	3.80±0.65^{c}
30	5.52±0.57^{a}	5.02±0.39^{ab}	4.58±0.49^{bc}	4.27±0.75^{c}	4.20±0.59^{c}

P0 = 0 ppm, P1 = 2000 ppm, P2 = 3000 ppm, P3 = 4000 ppm, P4 = 5000 ppm; Different superscript letters in the same row indicate significant differences in the results of Duncan's further test (P<0.05).

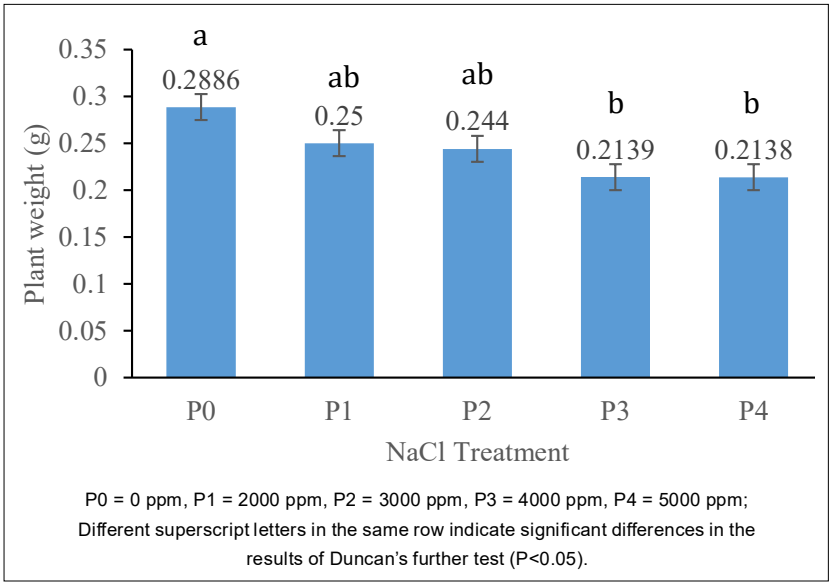


Fig 3: Fresh weight of *S. guianensis* under different salinity stresses (30 HST).

Table 4: Stress index of *S. guianensis* at different NaCl levels.

Parameters	P1	P2	P3	P4
	Stress tolerance index (STI) (Fernandez, 1992)			
Plant viability	0.11	0.16	0.26	0.42
Vertical height of the plant	0.18	0.26	0.30	0.33
Number of trifoliate leaves	0.50	0.55	0.64	0.73
Number of leaflets	0.32	0.39	0.44	0.51
Leaf length	0.18	0.26	0.30	0.31
Plant weight	0.13	0.15	0.26	0.26

P1 = 2000 ppm, P2 = 3000 ppm, P3 = 4000 ppm, P4 = 5000 ppm; Criteria: 0 - 0.25 : Mild stress, 0.26 - 0.50 : Moderate stress, 0.51 - 1.0: Extreme stress. STI: Stress tolerance index.

Table 5: Leaf color of *S. guianensis* under different salinity stresses.

Level NaCl									
P0		P1		P2		P3		P4	
Leaf Color	(%)	Leaf color	(%)	Leaf color	(%)	Leaf color	(%)	Leaf color	(%)
5GY 3/4	35.71	5GY 2/2	30.00	2.5GY 2/4	22.22	7.5Y 2/3	50.00	5GY 2/3	50.00
5GY 4/3	28.57	5GY 3/4	20.00	5GY 2/3	22.22	5Y 2/3	25.00	5Y 2/3	25.00
5GY 4/6	14.29	2.5GY 2/4	20.00	5GY 1/3	22.22	5GY 2/3	25.00	7.5Y 2/3	25.00
5GY 3/3	14.29	2.5GY 2/3	20.00	5GY 1/2	11.11				
5GY 2/3	7.14	10Y 2/4	10.00	10Y 2/3	11.11				
				10Y 3/4	11.11				

P0 = 0 ppm, P1 = 2000 ppm, P2 = 3000 ppm, P2 = 4000 ppm, P4 = 5000 ppm.



develop plant varieties that are more tolerant to salinity. The results of the calculation of the stress index in *S. guianensis* cv. Cook at different NaCl levels are presented in Table 4. Moderate stress began to appear at 3000 ppm salinity, which indicates *S. guianensis* cv. Cook is not tolerant to moderate salinity. The increase in index value is due to the high NaCl level damaging the cell membrane, thus disrupting the metabolism and physiology of *S. guianensis* cv. Cook.

Exposure to NaCl in the media also triggered changes in gene expression in *S. guianensis* cv. Cook and has an impact on the stress index value. This indicates that *S. guianensis* cv. Cook does not have the ability to adapt to medium-high salinity because it experiences a significant reduction in growth. According to Fuentes *et al.* (2010) *S. guianensis* cv. CIAT-184 shows moderate tolerance to salinity conditions, growth begins to be suboptimal in moderate to high salinity soils. Salinity stress in *S. guianensis* cv. Cook increased *peroxidase* and decreased root nodulation. Salinity stress inhibits root nodulation by suppressing the growth of rhizobium bacteria that are sensitive to saline areas (Yunus *et al.,* 2024). Based on Ghosh *et al.* (2025) reported that plants exposed to salinity stree need calsium and potassium supplementation to increase protein fraction.

Leaf color of *S. guianensis* under different salinity stresses

Leaf color is an important parameter to provide information on stress, health and nutrient content in plants. The observation results of *S. guianensis* leaf color at different NaCl levels are presented in Table 5. The number after GY/Y indicates the brightness of the leaf, where a higher number indicates a lighter color. The value after the slash represents chroma, with high numbers indicating a more saturated and intense color, while low numbers indicate a more muted color. The leaf color of *S. guianensis* (Table 5) in NaCl supplemented media showed green-yellow and dark yellow colors, while without NaCl was dominated by 5GY 3/4 at 36%, meaning that the leaf color has two shades, namely GY (Green-Yellow) with 3/4 which tends to be higher. The more chlorophyll in the leaves, the more green the color response is expressed (Prihantoro *et al.,* 2023). Exposure to NaCl in the media affects leaf color due to excess NaCl uptake, which inhibits water absorption and stimulates abscisic acid and anthocyanin production, causing leaf wilting.

CONCLUSION

Stylosanthes guianensis cv. Cook can survive in saline conditions up to 2000 ppm and at moderate stress (3000 ppm) has decreased significantly. Increasing NaCl in media causes a decrease in the growth of *S. guianensis* cv. Cook and changes in leaf color to yellow and darker. Further research can be carried out by providing genetic mutations with gamma rays and increasing salinity tolerance in *S. guianensis* cv. Cook.

ACKNOWLEDGEMENT

The present study was supported by the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia and the Directorate General of Higher Education, Research and Technology through the Master Program of Education Leading to a Doctoral Degree for Excellent Graduates (PMDSU). Research Implementation Assignment Agreement Number: 006/C3/DT.05.00/PL/2025. Additionally, the authors express their sincere appreciation to Thabed Tholib Baladraf for his valuable assistance in reviewing and improving the writing of this manuscript.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Chauhan, P., Singh, M., Sharma, A., Singh, M., Chadha, P., Kaur, A. (2024). Halotolerant and plant growth-promoting endophytic fungus *Aspergillus terreus* CR7 alleviates salt stress and exhibits genoprotective effect in *Vigna radiata*. *Front Microbiol.* **15(1):** 1-18. https://doi.org/10.3389/fmicb.2024.1336533.

Chen, W., Mou, X., Meng, P., Chen, J., Tang, X., Meng, G., Xin, K., Zhang, Y., Wang, C. (2023). Effects of arbuscular mycorrhizal fungus inoculation on the growth and nitrogen metabolism of *Catalpa bungei* C.A.Mey. under different nitrogen levels. *Front. Plant Sci.* 14(1): 1-18. https://doi.org/10.3389/fpls.2023.1138184.

Das, P., Manna, I., Sil, P., Bandyopadhyay, M., Biswas, A.K. (2019). Exogenous silicon alters organic acid production and enzymatic activity of TCA cycle in two NaCl stressed indica rice cultivars. *J. Plant Physiol.* **136(1):** 76-91. http://doi.org/10.1016/j.plaphy.2018.12.026.

Farooq, M., Gogoi, N., Hussain, M., Barthakur, S., Paul, S., Bharadwaj, N., Migdadi H.M., Alghamdi S.S., Siddique, K.H. (2017). Effects, tolerance mechanisms and management of salt stress in grain legumes. *J. Plant Physiol.* **118(1):** 199-217. https://doi.org/10.1016/j.plaphy.2017.06.020.

Feng, B.H., Li, G.Y., Islam, M., Fu, W.M., Zhou, Y.Q., Chen, T.T., Tao, L.X., Fu, G.F. (2020). Strengthened antioxidant capacity improves photosynthesis by regulating stomatal aperture and ribulose-1, 5-bisphosphate carboxylase/oxygenase activity. *J. Plant Sci.* **290(1):** 110245. http://doi.org/10.1016/j.plantsci.2019.110245.

Fernandez, George C.J. (1992). Effective Selection Criteria for Assessing Stress Tolerance. Shanhua, Taiwan: Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress. Asian vegetable Research and Development Center. pp 257-270. https://doi.org/10.22001/wvc.72511.

Fuentes, L., Pérez, Y., Alemán, S., Sosa, D., Sosa, M., González, S., Mesa, A.R. (2010). Physiological and biochemical response of *Stylosanthes guianensis* cv. CIAT-184 and Centrosema molle to sodium chloride stress. *Pastos y Forrajes*. **33(2):** 173-186.

Ghosh, S., Batra, D., Kumar, Y., Mata, N.K. (2025). Optimizing protein fractions in chickpeas: A salinity alleviation approach with calcium and potassium supplementation. *Agricultural Science Digest*. **45(1):** 153-159. https://doi.org/10.18805/ag.D-5911.

Hossain, M.A., Khatun, M.S., Hosen, M., Sayed, Z.I., Islam, M.R., Chowdhury, M.K., Iqbal, M.A., Al-Ashkar, I., Erden, Z., Toprak, C.C., Sabagh, A.E., Islam, M.S. (2024). Citric acid alleviated salt stress by modulating photosynthetic pigments, plant water status, yield and nutritional quality of black gram [*Vigna mungo* (L.) Hepper]. *Legume Research*. **47(12):** 2068-2076. doi: 10.18805/LRF-820.

Latief, M.F., Nohong, B., Nompo, S. (2020). The Effect of Phosphorus Fertilizer on Growth Characteristics and Dry Matter Production of *Stylosanthes guianensis*. IOP Conf. Ser.: Earth Environ. Sci. **492(1):** 1-5. https://doi.org/10.1088/1755-1315/492/1/012012.

Liu, Y., Feng, Y., Ding, X., Chen, Z., Huang, C., Dong, R. (2017). Synthetical evaluation of salinity tolerance of 67 *Stylosanthes Sw*. materials. *Chinese J. of Trop Crops*. **38(1):** 1-7. doi: 10.3969/j.issn.1000-2561.2017.11.008.

Liu, Y., Kong, D., Yang, H., Douxchamps, S., Atieno, M., Xu, B., Wang, W., Liu, G. (2022). A transcriptomic analysis of Stylo [*Stylosanthes guianensis (Aubl.) Sw.*] provides novel insights into the basis of salinity tolerance. *Front. Sustain Food Syst.* **6(1):** 1-18. https://doi.org/10.3389/fsufs.2022.725656.

Moussa, S.M., Khaled, A.M.A., Esh, A.M., Amer, E.A., Salama, E.A.A., El-wakil, H.E. (2022). Gamma radiation as a mutagenic agent for salinity tolerance improvement in some sweet sorghum, (*Sorghum bicolor* L.) genotypes. *Egypt. Acad. J. Biol. Sci.* **13(2):** 91-102. https://doi.org/10.21608/EAJBSH.2022.260524.

Palsaniya, D.R., Kumar, S., Shivkumar, B.G., Ram, S.N., Ghosh, P.K. (2017). Stylosanthes production, processing and utilization technologies for animal production-A review. *Anim. Nutr. Feed Technol.* **17(3):** 501-514. https://doi.org/10.5958/0974-181X.2017.00048.8.

Prihantoro, I., Karti, P.D., Aditia, E.L., Nisabillah, S. (2023). Quality of arbuscular mycorrhizal fungi (FMA) produced by different fortification and fertigation techniques on the growth of *Indigofera zollingeriana*. *J. Agric. Sci.* **28(3):** 377-385. https://doi.org/10.18343/jipi.28.3.377.

Putra, B., Warly, L., Evitayani, Utama, B.P. (2021). Fitoremediasi Lahan Tambang Emas Berbasis Fungi Mikoriza Arbuskula untuk Pengembangan Hijauan. Yogyakarta (ID): Deepublish.

Ramadhani, P., Ulpah, S. (2022). The effectiveness of vegetable herbicides from ketapang leaf extract (*Terminalia catappa* L.) against weed *Asystasia gangetica* L. *Dinamika Pertanian.* **38(2):** 155-162. https://doi.org/10.25299/dp.

Rizki, A., Karti, P.D.M.H., Prihantoro, I. (2022). The effectiveness of various arbuscular mycorrhizal fungi products in increasing productivity of *Stylosanthes guianensis* on acid soil. *J. Nutr. Feed Technol.* **20(3):** 89-94. https://doi.org/10.29244/jintp.20.3.89-94.

Santos-Garcia, M.O., Karia, C.T., Resende, R.M.S., Chiari, L., Vieira, M.L.C., Zucchi, M.I., Souza, A.P. (2012). Identification of *Stylosanthes guianensis* varieties using molecular genetic analysis. *AoB Plants*. **2012(1):** 1-14. https://doi.org/10.1093/aobpla/pls001.

Schultze-kraft, R., Wang, L., Peters, M., Miao, Y., Linjie. (2023). Development of transgenic composite stylosanthes plants to study root growth regulated by a β-expansin gene, SgEXPB1, under phosphorus deficiency. *Plant Cell Rep*. **42(3):** 575-585. https://doi.org/10.1007/s00299-023-02978-y.

Steel, R.G.D. and Torrie, J.H. (1995). Principles and Procedures of Statistics. McGraw-Hill Book Co.Inc. New York.

The Agriculture Research and Development Agency. (2020). Stylo (*Stylosanthes guianensis*) as Feed Ruminansia. Agroinovasi Edisi 22-28. Jakarta (ID): Balitbang Pertanian.

Tolib, R., Kusmiyati, F., Lukiwati, D.R. (2017). Effect of cropping system and organic fertilizer on agronomic characteristis of *Sebania grandiflora* and *Panicum maximum* at saline soil. *J. Agro Complex*. **1(2):** 57-64. https://doi.org/10/14710/joac.1.2.57-64.

Tome, A.H., Rahim, E.I., Arief, S.A., Pautina, A. (2024). Marine pollutions in Indonesia: Contradiction between regulations and settlement conditions. E3S Web Conf. 506(5004): 1-11. https://doi.org/10.1051/e3sconf/202450605004.

Veraplakorn, V., Nakorn, M.N., Kaveeta, L., Suwanwong, S., Bennett, I.J. (2012). Variation and long term regenerative capacity of two important tropical forage legumes: Cavalcade (*Centrosema pascuorum* cv. Cavalcade) and Stylo 184 (*Stylosanthes guianensis* CIAT184) *in vitro*. Afr. J. Biotechnol. 11(92):15843-15851. https://doi.org/10.5897/AJB11.2412.

Veraplakorn, V., Nakorn, M.N., Kaveeta, L., Suwanwong, S., Bennett, I.J. (2013). Variation in ion accumulation as a measure of salt tolerance in seedling and callus of *Stylosanthes guianensis*. *Theor. Expe. Plant Physiol.* **25(2):** 106-115. https://doi.org/10.1590/S2197-00252013000200003.

Wang, W., Zhang, P., Zhang, H., Grossiord, C., Pennington, S.C., Norwood, M.J., Li, W., Pivovaroff, A.L., Fernández-de-Uña, L., Leff, R. (2022). Severe declines in hydraulic capacity and associated carbon starvation drive mortality in seawater exposed Sitka-spruce (*Picea sitchensis*) trees. *Environmental Research Communications*. **4(3):** 1-13. https://doi.org/10.1088/2515-7620/ac5f7d.

Yunus, A.T.M., Chiu, B.S., Ghazali, A.H. (2024). *Vigna marina* as a potential leguminous cover crop for high salinity soils. *J. Trop. Agric. Sci.* **47(2):** 481-494. https://doi.org/10.47836/pjtas.47.2.10.

Zhao, B., Liu, Q., Wang, B., Yuan, F. (2021). Roles of phytohormones and their signaling pathways in leaf development and stress responses. *J. Agric. Food. Chem.* **69(12):** 3566-3584. https://doi.org/10.1021/acs.jafc.0c07908.