

## *Vigna marina* as a Potential Leguminous Cover Crop for High Salinity Soils

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### ABSTRACT

The beach bean (*Vigna marina*) exhibits robust growth in habitats characterised by sandy substrates, limited nutrient availability, and elevated saline levels. The utilisation of *V. marina*, a potentially beneficial leguminous cover crop, allows for its cultivation in regions characterised by soil salinity, hence facilitating the alleviation of environmental stress and the promotion of nitrogen fixation within the soil. A study assessed the feasibility of *V. marina* as a leguminous cover crop, in which this legume was cultivated in both coastal and inland soils. *Pueraria javanica* and *Mucuna bracteata*, widely recognised as established leguminous cover crops, were used as the control in this experiment. The observations involved were total plant biomass, nitrogenase activity, and leaf chlorophyll content of the host plants. The experiment consisted of five replicates arranged in a randomised complete block design, respectively. The effects of commercialised rhizobial compost on the development of the leguminous plants planted in both plots were also investigated. The results indicated that *V. marina* flourished in coastal and inland soils with the highest leaf chlorophyll concentration throughout the eight weeks of growth. It showed that *V. marina* has the potential to outperform the other two established leguminous cover crops when planted in highly salinised soils. The results also showed evidence that *V. marina* was an

excellent potential leguminous cover crop, especially for any agricultural plots of high salinity soils, compared to the other two well-established leguminous cover crops, *P. javanica* and *M. bracteata*.

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## INTRODUCTION

Due to high salt concentrations in the soil, most cereal plants are subjected to salinity stress conditions, and the area of land affected by the increase in salinity is expanding daily. Accordingly, soil salinity is one of the most detrimental environmental factors affecting agricultural plant productivity and food security (Sahbeni et al., 2023; Shrivastava & Kumar, 2015). Consequently, it may be the most influential ecological factor in worldwide agricultural productivity and food security (Sahbeni et al., 2023; Schwabe et al., 2006). Additionally, salinity is a significant hindrance to the growth of crop plants (Apse & Blumwald, 2002; Flowers, 2004; Munns & Tester, 2008; Witcombe et al., 2008). It also negatively impacts soil structure, nutrient availability, and plant growth, resulting in decreased cereal yields and, in extreme cases, desertification (Sahbeni et al., 2023; Shahid et al., 2018). Approximately one-third of all agricultural fields are becoming increasingly salinised, representing over a hundred countries with a wide range of climates (Khasanov et al., 2023). Estimates indicate that nearly one billion hectares of land are salinised globally, with 77 million hectares attributable to human activity (Khasanov et al., 2023; Squires & Glenn, 2011). Each year, up to two million hectares of land become salinised, substantially reducing agricultural productivity (Abbas et al., 2013; Khasanov et al., 2023). Due to its diverse climatic zones and physical terrain, Asia experiences many climate change effects, including sea level rise, saline water

intrusion and soil salinisation (Hasnat et al., 2018). In low-rainfall regions of Malaysia, saline soils are prevalent (Paramanathan, 2013). Approximately 50% of Peninsular Malaysia's land is deemed unsuitable for agriculture (Abubakar et al., 2023), referred to as problematic soils, of which five per cent are affected by salinity (Aminuddin et al., 2005; Nordin et al., 2015).

Coastal areas account for around 13% of Malaysia's overall landmass (total area of 4.43 million ha), whilst 57% of this land is employed for agricultural purposes (Shultana et al., 2021). Nevertheless, the primary limitation in coastal regions is salinity caused by seawater infiltration (Herman et al., 2015; Shultana et al., 2021). As such, extensive efforts are being actively implemented to utilise or remediate the high-salinity soils for agricultural activities. Among the measures is introducing plant varieties that can tolerate and establish under high salinity stress conditions. In addition, the application of microorganisms that are tolerant to increased soil salinity and subsequently involved in soil reclamation, improving the soil conditions, and enhancing plant growth was also involved. In addition, the cultivation of leguminous cover crops that are tolerant to high salinity conditions also has great potential for implementation in the plantation sector. The legumes may protect the soil from erosion and, at the same time, improve the soil conditions that are suitable for agricultural activity. Potential leguminous plants suited for this purpose are known as *V. marina*. This lesser-known legume grew on coastal soils

(voucher specimens [USM Herbarium 11782]) and was deposited in the Universiti Sains Malaysia (USM) herbarium. This leguminous plant is referred to as nanea, dune bean, notched cowpea, and beach cowpea (Singh et al., 2019). Moreover, according to Singh et al. (2019), *V. marina* is widely distributed in tropical regions, exhibiting a floral structure resembling the mung bean and urd bean. Singh et al. (2019) investigated its ability to withstand salinity stress and possess salt-tolerant genes. It is also recommended for beachfront properties as a ground cover to prevent coastal erosion (Singh et al., 2019). It may benefit the agricultural activity of economic crops planted in high-salinity soil conditions. Therefore, an experiment was conducted to observe and evaluate the growth performance of *V. marina* under salinity stress conditions compared with established leguminous cover crops, *P. javanica* and *M. bracteata*, under two different soil classifications, coastal and inland, respectively.

## MATERIALS AND METHODS

The seeds of *V. marina*, *P. javanica*, and *M. bracteata* provided by Agricultural Crop Trust (ACT) were germinated in bed using river sand as a medium under plant house conditions. Simultaneously, the seeds (according to respective treatments) were treated with 25 ml Commercial Rhizobial Compost (CRC) that contained rhizobial inoculum, provided by HUMIBOX (M) Sdn. Bhd. (Malaysia). After seven days of germination, the seeds were transferred to

new pot trays under plant house conditions for the seedling establishment of each legume for 30 days of growth. The seeds were treated accordingly with 25 ml of CRC on day 7. After 30 days of growth, the established seedlings were transferred to respective experimental plots: (1) coastal plot (5°25'33.4"N 100°19'37.4" E) (at beach site of Tanjung Bungah, Penang, Malaysia) with soil conductivity at 138.7 mS/cm, and (2) inland plot (5°21'29.4"N 100°17'38.7" E) (at experimental plots of USM, Minden, Penang Malaysia) with soil conductivity at 59.6 mS/cm. All plants of both plots were observed (growth and development) for 60 days of growth (D<sub>60</sub>). The seedlings were treated accordingly with 50 ml of CRC at day 37. Both experimental plots were supplied with rock phosphate (30% ± 1.0 P<sub>2</sub>O<sub>5</sub> [PK Fertilizers Sdn. Bhd., Malaysia]) at a rate of 20 g/plant at day planting (day 0) and day 49 (100 g/plant of rock phosphate). Additionally, at day 0, all seedlings were supplied with 10 g of black organic fertiliser (ANITA, Malaysia) with an NPK ratio of 8:8:8.

The experiment consisted of two treatments with ten replications for each tested leguminous plant. The treatments were as follows: (1) + CRC and (2) – CRC, and were arranged in a randomised complete block design (RCBD) for D<sub>60</sub>. Before the detrimental harvest at D<sub>60</sub> commenced, each seedling was examined to assess the chlorophyll content in their leaves. This assessment involved the utilisation of a soil plant analysis development (SPAD) meter known as the MINOLTA™ SPAD-502

(Japan), which quantifies leaf greenness (Neufeld et al., 2006). Determining leaf chlorophyll content was conducted by utilising a standard curve of chlorophyll meter values and the corresponding leaf chlorophyll content (Amir et al., 2001). A sample of leaves with different greenness (yellow, light green, and dark green) was selected for chlorophyll analysis (MINOLTA™ SPAD-502) (Japan) and total leaf chlorophyll content analyses (Amir et al., 2001; Coombs et al., 1985). As for the nitrogenase enzyme activity, the nodules of each tested legume and plots were sampled and incubated in an airtight vessel (7 ml) for acetylene reduction assay (ARA). Subsequently, a substitution of acetylene gas was performed, wherein 0.7 ml (equivalent to 10%) of the air present in the vessel was replaced (Reis et al., 2015). The quantification of ethylene was conducted after a 24-hr incubation period, during which 1 ml of gas was taken in triplicate for each sample. All samples were incubated at room temperature within a light-restricted environment. The assay was performed utilising the Shimadzu Gas Chromatography (GC-2014, Japan) instrument, which was outfitted with a Supelco Carboxen 1004 stainless steel micro packed column measuring 2 m in length and 0.76 mm in internal diameter, equipped with a flame ionisation detector (FID) in the setup. The flow rate of nitrogen, which is employed as a carrier gas, was 30 ml/min. In the experiment, the temperature of the column, injection, and FID were consistently held at 80°C (column

temperature) and 180°C (injection and FID temperature), respectively. The actual ethylene concentration was calculated by utilising the established standard curve of ethylene gas (C<sub>2</sub>H<sub>4</sub>, Supelco®, German) and analysing the corresponding peak region. The nitrogenase enzyme activities (µmol C<sub>2</sub>H<sub>4</sub>/g) were defined based on ethylene concentration (µmol C<sub>2</sub>H<sub>4</sub>) and the weight (g) of the tested nodules.

All treated seedlings were observed for plant biomass (fresh and dry weight) of leaf, above-ground, and root fresh and dry weight. The samples were oven-dried at 70°C to determine the dry weight (Ojo, 2001). Data collected were statistically analysed using IBM SPSS (version 26.0). Analysis of variance (ANOVA) was used to compute the *p*-value. At a significance level of *p* < 0.05, the Tukey honest significant difference (Tukey's HSD) test was utilised to determine the statistically significant difference between treatments. One-way ANOVA was performed to make comparisons between the means of the data.

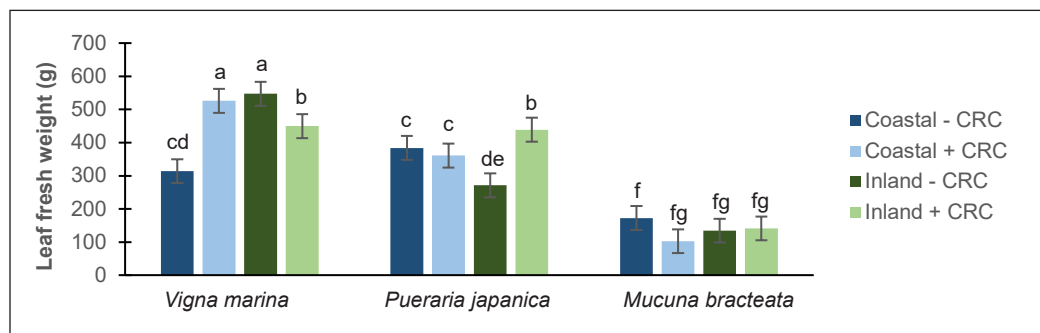
## RESULTS AND DISCUSSION

### Plant Growth Observations

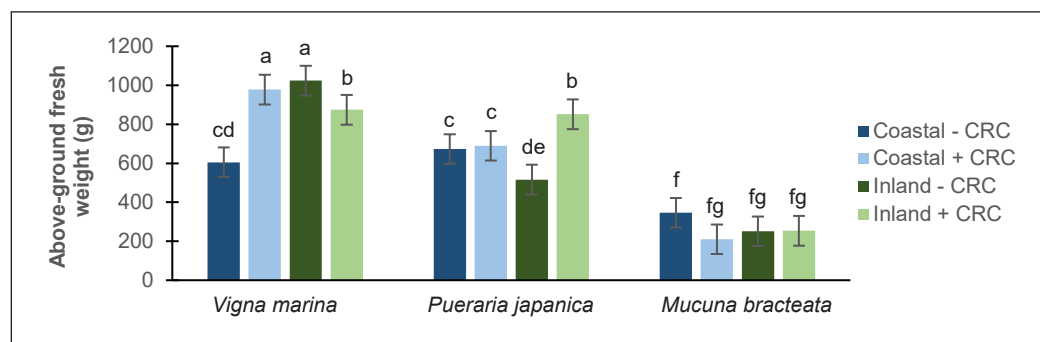
Treated leguminous cover crops were collected after D<sub>60</sub> for coastal and inland soil plots. The plant samples were observed for leaves, roots, and above-ground plant biomass. Treated *V. marina* seedlings had the highest leaf fresh weight among the other plants tested (526.1–547.2 g), whereas *M. bracteata* had the lowest leaf fresh weight (102.73–134.49 g) (Figure 1a). On the other hand, the results differed

for root fresh weight, where *P. javanica* with CRC in coastal soil had the highest root fresh weight among the plants, which was 99.3 g compared to both *V. marina* and *M. bracteata* (Figure 1b). Meanwhile, the highest above-ground fresh weight among

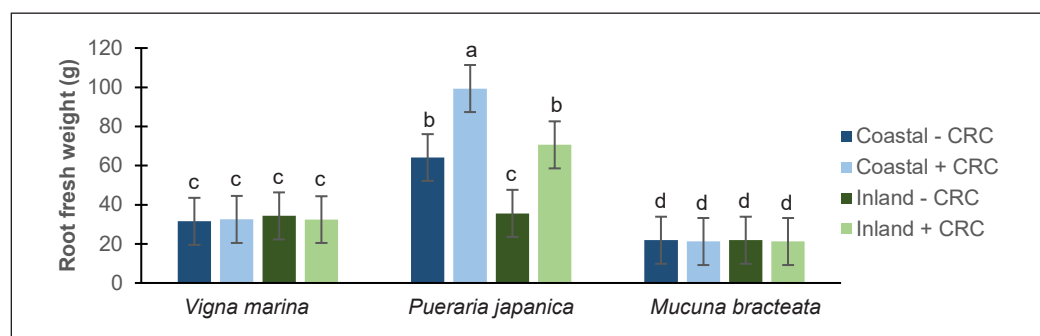
the plants tested was observed in *V. marina*, either treated with or without CRC for both inland and coastal soils (1,023.5 and 977.63 g, respectively). *M. bracteata* (with and without CRC in coastal and inland soils) had the lowest weight (Figure 1c).



(a)



(b)



(c)

Figure 1. Means ( $\pm$  SE) fresh weight of leaf (a), above-ground (b), and root (c) of three leguminous cover crops (*Vigna marina*, *Pueraria javanica*, and *Mucuna bracteata*) with and without commercialised rhizobial compost (CRC) grown in two different soils, coastal and inland for 60 days

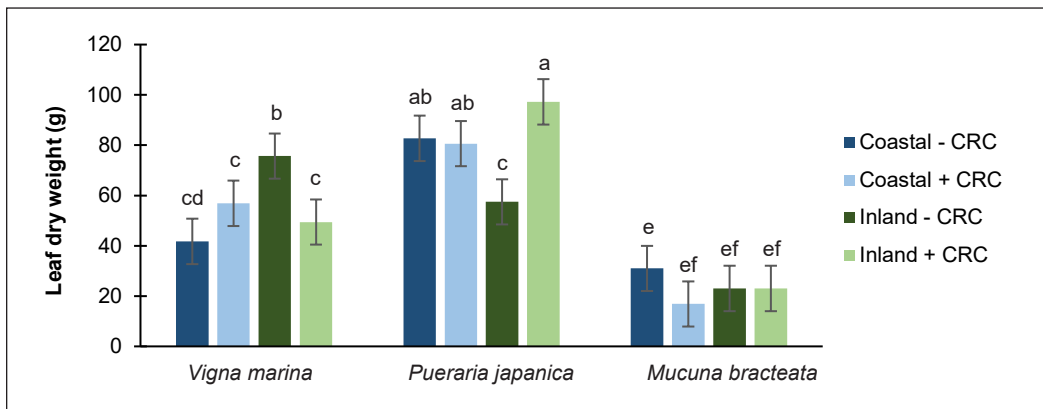
Note. Means with different letters are significantly different ( $p < 0.05$ ) based on Tukey's honest significant difference

The results showed that the leaf dry weight of *P. javanica* (+ CRC) on inland soils was the highest, followed by treated *V. marina*. The leaf biomass was recorded in a range of 40.1 to 97.25 g. The lowest leaf dry weight was recorded for *M. bracteata* in a range of 16.9–23.07 g (Figure 2a). Other than that, the root dry weight of *P. javanica* with CRC in coastal soil still had the highest weight among the plants, which was 52.65 g, while the least were both *M. bracteata* with and without CRC in inland soil, which was 4.12 and 3.44 g, respectively (Figure 2b). Subsequently, the highest above-ground dry weight was recorded in *P. javanica* treated with CRC, followed by *V. marina* for coastal and inland soils tested (174.24–138 g) (Figure 2c).

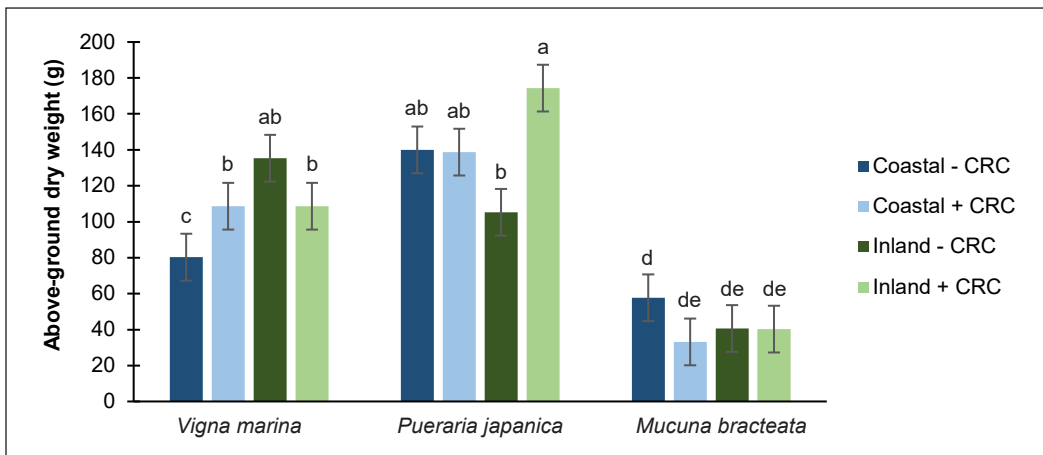
The findings demonstrate that abiotic stress can inhibit plant growth and development, reduce yield, and, in extreme cases, cause plant death (Gechev & Petrov, 2020; Krasensky & Jonak, 2012). The global climate's continual deterioration and the rise in agrochemical utilisation and industrialisation contribute to the escalating severity of abiotic stressors on a global scale (Naing & Kim, 2021). Salinity is one of the most significant abiotic stresses that limits crop growth and productivity, as well as one of the world's earliest and most widespread environmental problems (Safdar et al., 2019). Due to high osmotic potential and specific ion toxicity, soil salinity can substantially inhibit seed germination and seedling growth (Rajabi et al., 2020). The presence of salinity has several effects on plants. One notable

impact is reducing water availability to the roots due to the salt content in the soil solution (Safdar et al., 2019). Consequently, this hinders the plants' capacity to uptake water and essential minerals like potassium ( $K^+$ ) and calcium ( $Ca^{2+}$ ) (Yadav et al., 2020).

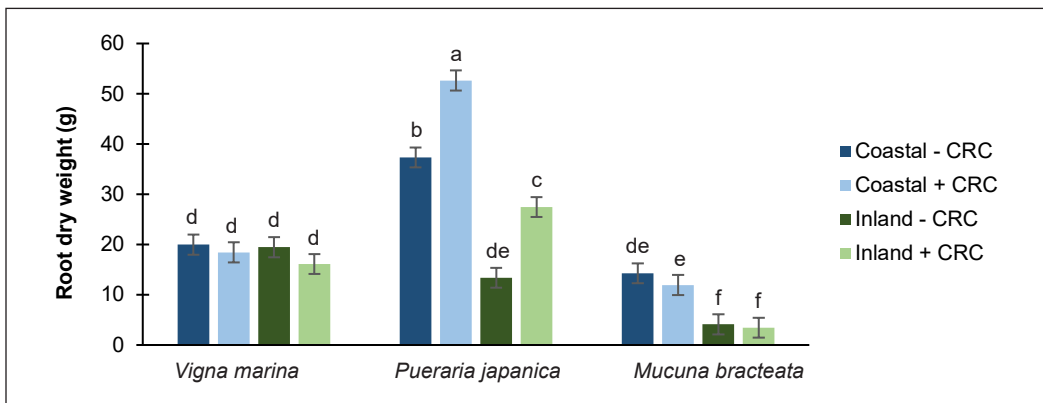
Additionally, according to Safdar et al. (2019), salt accumulation in different plant tissues can reach dangerous levels, hindering plant functionality and metabolic processes. The infiltration of sodium ( $Na^+$ ) and chloride ( $Cl^-$ ) ions into cells has been observed to have adverse effects on the integrity of the cell membrane and disrupt metabolic activities occurring within the cytosol (Yadav et al., 2020). Salinity inhibits seedling establishment by decreasing endogenous phytohormone levels (Egamberdieva & Kucharova, 2009; Ullah et al., 2021). Subsequent stages of shoot growth will be affected if seeded during the initial phase of salinity (El Sayed, 2011; Ullah et al., 2021; Wakeel et al., 2011). Although the seedlings are vigorous and generally grow during the second phase of salinity, the final yield will be affected (Ullah et al., 2021). Due to decreased stomatal conductivity, the plant's carbon fixation capacity is reduced, interfering with the catalytic activities of enzymes that fix carbon and destroy photosynthetic pigments (Omoto et al., 2012; Ullah et al., 2021). As a result, the shoot and root biomass of plants established in saline soil decreased significantly (Genc et al., 2019; Kalhor et al., 2016; Ullah et al., 2021).



(a)



(b)



(c)

Figure 2. Means ( $\pm$  SE) dry weight of leaf (a), above-ground (b), and root (c) of three leguminous cover crops (*Vigna marina*, *Pueraria japonica*, and *Mucuna bracteata*) with and without commercialised rhizobial compost (CRC) grown in two different soils, coastal and inland for 60 days

Note. Means with different letters are significantly different ( $p < 0.05$ ) based on Tukey's honest significant difference

### Nitrogenase Activity

The results showed that treated *P. javanica* with CRC in inland soil had the highest nitrogenase enzyme activity among tested seedlings, which was 110.26  $\mu\text{mol C}_2\text{H}_4/\text{g}\cdot\text{h}$  compared to seedlings planted on coastal soil. In comparison, *V. marina* and *M. bracteata* recorded the lowest nitrogenase enzyme activity for both inland and coastal soils (Figure 3).

At the same time, similar findings were observed earlier by Abd-Alla et al. (2019), who stated that soil salinity negatively affects root colonisation, root filament infection by rhizobia, and the growth and viability of these bacteria. These abiotic elements have the potential to exert a detrimental impact on the advantageous characteristics and effectiveness of the inserted plant growth-promoting rhizobacteria (PGPR) inoculants (Egamberdieva et al., 2017). Eventually, this will hinder the ability of legumes to form

and maintain nitrogen-fixing nodules in salt-affected soils as it inhibits the formation or development of nodules, thus reducing the performance of wholly developed nodules, resulting in a severe reduction in legume production (Rao et al., 2002; Wan et al., 2023). In addition, it has been observed that salt stress has a detrimental impact on the nutrient adsorption capacity of plants, specifically affecting the ability to adsorb nitrogen, leading to a decrease in plant development (Wan et al., 2023). Nitrogen may improve the plants' water utilisation and stem structure (Agami et al., 2018). The legumes relied on effective nodules where atmospheric nitrogen is converted to nitrate ion ( $\text{NO}_3^-$ ) and ammonium ion ( $\text{NH}_4^+$ ) and absorbed by plants (López et al., 2018).

### Leaf Chlorophyll Content

Treated plants had improved the leaf chlorophyll content over eight weeks of

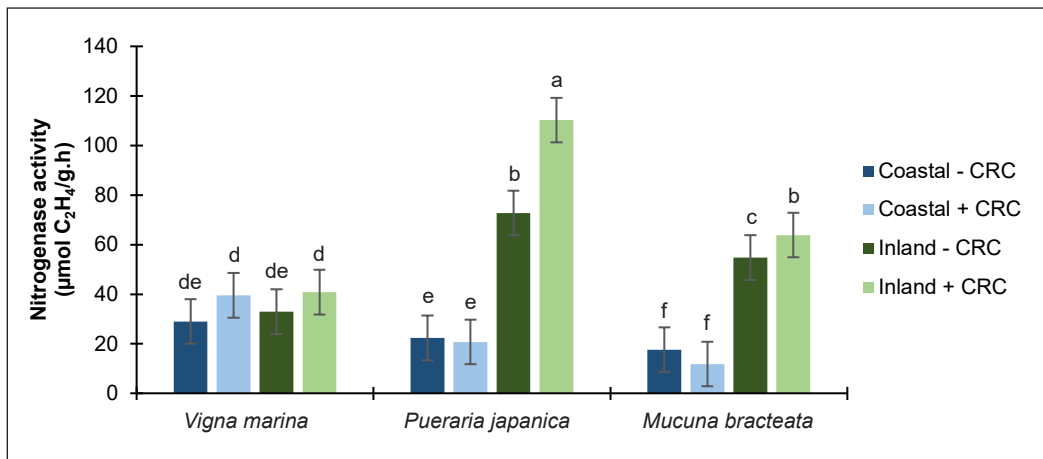


Figure 3. Means ( $\pm$  SE) nitrogenase activity of three leguminous cover crops (*Vigna marina*, *Pueraria javanica*, and *Mucuna bracteata*) with and without commercialised rhizobial compost (CRC) grown in two different soils, coastal and inland for 60 days

Note. Means with different letters are significantly different ( $p < 0.05$ ) based on Tukey's honest significant difference



observation. The highest leaf chlorophyll content was observed on *V. marina* (-CRC) in coastal soil (0.95 mg chlorophyll/mg leaf). In contrast, the lowest leaf chlorophyll content was shown on *M. bracteata* with CRC at 0.65 mg chlorophyll/mg leaf (Figure 4). Similar observations were recorded for the seedlings grown on inland soils. The highest leaf chlorophyll content was observed on *V. marina* (+ and -CRC) in coastal soil (0.85 and 0.89 mg chlorophyll/mg leaf). At the same time, the lowest leaf chlorophyll content was depicted on *M. bracteata* at 0.65 mg chlorophyll/mg leaf (Figure 4).

The results have clearly shown that *V. marina* may overcome the high salinity conditions of the soil and maintain higher leaf chlorophyll content. The results also showed that the leaf colour of *M. bracteata* was considerably less vibrant than that of both *V. marina* and *P. javanica*. Increasing nitrogen accelerated the rate of photosynthesis, resulting in an increase in chlorophyll and a verdant leaf (Li et al., 2020; Mooney et al., 1995; Piccoli & Bottini, 2013; Wang et al., 2021). Since *M. bracteata* was less verdant than both *V. marina* and *P. javanica*, this explains why *M. bracteata* had the lowest leaf

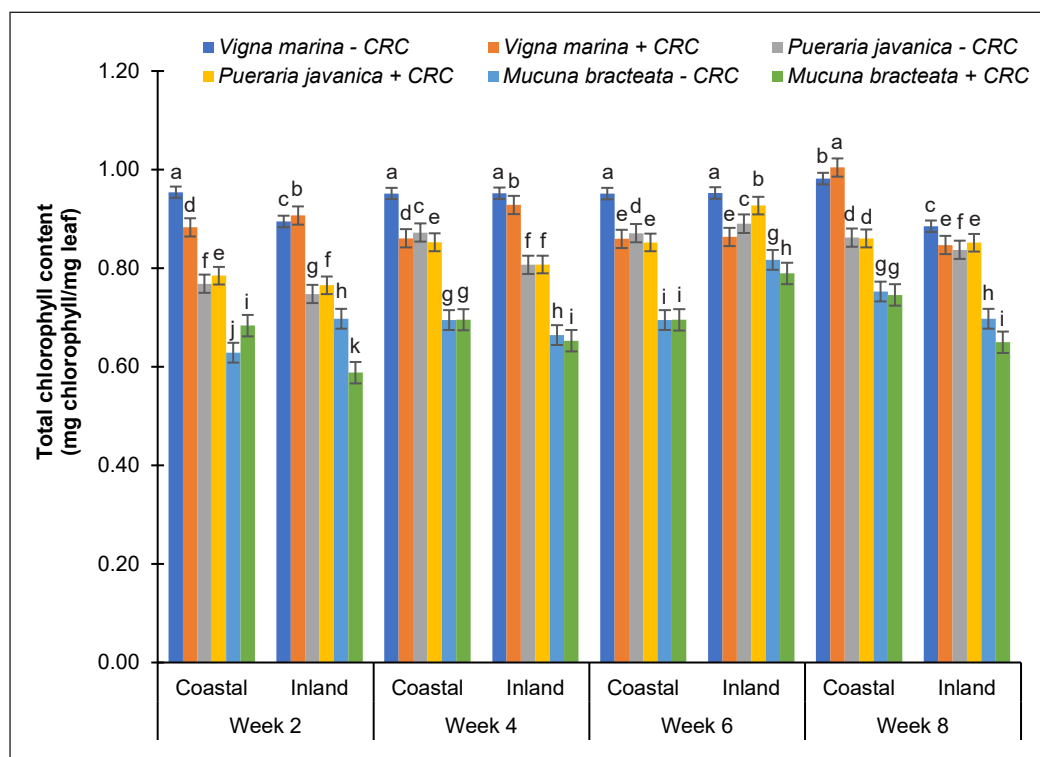


Figure 4. Means ( $\pm$  SE) leaf chlorophyll content of three leguminous cover crops (*Vigna marina*, *Pueraria javanica*, and *Mucuna bracteata*) with and without commercialised rhizobial compost (CRC) grown in two different soils, coastal (a) and inland (b) for 60 days

Note. Means with different letters are significantly different ( $p < 0.05$ ) based on Tukey's honest significant difference

chlorophyll content. Earlier findings by Ali et al. (2004) and Muhamad Hassan et al. (2022) indicated that salinity decreased leaf chlorophyll content. Loss of chlorophyll due to salt stress may be associated with photoinhibition or reactive oxygen species (ROS) formation (Heidari, 2012; Kato & Shimizu, 1985; Kesawat et al., 2023).

Nounjan et al. (2020) observed that the impact of salt stress on thylakoid stacking results in a decrease in the chlorophyll *a:b* ratio. The loss of chlorophyll levels in response to saline stress is a widely seen occurrence attributable to multiple mechanisms. These factors include the inhibition of chlorophyll biosynthesis, produced by the activation of the chlorophyllase enzyme, as well as the destruction of cellular membranes due to chlorophyll breakdown mediated by salinity (Muhamad Hassan et al., 2022). The adverse effects of salt stress on plants are evident in their morphology, physiology, and biochemical properties: morphologically, plants experience stunted growth, chlorosis, and impaired seed germination; physiologically, salt stress inhibits photosynthesis and disrupts nutrient balance; biochemically, plants undergo oxidative stress, electrolyte leakage, and membrane disorganisation (Balasubramaniam et al., 2023). The decrease in photosynthesis under saline soils caused a reduction in chlorophyll content, as the chlorophyll index was shown to be reduced in sensitive genotypes under salt stress conditions, but it was observed to be raised in tolerant genotypes (Heidari, 2012; Masarmi et al., 2023).

## CONCLUSION

The growth efficacy of *V. marina* was compared to that of two other well-established leguminous cover crops, *P. javanica* and *M. bracteata*. Although planted on coastal soils, *V. marina* performed exceptionally well as a potential leguminous cover crop with the highest leaf chlorophyll content and biomass compared to conventional leguminous cover crops, *P. javanica* and *M. bracteata*. Additionally, *V. marina* was also able to flourish in both coastal and inland soils. With the growing problem of saline soil in agricultural fields such as oil palm and rubber plantations, *V. marina* showed promising potential as the leguminous cover crop for selected agricultural fields and warranting further investigation.

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