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Potential uses of Marine Bean (*Vigna marina* Burm.) as salt tolerant Legume in coastal salty land, Southeast Sulawesi, Indonesia

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Abstract. *Vigna marina* has potential to use as productive legume in salty areas of southeast Sulawesi. The study aimed to know the adaptation of *V. marina* grown under higher salinity condition, and elucidate their potential uses as salt tolerant legume in salty land. The germinated seeds were placed in polybags. Five polybags were taken as control, while five polybags watered by NaCl 200mM, and five polybags watered by NaCl 400mM for every day during 2 months. The dry biomass, chlorophyll and antioxidant (Vitamin C, anthocyanin and alkaloid) contents were determined. The results showed that dry biomass of marine bean was not significantly different among treatment indicating similar growth ability of marine bean at different treatment. On the other hand, the contents of chlorophyll *a* and chlorophyll *b*, as well as antioxidant were significantly higher at salinity concentration of NaCl 400mM, indicating higher salt concentration induced the marine bean to produce higher chlorophyll and antioxidant contents as mechanisms to grow and withstand in the salty land. Therefore, the results of this study indicated that this legume is salt tolerant and is potential to use as productive legume at coastal salty land.

1. Introduction

Increased salinity of cultivated and irrigated lands is becoming the most serious problem in agriculture sector of the world and is happen rapidly today [1, 2, 3]. Soil salt condition is one of among the stresses of environmental factors that reduced the land quality and influence the crop plants growth and productivity [4] because salt affects many physiological and reproductive processes in plants. As an increasing of global world population, the food demand will continue to increase along with population growth and increasing human needs, while most of crop plants are salt sensitive. Therefore, looking for salt tolerance crops is very important to ensure food sustainability and fulfilment of human life needs.

Many legume plants, such as soy bean, mung bean, common bean, faba bean, chick pea, cowpea etc. are known to play a very important role in the world because of their significance in human nutrition. Moreover, most of legumes are salt sensitive plants, and therefore, we need to find out the more salt tolerant of legumes. The marine bean (*Vigna marina*), a tropical legume that grow in the coastal sandy land with salt conditions and nutrient-poor and is salt tolerant legume [5]. This legume is one of most potential marine plants that found in coastal area of Southeast Sulawesi. This marine bean grows in



sandy beach, and produce very abundant seeds, and therefore, it has potential as a source of renewable food source.

Southeast Sulawesi coastal land is potential area for cultivation of bean salt tolerant, because consists of wide area of sandy beach, and far away from human activity. The present of marine bean in this region is less attention by people, while their potential to use as a productive legume at salty land has not been examined till now. Therefore, the objectives of this study were (1) to know the adaptation strategy of marine bean (*V. marina*) grown under higher salinity condition, and (2) to elucidate the potential uses of marine bean as salt tolerance legume that could be used by local communities as productive plant in coastal salty land, Southeast Sulawesi, Indonesia.

2. Materials and Methods

2.1. Study site and seeds collection

The seeds of marine bean *Vigna marinawere* collected from Toronipa beach, which is located at the northern part of Kendari city, Southeast Sulawesi, Indonesia (Figure 1). The *Vigna marina* grow along the beach with sandy and far away from tourism activity. This plant covered about 1000 m² and show nice panorama in the beach. This plant produce abundant leaves and fruits at rainy season, while they have few leaves only at dry season.

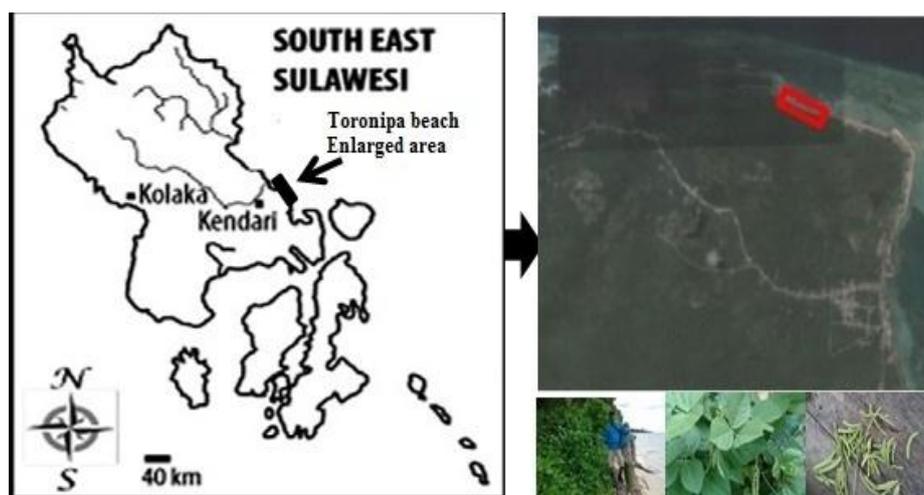


Figure 1. Study site of Toronipa beach, Southeast Sulawesi (left), and sampling sites of marine bean plant with leaves and fruits (right).

2.2. Experimental setting

The high-quality seeds were selected and then stored in seeds desiccators. Seeds of almost uniform size were germinated on Petridis. The seeds were pierced with needles to facilitate germination because the shell of seeds is very hard. After the seeds were germinated for 1 week and then transferred to 25 plastic polybags that contained 250 g of soil in each polybag. Five polybags were taken as control, while five polybags were watered by NaCl 200mM, and another five polybags were watered by NaCl 400mM. Watering were done for every day with 20 ml in each polybag for 2 months.

2.3. Chlorophyll and antioxidant measurement

The oldest of uniform leaves of marine bean were picked and selected for measurement of chlorophyll and antioxidant (Vitamin C, anthocyanin and alkaloids) contents. The analysis of chlorophyll content begins with weighing a 0.2 g of leaves samples, then chopped and extracted with 20 ml of 80% acetone and finally grinded in a mortar. The extract then filtered with Whatman 41 filter paper and putted into a 25 ml measuring cup. The solution was centrifuged for 15 minutes at a speed of 2500 rpm. After that,

10 ml of filtrate was taken and observed at the absorbance of 663 and 645 nm wavelengths. In addition, the analysis of vitamin C content begins with the extraction of leaves in water solvent: citric acid (9: 1) which was previously cut into pieces. Extraction was carried out for 30 minutes, and then centrifuged for 10 minutes and finally filtered using Whatman41 paper. About 10 ml of filtrate was then taken and its absorbance was seen at a wavelength of 269 nm.

The extraction of anthocyanins begins by weighing 2 g of fresh leaves of *V. marina* and mixed with a half portion of 25 % acetic acid solution (100 mL) and then transferred to a test tube. The extraction was centrifuged for 10 minute and filtered by using Whatman 41 paper. The filtrate samples were analyzed using spectrofotometer on wave length of 510 nm. The total content of anthocyanin was calculated. Each sample was dissolved in potassium chloride-hydrochloride acid buffer solution pH 1.0 and sodium acetate trihydrate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$) buffer solution pH 4.5. The absorbance was determined at 510 nm in a UV-Visible spectrophotometer. The dilution factor for each sample was firstly determined by dissolving the sample in KCl buffer pH 1.0 until its absorbance at 510 nm obtained less than 1.2 versus the KCl buffer pH 1.0 as blank. Sample was then dissolved in KCl buffer pH 1.0 (allowed to stand for another 15 min.) and $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ buffer pH 4.5 (allowed to stand for another 5 min.) based on the dilution factor. The absorbance for each sample was then read versus the buffer solution pH 1.0 and buffer solution pH 4.5 as the blank at $\lambda = 510$ nm (for the cyanidin 3-glucoside) and $\lambda = 700$ nm (for correction factor). The final absorbance (A) was calculated by using the formula as follows:

$$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5} \quad (1)$$

Total anthocyanin concentration was calculated by using the following formula:

$$\text{TAC} = \frac{A}{\epsilon L} \times \text{MW} \times \text{DF} \times \frac{V}{\text{Wt}} \times 100\% \quad (2)$$

where TAC is total anthocyanin content (mg. 100 g^{-1} of sample); ϵ is molar absorption coefficient of cyanidin 3- glucoside ($226,900 \text{ L (mol.cm)}^{-1}$); L is width of cuvette (1 cm); MW is molecular weight of cyanidin 3-glucoside ($449.2 \text{ g. mol}^{-1}$); DF is dilution factor; V represents final volume or sample volume after dilution (L); and Wt is weight of original extract (g).

Alkaloids extraction for determination of the content of *V. marina* was done by crushing 0.5 g of and then added with 40 mL of 10 % acetic acid in ethanol and covered for 4 hours. After the solution was filtered, then the extract was concentrated on a water bath to 1/4 of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitation was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.4. Growth Analysis

Biomass of marine bean was determined by drying of *V. marina* seedlings sampled at 100°C for two days using an oven. Each plant organ (leaf, stem and root) were putted into the oven until a constant weight and then weighed using an analytical scale.

3. Results and Discussion

3.1. Total biomass and chlorophyll content

Total biomass and chlorophyll content of seedlings of marine bean grown different salinity concentration is represented by Table 1. The total biomass of marine bean seedlings was not significantly different among treatment indicating the similar growth ability of marine bean under different salt concentration. On the other hand, the contents of chlorophyll *a* and chlorophyll *b* in leaves were significantly different among treatment ($P < 0.05$). The higher chlorophylls *a* and *b* content in leaves of *V. marina* were found at salinity concentration of NaCl 400mM, while the lower was found at control

or without NaCl. It seemed that higher salt concentration induced the seedling to produce much higher chlorophyll content that higher capacity of this bean does photosynthesis.

Table 1. Mean and Standard error (SE), of biomass of dry weigh, chlorophyll *a* and chlorophyll *b* contents in leaves of marine bean (*Vigna marina* Burm.) Similar letters in the same column indicate no significantly different at 5%.

No	Treatment	Biomass (g)	Chlorophyll <i>a</i> (mg/g)	Chlorophyll <i>b</i> (mg/g)
1	Control	0.80± 0.0111a	21.801±9.84a	9.485±3.26a
2	NaCl 200 mM	0.75 ± 0.0127a	33.549±2.33b	12.842±1.57b
3	NaCl 400 mM	0.88± 0.027a	41.591±3.62b	15.608±0.40b

This study has confirmed that the dry weigh of biomass of *V. marina* seemed to increase as an increasing concentration of NaCl, indicating that this legume is able to grow and withstand in the high salinity condition. Moreover, the chlorophyll content in seedlings of *V. marina* showed increasing trend as they grown in high salinity, indicating higher photosynthesis activity. It has reported by [5] that high photosynthetic activity of *V. marina* when growth at high salinity, while *Vigna unguicularis* and *V. radiata* were unable to growth. However, [6] reported the decreasing of chlorophyll *a* content of *Vicia faba* L as an increasing NaCl concentration. Thus, the results of this study suggest that the *V. marina* have high ability to cope with saline condition. Although, several studies reported that high salinity conditions of soil affect the plant growth, osmotic stress and nutrients deficiency [7, 8, 9]. Moreover, [10] mentioned that plants develop several mechanisms to cope with high salt concentration such as, ion homeostasis mechanism, antioxidant compounds production, nitric oxide (NO) production, etc.

3.2. Antioxidant content

Antioxidant content (vitamin C, anthocyanin and alkaloids) in seedlings leaves of marine bean grown different salinity concentration is described in Table 2. The vitamin C in leaves of marine bean was significantly different among treatment ($P < 0.05$), which was significantly higher at treatment of 400mM NaCl than control as well treatment of 200 mM of NaCl ($P < 0.05$), but it was not significantly different between control and 200 mM of NaCl concentration. Moreover, alkaloids content was significantly different among treatment ($P < 0.05$). The seedlings grown without NaCl were contained much lower alkaloids, while they contained much higher alkaloids when they grow at treatment of 200mMNaCl and of 400 mM NaCl. Therefore, antioxidant content in leaves of *V. marina* seedlings seemed to increase as an increasing of NaCl concentration, indicating its protection role to salt condition. Thus, higher salt concentration induced the production of secondary metabolic compound that plays an important role for the growth and development of *V. marina* under salt stress or other environmental stressed.

Table 2. Mean and Standard error (SE), of vitamin C, anthocyanin and alkaloids contents of marine bean (*Vigna marina* Burm.). Similar letters in the same column indicate no significantly different at 5%.

No	Treatment	Vitamin C (mg/g)	Anthocyanin (%)	Alkaloids (%)
1	Control	614.14±3.56a	0.0423 ± 0.0212a	0.0217 ± 0.002a
2	NaCl 200 mM	649.69±4.45ab	0.0634 ± 0.0312ab	0.0283 ± 0.001b
3	NaCl 400 mM	691.55±39.16c	0.1221 ± 0.0343c	0.0457 ± 0.003c

The seedlings of *V. marina* might be able to growth under salt stress condition because of production antioxidant as a defense mechanism. Antioxidants play an important role in the adaptation of plants to abiotic pressure (high salinity) because of the ability of plants to overcome the high salinity associated with oxidative defense systems which include antioxidant compounds. Vitamin C is one of the antioxidants that can capture free radicals and thereby reducing oxidative. An increasing in vitamin C content in high salinity can protect oxidative changes due to free radicals. Several studies reported that

salinity induces the production of reactive oxygen species (ROS) in plant cells ([11, 12] because ROS is acting as hinting molecules that mediates important physiological processes of many salt tolerance plants to adapt growth under salty condition [10]. Nevertheless, as shown in Table 2 that anthocyanin, ascorbic acid and alkaloids contents increased as increasing salt concentration. [10] reported many salt tolerant plants produce anthocyanin and ascorbic acid to adapt growth under high salinity. The anthocyanins may act as agents to reduce peroxidase in the cytoplasm and prevent DNA damage of salt tolerant plants.

Meanwhile, according to [13] that antioxidants play an important role in the adaptation of plants to high salinity pressure. Alkaloids might play a role in detoxification of harmful compounds that make salt tolerant plants able to growth better in higher salt concentration. Alkaloids also may act as deposit compounds that provide nutrients for plants [14]. Thus, the *V. Marina* has high capability to cope with higher salt condition of land due to production of secondary metabolic compounds. Therefore, the finding of this study realized the potentiality of marine bean as source of productive legumes to salt land, and that is high prospective to use for future agriculture development of salt land in coastal area of Southeast Sulawesi.

4. Conclusion

The present results showed the potential uses of marine bean of marine bean (*V. marina*) as salt tolerance legume that high possibility to use as productive legume in the coastal salty land of Southeast Sulawesi, Indonesia. This legume is able to grow and withstand in the high salinity condition, because they are able to produce antioxidant compounds. Thus, the finding of this study mentioned the promising marine bean as salt tolerance legume, which will be useful for cultivation in the coastal salty land, and have high promising for coastal communities as alternate food source in Southeast Sulawesi, Indonesia.

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