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Research Article
Response of Shoot and Root *in vitro* Cultures of Banana Plant (*Musa acuminata* L.) cv Barangan to Salinity Stresses

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Abstract

Background and Objective: Banana plants are agricultural crops, particularly horticulture, mostly consumed by many people. Banana plants are considerably influenced by biotic and abiotic environmental factors. Stresses caused by abiotic factors, is one of which caused by salinity, will result in decreased production of bananas, because these plants are very sensitive to salinity. This study aimed to investigate the response of banana plants to salinity stresses *in vitro* culture. **Materials and Methods:** Explants of banana *Musa acuminate* L. var. Barangan and basic medium murashige and skoog, benzyl amino purine, sugar, jelly, NaOH, HCl were used. Statistic analyzed with analysis of variance and Duncan test. The experimental design used was randomize complete design with two factors, factor I: Sodium chloride (NaCl) treatments of various concentrations, i.e., 0, 50, 100, 150 and 200 mM and factor II: Time after culture, in 1, 2, 3 and 4 weeks after culture. Observations over growth parameters were performed on height, wet weight and dry weight of banana plantlets at 1, 2, 3 and 4 weeks after cultures. Statistical analysis was done by one way analysis of variance. **Results:** The statistical analysis showed no significant differences in the plant height, salinity inhibits the growth of banana plantlets in term of the parameters of height, wet weight of shoots and roots, dry weight of shoots and roots of the banana plantlets. A treatment of 200 mM NaCl indicated that plantlet growth is inhibited in term of height (2.6500 cm), wet weight of shoots (0.0917 g) and roots (0.1957 g) and dry weight of shoots (0.0096 g) and roots of banana plantlets (0.02009 g). **Conclusion:** Plant growth in saline stressed concentration of NaCl 50, 100 and 150 mM.

Key words: *In vitro* culture, *Musa acuminate* L., salinity, shoot and root, plantlet

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Banana plant is cultivated through the humid tropics and sub-tropics in Americas, Africa and Asia included Indonesia. Bananas are widely consumed in Indonesia, but despite their high economic value, they don't make Indonesia one of the world's largest banana exporters. However, the climate is very supportive for the growth of banana and its development and hence would make Indonesia one of the world's major banana exporters[1]. One attempt to increase the production of banana in Indonesia is through extensification, i.e., by expanding the planting areas. Indonesia increasing population is a constraint to the attempt for extensification, leading to decrease in the productive lands for agriculture. Marginal lands become an alternative for agricultural land expansion despite the fact that they have a very low potential for agricultural production. Such types of marginal lands are found in tidal lands where soil salinity is quite high. In other case, human induced processes that result in the accumulation of dissolved salt in the soil. High salt levels in the soil can cause plasmolysis in plants as the osmotic pressure in the soil is higher than that in the plant cells[2]. Plant growth due to high salinity also posses the following (1) A low water potential in plants, (2) Toxic effects of Na^+ and Cl^- ions and (3) Unbalanced nutrients in the plant. A possible attempt that can be done is through research using sophisticated and accurate techniques, one of which is tissue culture or *in vitro* tissue culture which has extensively contributed to the regeneration of plants[2-4].

In vitro tissue culture technique is a culture of plant parts such as seeds, embryos, plant organs, explants, tissues, cells and protoplasts in a medium under sterile conditions in a container such as a glass or bottle[5-9]. The tissue culture technique can accelerate plant growth sand these growths are multiplying genetically uniform reported[7]. The term growth means increase in size, weight and number of cells. Measurement of growth can be performed by measuring the height, wet weight and dry weight of the plants. This study was conducted to determine responses of *Musa acuminata* L. cv Barangan to salinity under various concentrations of NaCl *in vitro* culture.

MATERIALS AND METHODS

Plant materials: This study used a source of explants of *in vitro* banana shoots cultivar Barangan from PT. Multi Agro Kultura, Tangerang, Indonesia. The medium used was Murashige and Skoog basic medium (MS) 4.4 g L^{-1} with the addition of growth regulator 1.5 mg L^{-1} BAP (benzyl amin purine) of, sugar 30 g, Jelly 8 g, NaOH, HCl.

***In vitro* culture of banana shoots:** The explants were derived from *in vitro* culture of banana shoots (±3-4 cm high) and the medium of Murashige and Skoog medium (1962) with an addition of growth regulator 1.5 mg L^{-1} benzyl amino purine (BAP). To preserve the culture, sub-cultures were performed every 2 weeks. A culture contaminated with fungi or bacteria would be removed and replaced with a sterile culture and until the culture could be taken at 1, 2, 3 and 4 weeks after culture.

Experimental design: The experimental design used a completely randomized design with two factors, namely various concentrations of sodium chlorate (NaCl) salt and culture age repeated 3 times. The first factor consisted of sodium chlorate (NaCl) concentrations of 0 mM (n_0), 50 mM (n_1), 100 mM (n_2), 150 mM (n_3) and 200 mM (n_4). The second factor consisted of culture ages of 1 week (m_1), 2 weeks (m_2), 3 weeks (m_3) and 4 weeks (m_4).

Statistical analysis: The statistical analysis was done by one way analysis of variance, if significance continued to Duncan test level 5%. The parameters measured were the height of the banana plantlets, wet weight of shoots and roots of the banana plantlets and dry weight of the shoots and roots of the banana plantlets (F-value, level 0.05).

RESULTS AND DISCUSSION

Effects of salinity on the growth of banana plantlets: Salinity greatly affects the growth of the banana plantlet *in vitro* culture. It was morphologically marked, especially in leaves, by a brown color and dark brown at the roots (Fig. 1). This is due to the concentration of Na^+ ions which has inhibited high intake of K^+ ions into the plant. Normal plant cells have K^+ concentration higher than Na^+. The functions of the potassium ions among others are to maintain osmotic pressures in the cells, regulate the opening and closing of stomata, synthesize proteins and serve such as pyruvate kinase thus, the low concentration of K^+ in the cells causes chlorosis and necrosis[3,10,11].

Height of banana plantlets: The measurement of plant growth was done by measuring the height of the banana plantlets at the beginning phase of the culture until the age of 4 weeks after culture. Based on the analysis of variance

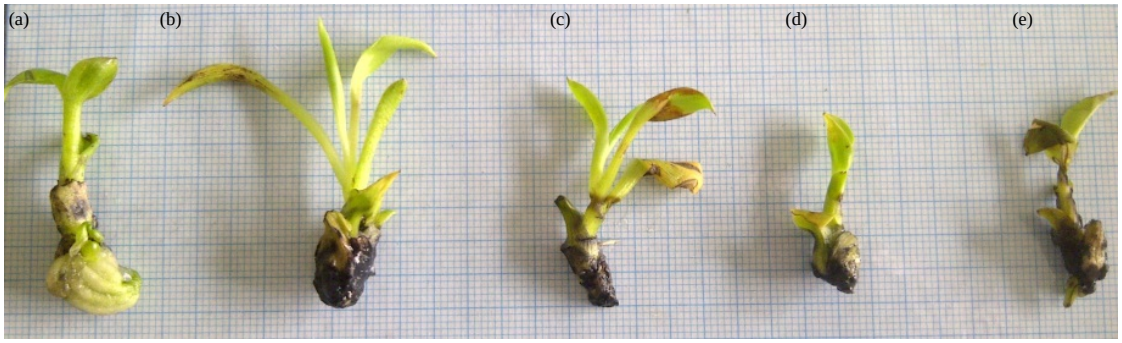

Fig. 1: Various NaCl treatments to banana plantlets, (a) NaCl 0 mM, (b) NaCl 50 mM, (c) NaCl 100 mM, (d) NaCl 150 mM and (e) NaCl 200 mM

Table 1: Effect of NaCl treatments to characters observed of banana plantlet

Culture age (week)	Height plantlets	Wet weight		Dry weight	
		Shoot	Root	Shoot	Root
m_1	2.6533^a	0.0937^a	0.1653^a	0.0075^a	0.01689^a
m_2	2.9444^a	0.1164^{ab}	0.2552^b	0.0099^{ab}	0.0232^{ab}
m_3	3.0022^a	0.1544^b	0.2614^b	0.0118^{ab}	0.02677^{ab}
m_4	3.4156^a	0.1604^b	0.3151^b	0.0138^a	0.02940^b
NaCl treatment					
n_0	3.3722^a	0.1609^b	0.3326^b	0.0116^a	0.02664^a
n_1	3.1250^a	0.1483^b	0.2891^{ab}	0.0111^a	0.02549^a
n_2	3.0250^a	0.1356^{ab}	0.2205^b	0.0107^a	0.02466^a
n_3	2.8522^a	0.1196^{ab}	0.2090^a	0.0109^a	0.02345^a
n_4	2.6500^a	0.0917^a	0.1957^a	0.0096^a	0.02009^a

Mean number of following treatment equal in column direct is no significant base on Duncan's in 5%

(Table 1), shown culture age increased and no significant differences are shown in culture age 2, 3 and 4 weeks compared with controls. In contrast NaCl treatments caused the height of banana planlet was lowest. Treatment without NaCl (control) shows fairly good growth, while treatment NaCl 200 mM there was decreased after a period of 4 weeks.

This shows that the effect of salinity has inhibited the height of banana plantlets. A high NaCl concentration in the growth medium has resulted in hyper ionic and hyper osmotic conditions in the plants. Excessive sodium ions in the medium will compete with potassium ions in order to be able to get into the plant. For plants susceptible to the condition of NaCl (glycophytes), this condition will certainly influence their homeostatic system. An increased osmotic pressure in plants can lead to difficulty for water to get into the plant tissue. Eventually, the ability of the plant to grow will decrease. A plant's responses to salinity can inhibit its growth due to osmotic stresses, high osmotic pressure causes the inability of the plants to fully absorb water from the growth medium. In addition, there is also toxicity of ions or ion accumulation in the plant tissues. The number of ions can cause a disruption to the plant homeostatic process, resulting in a lot of proteins needed in the metabolism of the plants that are not well expressed[3,12,13].

Wet weight shoot of banana plantlets: Salinity (NaCl) had effect to grow of *Musa acuminate* L. on character of wet weight shoot. The wet weight of shoots of the plantlets was one of the variables of plant growth. The results of the analysis of wet weight shoot of banana plantlets showed that the treatment significantly increased (F>0.05) character of wet weight shoot. The treatment of NaCl 200 mM (n_4) decreased on character of wet weight 0.0917 g of shoots had significant effect (F>0.05) compared controls and the other treatment n_1, n_2, n_3 were no significant with controls (n_0). Culture age weeks had significant with control (F>0.05). The highest characters of wet weight shoot of plantlets was culture age 4 weeks obtained 0.1604 (m_4) and had no significant with other treatment m_1, m_2, m_3 (Table 1). Salinity affect to water potential and the more high salinity will decrease it, whereas, stomata will closed and photosynthesis process hampered[14]. Metabolism process is affected by salinity in plant.

Wet weight root of banana plantlets: *In vitro* culture used of MS basic medium with the growth regulator of BAP 1.5 mg L^{-1}, the cells in the culture continued to grow with the presence of NaCl the plantlet shoots decreased, while the roots increased as the roots were in a medium other than NaCl. Also, there were nutrients that caused of cell divisions at the lower part of the plantlets. Wet weight of plantlets was increased in culture age had significant with control (F>0.05). The highest wet weight root of plantlets was culture age 4 weeks (Table 1).

The treatment of NaCl 50 mM (n_1), 100 mM (n_2), 150 mM (n_3) and 200 mM (n_4) were not significant ($F<0.05$). The treatment NaCl 200 mM on character wet weight root was lowest. Salinity stress can cause supply of K^+ decreased and cell divided, growth of root[15,16]. High salinity caused membrane activity reduced. There are interaction between ion Na^+,Ca^{2+} with growth root on membrane[17].

Wet weight shoot of plantlets culture age in week 4 was high, but NaCl treatment 200 mM was low about 0.0917 g. Affected of salinity decreasing of potential water will inhibition of photosynthesis process.

Wet weight of shoots and roots suggests that the effect of salinity (NaCl) of 50, 100, 150 and 200 mM were not significant difference when compared to that with control. Salinity inhibits the growth of banana plantlets in term of the parameters of height, wet weight of shoots and roots, dry weight of shoots and roots of the banana plantlets, however, they are different in every treatment and time after culture. A treatment of 200 mM NaCl indicates that plantlet growth is inhibited in term of height, wet weight of shoots and roots and dry weight of shoots and roots of banana plantlets.

Dry weight of banana plantlets shoots and roots: The statistic analysis result showed that culture age week 4 (m_4) had significant differences with control, but no significant with culture age week 2 (m_2) and culture age week 3 (m_3). Culture age week 2 (m_2) and week 3 (m_3) have no significant to controls (Table 1). The highest dry weight of shoot by treat sodium chloride obtained by controls and the lowest was NaCl 200 mM (n_4). Early affected of salinity was osmotic effect causing water potential decreased, cell dried and plant growth inhibition. Increase toxicity causing leave senescence, so that assimilate reduced[10]. The dry weight shows net photosynthesis results of the plants and is an actual measure of the biomass.

The result statistic analysis dry weight root of banana plantlets of culture age and NaCl treatment showed in culture age in week 4 (m_4) was significant with controls ($F>0.05$), but no significant different with culture age in week 2 (m_2) and week 3 (m_3). Culture age treat in week 2 (m_2) and week 3 (m_3) was no significant to controls.

The highest on character dry weight root obtained from controls, the lowest was NaCl 200 mM (n_4). Sodium chloride treat 50 mM (n_1), 100 mM (n_2), 150 mM (n_3) and 200 mM (n_4) were no significantly different to controls. Potassium (K^+) has more important to protein and turgor regulation in cell. High of sodium chloride caused K^+ decreased of phosphate. Sodium chlorate treatment 200 mM (n_4), membrane use hampered, root grow relations with Ca^{2+}/Na^+ ion[8]. The dry weight of the shoots but the dry weight roots has showed the biomass of the samples at a given time instead.

CONCLUSION

The effect of salinity could influent to growth *Musa acuminata* L.cv Barangan *in vitro* culture. The treatments of NaCl 200 mM for character height lower than controls. The height of banana plantlets after culture age in week 4 is decreased, the salinity can to height plantlets. The NaCl treatment can be used for simulation salinity tolerant for banana plant *in vitro*.

SIGNIFICANCE STATEMENTS

This study discovered the salinity effect to grow plant and culture age of *Musa acuminata* L. that can be beneficial for optimum obtained of concentration saline (NaCl). This study will help the researcher to find the critical areas of environmental stresses such as salinity that many researchers were not able to explore. Thus a new theory on optimization of saline stresses may be arrived at for the benefit of plant growth and development of *Musa acuminata* L.

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