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Effect of Various Concentrations of Humic Acid on Growth and Development of Eggplant Seedlings in Tissue Cultures at Low Nutrient Level

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***Abstract*—**Humic acids (HAs) have been shown to activate some ion uptakes along with stimulating the lateral roots at effective concentration of micronutrients. However, the effects of HA on ion adsorption by plant roots are not easily explainable due to the varieties of HAs that differ from origins. Therefore, this study was aimed to investigate the effect of various concentrations of HA obtained from the compost derived from mix manures and some agricultural wastes on the growth of eggplant seedlings (*Solanum melongena* L. cv. Chao Praya) in tissue cultures at low nutrient level. Egg plant seeds were surfaced sterilized and germinated in ½ Murashige and Skoog medium (MS) without HA added or in ¼ MS supplemented with 0, 25, 50, 75 and 100 ppm of HAs. Then, they were cultured for 4 weeks under the controlled environment. The results showed that seedlings grown on ¼MS supplemented with HAs at the concentration of 25 and 50 ppm had the average plant heights (2.49 and 2.28 cm, respectively) higher than the other treatments. Both treatments also significantly showed the maximum average fresh and dry weights ($p<0.05$). Also the later yielded the highest average number of leaves and the longest average root length ($p<0.05$). However, there was no statistically different in the number of roots among treatments ($p>0.05$). This suggested that HAs at the concentration of 25 and 50 ppm could improve the growth of egg plant seedlings in tissue cultures at low nutrient level (¼ MS).

***Keywords*—**growth, seedling, humic acid, fresh weght, dry weight, tissue culture

I. Introduction

HUMIC acids (HAs) have long been recognized that play a major role in produce morphological and physiological effects on higher plants [1-2]. It has been reported that HAs are able to stimulate or inhibit plant growth depending on their differences in origin, nature and concentration. The application of humic substances to nutrient solution, to soil or sand has been documented and the results showed that they enhanced significant growth responses [3]. The mechanisms showed that humic substances enhanced the growth by increasing the uptake of micronutrient [4]. Foliar sprays of HA promoted growth in many plants such as tomato, cotton and grape [5]. However, there have been scarce reports on the effect of HA on plant growth and development in vitro propagation, especially at low nutrient level in which the sources of plant food were limited. Since HAs were different in origin, the HA that derived from mix manures and some agricultural wastes in this study was considered unique. Therefore, the objective of this study was to investigate potential effects of HA and its optimal concentrations on eggplant seedlings cultured in vitro propagation at the low concentration of Murashige and Skoog medium (MS) [6].

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II. Materials and Methods

A. Humic Acid Preparation

HA in this study was extracted from the compost that was prepared with bat, chicken, pig and cow manures, 2.13%, 8.5%, 17%, and 17%, respectively mixed with chopped *Leucaena leucocephala* de Wit 10.63%, bran of rice 25.5%, dolomite 10.63%, molace 2.3%, fish fermented juice 2.13% and micronutrients 4.25%. The total weight of the mixed compost was 470.5 Kg. After monitoring the change of temperature, the end of active phase of the compost was at day 35. Ten samples were randomly collected on this day and mixed together. Then, it was sieved through 2 mm mesh and extracted for HA after the method of International Humic Substance Society (IHSS) [7]. Freeze dried HA was kept for further experiments.

B. Plant Materials And Growth Condition

Eggplant seeds were surfaced sterilized by using 15% Clorox® Ultra household bleach solution (5.25 % NaOCl, Clorox, Sdn, Bhd, Malaysia) added with 0.1 % Tween-20 for 10 min. and then rinsed three times with sterile distilled water. Seeds were germinated on ½MS without HA added or ¼MS supplemented with HA at the concentration of 0 (control), 25, 50, 75 and 100 ppm. All cultures were incubated at 25 ± 2 ^{o}C under 16-h photoperiod of 35 $\mu molm^{-2}s^{-1}$ obtained from daylight fluorescent lamps.

C. Data Analysis

Ten replicates were carried out for each treatment. After in vitro propagating for 4 weeks, plant height, total fresh and dry weights, number of leaves, leave area, number of roots and root length were examined. The data were analyzed by SPSS for windows version 18 using one-way ANOVA analysis followed by Duncan test or Kruskal-Wallis test,depending on

whether the normality assumptions were met. Differences of means were calculated to compare significant effects at the 5% significant level.

III. Results and Discussions

The effects of HA concentrations on the growth of eggplant seedlings showed that after cultured for 4 weeks, ¼MS supplemented with HA at 25 and 50 ppm statistically induced plant height and total fresh and dry weights than any other treatments ($p<0.05$). The average plant heights of both treatments were 2.49 and 2.28 cm, respectively (Fig.1). The average total fresh weights both were 0.1000 g (Fig. 2) and the average total dry weights were 0.0126 and 0.0117 g, respectively (Fig. 3). Seedling grown on ¼MS supplemented with 50 ppm of HA had the highest average number of leaves (Fig. 4). However, there was only one root of each treatment. Thus, no statistically difference was shown. The longest root length was found on ¼MS medium supplemented with 50 ppm of HA (Fig. 5). Similar results were found in maize roots applied with HA extracted from vermicomposts that the HA increased root elongation [8].

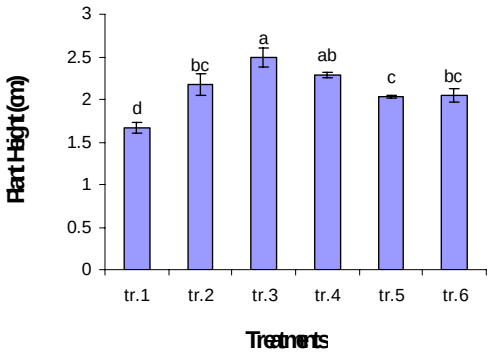

Fig.1 Effect of various concentrations of HA + ¼MS on the heights of eggplant seedlings cultured for four weeks in comparison with ½MS

tr.1 = 1/2MS, tr.2 = 1/4MS, tr.3 = 1/4MS+25 ppm HA,
tr.4 = 1/4 MS+50 ppm HA, tr.5 = 1/4 MS+75 ppm HA,
tr.6 =1/4 MS+100 ppm HA

Nevertheless, exceeding optimum concentrations of HA might not be appropriate for plant growth. As shown in this study that ¼MS supplemented with HA concentrations higher than 50 ppm decreased the growth of the eggplant seedlings. This was found the same with another study by Atiyeh et al.[9] that the general pattern of plant growth was increased in response to treatments of low application rate of HA but decreased significantly when the exceeding optimum concentrations of HA were applied.

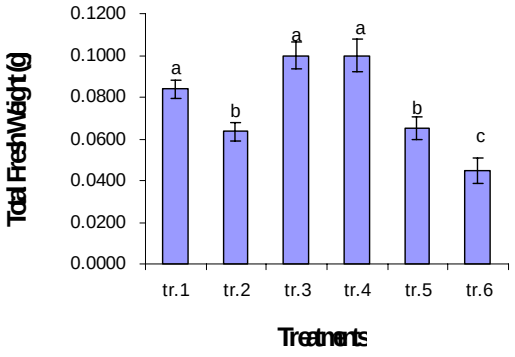

Fig. 2 Effect of various concentrations of HA + ¼MS on the total fresh weights of eggplant seedlings cultured for four weeks in comparison with ½MS

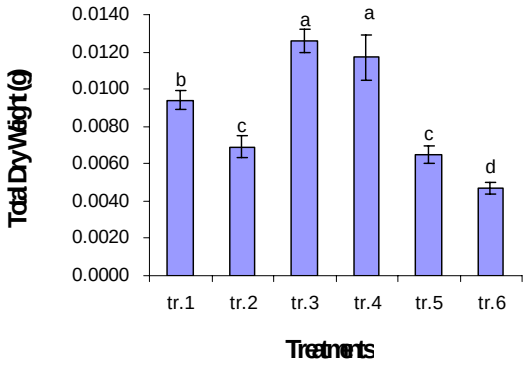

Fig. 3 Effect of various concentrations of HA + ¼MS on the total dry weights of eggplant seedlings cultured for four weeks in comparison with ½MS

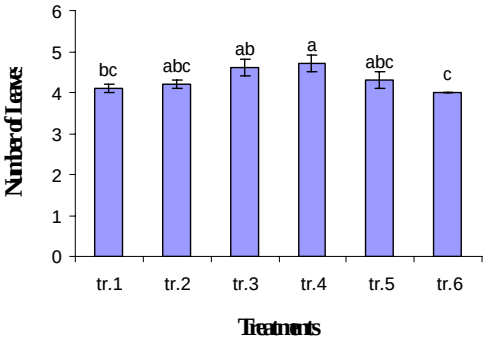

Fig. 4 Effect of various concentrations of HA + ¼MS on the numbers of leaves of eggplant seedlings cultured for four weeks in comparison with ½MS

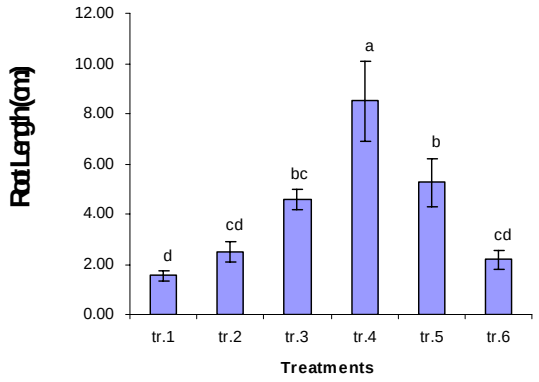

Fig. 5 Effect of various concentrations of HA + ¼MS on the root lengths of eggplant seedlings cultured for four weeks in comparison with ½MS

The suggested mechanisms might be the interfering of hormone-like activities of HA, such as an auxin-like activity [1, 10]. Since plant hormones such as auxin when was applied at high concentration (beyond an optimum level) could reduce the growth and development of plants [10].

IV. Conclusion

One fourth of MS supplemented with HAs at 25 and 50 ppm cultured for four weeks induced plant height and total fresh and dry weights than any other treatments. Even the seedling grown on ½MS could not compete. Although, there was no statistically difference on the numbers of roots among treatments, seedling cultured on ¼MS supplemented with 50 ppm HA had the longest average root length and the highest average number of leaves. The results showed that appropriate amounts of HA could be used as growth enhancement for eggplant especially when the nutrients were limited which in this case, at low concentration of medium. On the other hand, it implied that HA could be applied to help improve soil quality especially at poor soil quality areas. Field experiments to determine appropriate amounts among different types of HAs still challenge research scientists of this area.

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