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Ananas comosus L. Multiplication on Reduced MS Media Concentrations Supplemented with a Nanofertilizer

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Abstract: The present study consists of two experiments aiming at investigating the efficiency of pineapple (*Ananas comosus* L.) cv "Smooth Cayenne" micropropagation on reduced MS media concentrations supplemented with NPK nanoparticles. In this trial, explants used were shoot tips and subcultures were conducted every three weeks. In the first experiment, shoot tips were cultured on solid MS media supplemented with 3% sucrose, 3 mg/ L BA and different concentrations (0, 2, 4 and 8 mg/ L) of NPK 9- 0- 6 nanoparticles. Results revealed that the 4 mg/ L NPK nanoparticles treatment recorded the best results with regard to number of shoots and number of leaves, though shoots were compact. In the latter study, MS media with different strengths (25 %, 50, 75% and 100% MS media) were supplemented with 3% sucrose, 3 mg/ L BA and the NPK nanoparticles concentration that promoted multiplication the most (4 mg/ L). Results showed that 75 % strength MS medium produced the highest number of shoots and leaves while 25 % strength MS medium produced the longest shoots.

Key words: Pineapple · Micropropagation · Nanofertilizer · MS

INTRODUCTION

Agricultural researchers face great challenges in their work aiming at developing efficient procedures for sufficient agricultural production with regard to quantity and quality to feed the increasing global population without degrading soil health and agroecosystems [1]. Among the technological advancements that emerged to overcome this challenge is agrinanotechnology. Nanotechnology in agriculture touched several fields including food industry, plant protection, plant productivity and plant nano- nutrition [2], which is of special interest in this study. Crucial need for the development of nanofertilizers has emerged to reduce production costs resulting from the usage of low efficiency traditional chemical fertilizers and to reduce associated environmental pollution [3]. In this regard, it is worth mentioning that nanofertilizers have unique physicochemical properties which gives them potential to boost plant metabolism [4, 5]. Nevertheless, interactions between plants and engineered nanomaterials are also of particular importance, as plants directly interact with soil, water and the atmosphere and serve as a potential pathway of these nanoparticles exposure for higher species through the food chain [5, 6]. Moreover, additional research on the toxicity of newly developed nanofertilizers must be conducted to alleviate the public fears about the associated nanotoxicity especially that crops need only trivial amounts of nutrients to maintain normal physiological activities while extremely high concentrations of any nutrient would cause phytotoxicity [7]. Few research and systematic studies on the effects and advantages of applying micronutrient nanofertilizers under field conditions are available because development and application of nanofertilizers are still at initial stages [7]. Meanwhile, evaluation of nanofertilizers *in vitro* is considered at its preliminary stages, but nanotechnology has potential to provide opportunities for plant science researchers to develop new tools for incorporation of nanoparticles into plants that could augment existing functions and add new ones [8, 5].

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In this study, pineapple (*Ananas comosus* L.) which has been successfully multiplicated *in vitro* on media enriched with BA alone will be challenged with reduced media concentrations in the presence of NPK 9- 0- 6 nanoparticles [9]. We believe that accelerating growth and/ or enhancing *in vitro* productivity with the aid of nanofertilizers can open new perspectives in agricultural practice, though responses will vary according to plant species, growth stages and nature of nanomaterials. The objective of this study is to evaluate the amount of growth in terms of number of shoots and leaves and shoot length that could be obtained from single explants cultured on MS media with different concentrations in the presence of NPK 9- 0- 6 nanoparticles.

MATERIALS AND METHODS

This investigation was carried out in the Central Laboratory for Date Palm Research and Development, Agricultural Research Center, during 2016. In the first stage of this study, solidified agar (7 g/ L) MS medium [10] was prepared, supplemented with 3 % sucrose and enriched with 3 mg/ L BA and divided into four beakers, 300 ml each. Medium in the first beaker was NPK nanoparticles- free (Control), while media in the second, third and fourth beakers were supplemented with 2, 4 and 8 mg/ L NPK 9- 0- 6 nanoparticles, respectively. Media pH was adjusted to 5.7 and was dispensed equally into 15 glass jars (20 ml/ gar). Jars were lid- covered and autoclaved at 121°C under 15 psi for 15 min. Stock cultures of cv "Smooth Cayenne" shoot tips sub- cultured twice earlier, every 21 days on MS medium supplemented with 3 % sucrose and 3 mg/ L BA were the source of explants for this study. A single shoot was cultured in each jar under aseptic conditions and jars were incubated at 26°C and a 16 h photoperiod. Number of shoots, shoot length and number of leaves/ shoot were recorded at the following three subcultures that took place every three weeks.

In the second stage of this study, single shoots from the same stock culture used in the first stage were cultured individually in glass jars on solidified agar (7 g/ L) MS medium with different strengths supplemented with 3 % sucrose and enriched with 3 mg/ L BA. MS strengths investigated were 100, 75, 50 and 25 %. Incubation conditions, subcultures and data recorded were similar to those of the first stage in this trial. Both experiments were laid out using a Completely Randomized Design (CRD) with three replicates (each represented by 5 jars) per treatment. Experimental data obtained was treated with Analysis of Variance (ANOVA) at confidence level of 95% and the differences between means were detected using least significant difference (LSD) at $P< 0.05$ and data was analyzed using statistical software (MSTATC 2.10, Russell D. Freed).

RESULTS AND DISCUSSION

Because several shoots developed from each shoot tip in the preliminary stage, we concluded that they were of adventitious origin.Results for the first experiment in this study are presented in Figure 1 (a, b and c). As shown, supplementing MS medium with different concentrations of nano NPK had a significant effect on the number of shoots, average shoot length and number of leaves/ shoot recorded throughout the whole experiment. As shown in Figure 1 a, 4 mg/ L nano NPK resulted in a significantly high propagation rate compared to the control and other concentrations investigated. It was also noticed that the control and the 2 mg/ L nano NPK treatment recorded insignificant differences in-between in favor of the 2 mg/ L nano NPK treatment. Moreover, supplementing MS medium with 8 mg/ L nano NPK increased the number of shoots produced compared to supplementing the medium with 2 mg/ L, though statistical significance was recorded in the first subculture only. On the other hand and as presented in Figure 1 b, nano NPK generally inhibited shoot elongation. This effect was most evident on shoots grown on MS medium supplemented with 4 mg/ L nano NPK. Shoots cultured on 4 mg/ l nano NPK- supplemented MS medium were significantly shorter than those cultured on nano NPK- free and 2 mg/ L nano NPK- supplemented MS media, between which, insignificant differences were detected. As for the number of leaves/ shoot as affected by nano NPK concentration, values were recorded and presented in Figure 1 c. As shown, the 4 mg/ L nano NPK treatment produced shoots with the highest number of leaves, followed by the 8 mg/ L, 2 mg/ L and the control, respectively.

Results of the second experiment of this trial are presented in Figure 2 (a, b and c). As shown in Figure 2 a, the highest numbers of shoots were recorded for 75 % MS medium supplemented with 4 mg/ L nano NPK at all three investigated subcultures. It was also noticed that reducing MS medium strength from 100 to 50 % insignificantly affected the number of proliferated shoots when media were supplemented with 4 mg/ L nano NPK. Contrarily, shoots proliferated on 75 % MS medium were

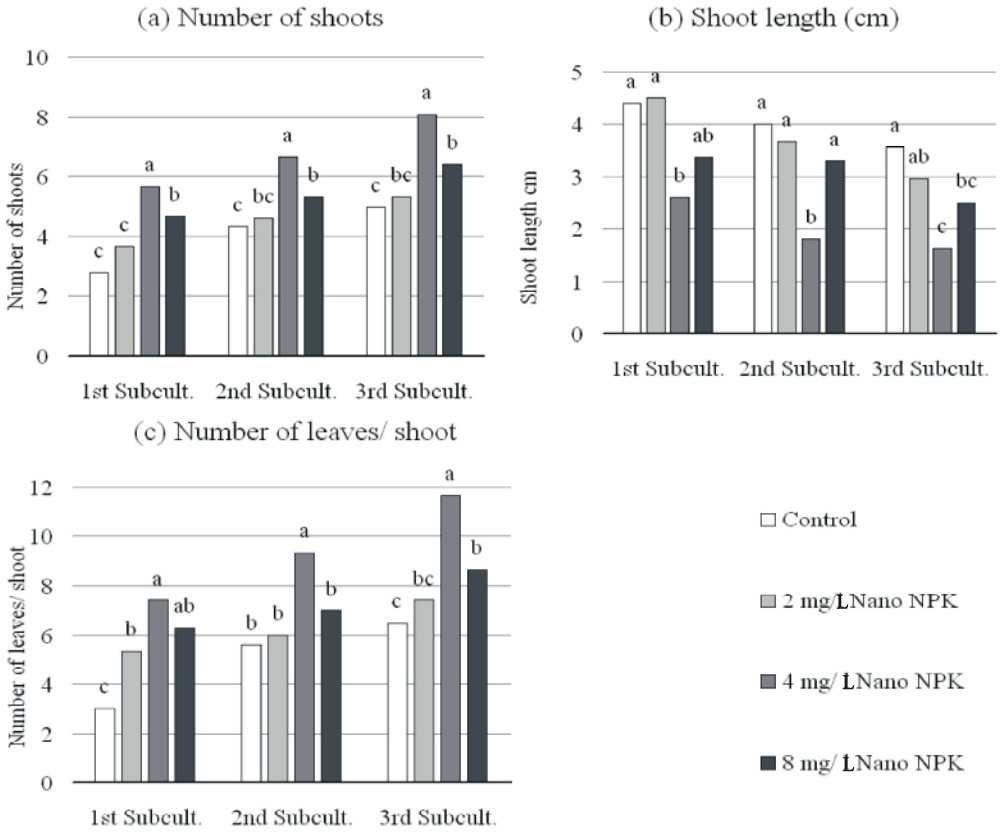

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Fig. 1: Effect of different concentrations of nano NPK 9- 0- 6 on (a) number of shoots, (b) shoot length and (c) number of leaves/ shoot of pineapple cv "Smooth Cayenne" on MS mediumfor three, 21- day subcultures

the shortest compared to shoots cultured on MS media with other strengths. As shown in Figure 2 b, results followed identical numerical and statistical trends at all three subcultures. The highest values were recorded for 25 % strength MS medium treatment, followed by 50, 100 and 75 % strengths MS media, respectively. Neither differences between 100 % and 75 % strength MS medium nor the differences between 100 % and 50 % strength MS medium were statistically different. Finally, the highest number of leaves/ shoot were recorded for 75% MS medium, followed by 100 %, 50 % and 25 %, respectively, as shown in figure 2 c. At the 1st subculture only, reducing medium strength from 100 to 50 % resulted in an insignificant reduction in the number of produced leaves/ shoot. In the following subcultures, reductions were statistically significant. It was also noticed that reducing MS medium strength from 50 to 25 % was always correlated with statistically significant reductions in the number of leaves/ shoot.

Enhanced growth and multiplication on reduced MS media concentrations found in this study is in harmony with what Jinghua, [11] reported regarding enhanced uptake and use of nutrients in response to the application of a nanoengineered composite consisting of N, P, K micronutrients, among other compounds. Moreover, several other researchers reported that nanomaterials can promote plant growth [12, 13]. This is probably attributed to particular properties nanomaterials possess (surface effect, volume effect and quantum size effect) and macronutrients lack [14]. Nanoparticles have high reactivity because of more specific surface area, more density of reactive areas, or increased reactivity of these areas on the particle surfaces. These features in nano-scale simplify their absorption in plants [15]. Maintaining vigor on reduced MS media concentrations supplemented with nano- NPK might be attributed to promoted water and nutrient absorption and improved photosynthesis [16]. In other words, nano-NPK can be

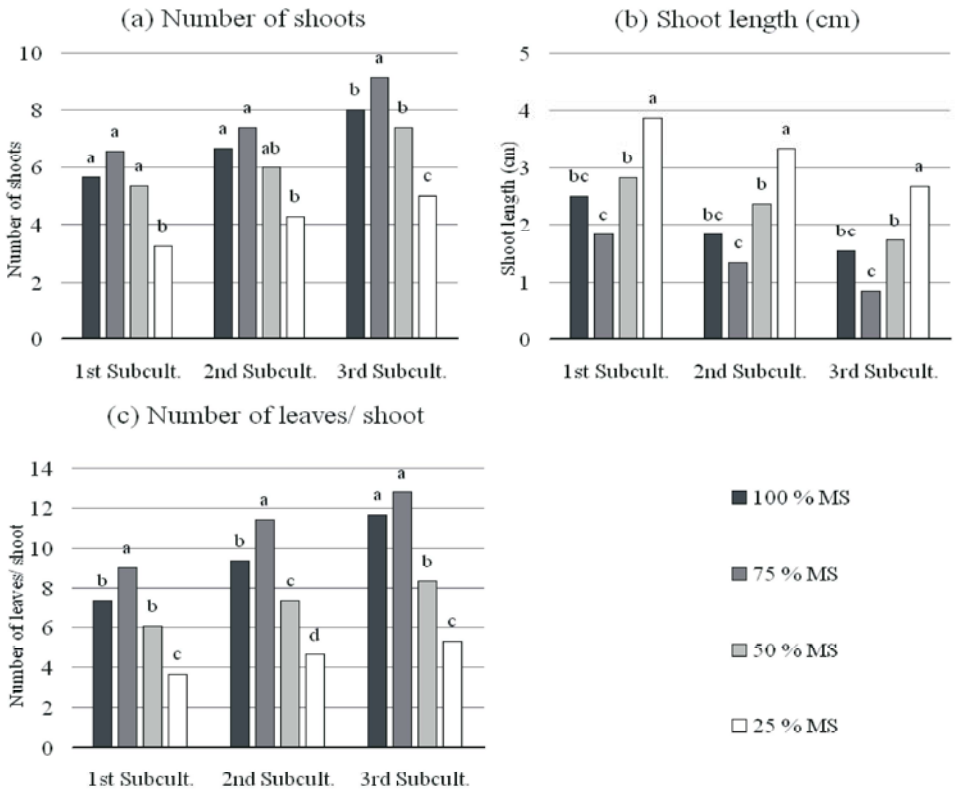

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Fig. 2: Effect of supplementing different strengths of MS medium (100, 75, 50 and 25 % MS medium) with 4 mg/ l nano NPK 9- 0- 6 on (a) number of shoots, (b) shoot length and (c) number of leaves/ shoot for pineapple cv "Smooth Cayenne" for three, 21- day subcultures.

considered the biological pump for the plants to absorb nutrients and water [17]. Moreover, it was found that nanoparticles induce the formation of new larger pores in the plant cell walls to allow the entrance of large nanoparticles that are afterwards transported to the vascular system of various shoot systems [18-21].

CONCLUSION

Considering the results obtained in this study, it can be concluded that nano NPK 9- 0- 6 proved to be beneficial in maintaining *in vitro* high multiplication rates of Smooth Cayenne pineapple on reduced MS media concentrations. This is an environment- friendly application for the use of nanofertilizers in reducing *in vitro* micropropagation costs which may open new perspectives for *in vitro* agricultural practices.

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