VOL. 11, NO. 5, MAY 2016 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

IMPROVED ROOTING IN CAVENDISH BANANA CV. GRANDE NAINE USING COCONUT SHELL ACTIVATED CHARCOAL AND NAA SUPPLEMENTS

Remedios S. Flamiano[1], Lordy Grace C. Itang[1], Pablo B. Flamiano[1], Rosemarie S. Diamonon[2], Renan B. Gubalani[1] Jeanette B. Madas[1], Erna S. Bautista[2], Livilla Donah V. Ligan[2] and Rebecca S. Casido[2]
[1]Department of Science, College of Natural Sciences and Mathematics, Mindanao State University, General Santos City, Philippines
[2]Surallah National Agricultural School, Philippines
E-Mail: rflamiano@yahoo.com

ABSTRACT

The present study was done to evaluate the efficacy of using coconut shell activated charcoal in combination with napthalene acetic acid as rooting amendment tofull strength Murashige and Skoog Medium for Cavendish cv. Grande Naine. Concentrations of napthalene acetic acid from 1-5 ppm, and coconut shell acivated charcoal from 1% to 3% were added to full strength MS medium. Regenerated microshoots of Cavendish banana were allowed to root for 21 days in the different MS amended media. Results of this study revealed that rooting of Cavendish banana cv. Grande Naine was best in full strength MS medium amended with 3.0% coconut shell activated charcoal with2 ppm NAA,witheach plantlet developed anaverage 26 roots having the longest roots of about 92.22 cm. Higher concentrations of napthalene acetic acid at 4 ppmand 5 ppm combined with 3% activated charcoalwere shown to have inhibitory effects on the number and length of roots. Full strength ,hormone-free MS medium had also been shown to support rootingas with the different charcoal-amended MS medium. Addition of 3% coconut shell activated charcoal to full strength MS Medium further increased the number ofmicroshoot roots although the increase was not significant from the hormone-free full strength MS Medium.

Keywords: cavendish, rooting, naphthalene acetic acid, activated charcoal.

INTRODUCTION

Banana is an important and widely grown fruit crop in the tropical and subtropical regions of the world (Darvari *et al*., 2010; Rahman *et al*., 2013). In the Philippines, it is the number one fruit commodity both in production and hectarage with Cavendish bananas as the most cultivated variety. In 2014, Cavendish bananas were planted to about 83,729.71 has land area (PSA, 2014). Currently, area planted to Cavendish has further increased due to escalating global demands for fresh bananas mainly from Japan, China, South Korea, UAE, Singapore and recently from USA. Philippines is presently the third largest exporter of bananas worldwide with$1.1 billion or 10.3% of total banana export following Belgium with$1.3 billionor 12.1% of total banana export and Ecuador with 2.6 billion or 23.7% of total banana exports (Workman, 2015).

Commercial plantations of Cavendish bananas have beendependent on tissue-culture technology for the production of multiple true-to-type seedlings for planting. This technology allowed the mass production of banana planting material in Murashige and Skoog medium (Murashige and Skoog, 1962) amended with different concentrations of auxins and cytokinins (Ngomuo *et al.,* 2014). Auxins that arecommonly used for banana tissue culture media include indole-3-acetic acid (IAA) (Al-Amin *et al.,* 2009; Jaisy and Ghai, 2011; Saad and Elshahed, 2012; Ngomuo *et al.,* 2014; Uzaribara*et al.,* 2015), indole-3-butric acid (IBA) (Al-Amin *et al.,* 2009; Jaisy and Ghai, 2011; Saad and Elshahed, 2012; Ngomuo *et al.,* 2014), 2, 4-dichlorophenoxy-acetic acid (2, 4-D) (Saad and Elshahed, 2012) and naphthalene-acetic acid (NAA) (Al-Amin *et al.,* 2009; Saad and Elshahed, 2012;Uzaribara *et al.,* 2015) while cytokinins include BAP (6- benzyloaminopurine) (Muhammad *et al.,* 2004; Al-Amin *et al.,* 2009; Saad and Elshahed, 2012; Ngomuo *et al.,* 2014; Uzaribara*et al.,* 2015; Qamar *et al.,* 2015), 2iP (6- dimethylaminopurine) (Saad and Elshahed, 2012), kinetin (N-2-furanylmethyl-1H-purine-6-amine) (Saad and Elshahed, 2012), Zeatin (6-4-hydroxy-3-methyl-trans-2-butenylaminopurine) Saad and Elshahed, 2012) and TDZ (thiazuron-N-phenyl-N-1, 2, 3 thiadiazol-5ylurea) (Shirani *et al*., 2009; Saad and Elshahed, 2012). These hormones are either used singly or in combination with other plant hormone to elicit response, and are found to be specific per variety.

Several studies have proved the effectiveness of auxins IBA (Jaisy and Ghai, 2011), NAA (Al-Amin *et al.,* 2009; Uzaribara *et al.,* 2015) and activated charcoal (Jaisy and Ghai, 2011; Uzaribara *et al.,* 2015) for rooting. The utilization of each hormone for rooting needs to be determined per plant so as to maximize its use. Synthetic hormones are not only expensive but they may also contribute to increasing incidence of somaclonal variation at high concentration. In the present study, we evaluated the efficiency of using coconut shell charcoal alone and in combination with NAA as rooting amendment to MS medium for root formation in Cavendish banana cv. Grande Naine.

MATERIALS AND METHODS

To identify the optimum supplement for rooting tissue-cultured Cavendish banana cv Grande Naine, well defined regenerated microshoots from subculture 5 with 2-3 leaves of about 3-5 cm in length were used. Eight (8) microshoots of more or less same sizes were inoculated in

250-ml bottle containing about 20 ml sterile full strength Murashige and Skoog supplemented with 3 different levels (1%, 2% and 3%) of activated charcoal and NAA (α-naphthalene acetic acid) viz. 1, 2, 3, 4 and 5 mg/L. The experiment was the carried out in a Complete Randomized Design (CRD) with 3 replications, each unit of bottle was considered as a replicate. Bottles were arranged randomly in a growth room with a temperature of about 26°C and lighted 8 hours a day. The number of roots and root length of each microshoots were recorded 21 days after incubation in the different modified full strength MS Medium. Following data collection, the bottles containing the rooted microshoots were hardened under a shade for 7 days and individually were planted in a 0.002 6x6 polyetheylene (PE) black plastic bag.

RESULTS

Table-1. Mean number of roots and root length (cm) ofCavendish banana cv. Grande Naine21days after inoculation of regenerated microshoots in the different Murashige and Skoog amended media.

Treatments	No. of roots n=9	Root length (cm) n=9
MS Medium alone	8.778^{D}	52.333^{B}
MS Medium +1% charcoal	9.00^{D}	53.67^{B}
MS Medium +1.5% charcoal	10.00^{D}	53.89^{B}
MS Medium +2.0% charcoal	11.00^{D}	52.11^{B}
MS Medium +3.0% charcoal	12.007^{D}	48.33^{B}
MS Medium +3.0% charcoal +1 ppm NAA	13.00^{CD}	51.00^{B}
MS Medium +3.0% charcoal +2 ppm NAA	26.00^{A}	92.22^{A}
MS Medium +3.0% charcoal +3 ppm NAA	24.00^{AB}	70.11^{AB}
MS Medium +3.0% charcoal +4 ppm NAA	18.00^{BC}	69.33^{AB}
MS Medium +3.0% charcoal +5 ppm NAA	9.00^{D}	54.44^{B}
	cv=28.44	cv=30.00

Means having similar letter are not significant at 5% level.

Results of this study revealed that regenerated microshoots of Cavendish banana cv. Grande Naine produced the most number of roots with longest root length in full strength MS medium supplemented with 3.0% charcoal and 2 ml/L NAA (Figure-2). This was combination found most optimal for root formation in Cavendish banana cv. Grande Naine. The findings in the present investigation was different from those obtained in trial using red banana (*Musa acuminata*) where they got lower NAA concentration optimal for rooting. The greatest number of roots and longest roots were induced in MS medium supplemented with 1.5 mg L^{-1}NAA and 2.0 g L^{-1} activated charcoal while least number of roots and shortest roots were observed with MS medium + 1.0 mg L^{-1} NAA + 2.0 g L^{-1} activated charcoal and MS medium + 0.5 mg L^{-1} NAA + 2.0 g L^{-1} activated charcoal respectively (Uzaribara *et al.,* 2015). In a similar study, Uzaribara *et al.* (2015) demonstrated that rapid increase in root length was observed in MS medium amended with 2.0 g/L^{-1} activated charcoal with 1.5 mg L^{-1} IBA. In the present study, we have demonstrated that increasing NAA concentration up to 3 ppm in full strength MS medium with similar amount of activated charcoal (3%) had similar effect as 2ppm NAA although a slight decrease in the number of roots and root length was observed. However, when NAA was further increased to 4 ppm, root formation was significantly reduced to an average of 18 roots per plantlet although root length was not significantly reduced. Further, this study showed that4 ppm NAA combined with 3% activated charcoal was found to have negative effect on root formation of Cavendish cv. Grande Nainemicroshoots which was in contrast to the findings of Rahman *et al.* (2013) on rooting where they demonstrated that 4 ppm NAA was best for rooting banana cv. Sabri. Higher NAA concentration of5ppm NAA in combination with 3% activated charcoal was found to have remarkable inhibitory effect on rooting of Cavendish cv. Grande Naine.

Rooting of Grande Nainemicro shoots may however be initiated in a hormone-free or in an activated charcoal-free full strength MS Medium (Figure-1). Thus, in the absence of these supplements, rooting of Cavendish banana Grande Naine may still be possible. Moreover, this study has shown that addition of activated charcoal of up to 3% in full MS medium had increased slightly the number and length of microshoot roots 21 days after inoculation in the medium compared to the microshoots rooted in activated charcoal-free medium although the increase was not found to be significant indicating that addition of up to 3% coconut shell activated charcoal may have improved rooting but had little effect on root formation of Cavendish cv. Grande Naine.

Figure-1. Cavendish meriplants in full strength MS Medium amended with different concentration of activated charcoal. T1 as the control (MS Medium only, T2 with 1% charcoal, T3 with 1.5% charcoal, T4 with2.0% charcoal, and T5 with3.0% charcoal.

Figure-2. Cavendish banana microshoots 21 days after inoculation in full strength MS Medium amended with 3.0%coconut shell activated charcoal combined with different concentrations of NAA (T6 with 1 ppm, T7 with 2 ppm, T8 with 3 ppm, T9 with 4 ppm and T10 with 5 ppm NAA). Note heavy root formation in T6, T7 and T8 and remarkable root inhibition in T10 (5 ppm NAA).

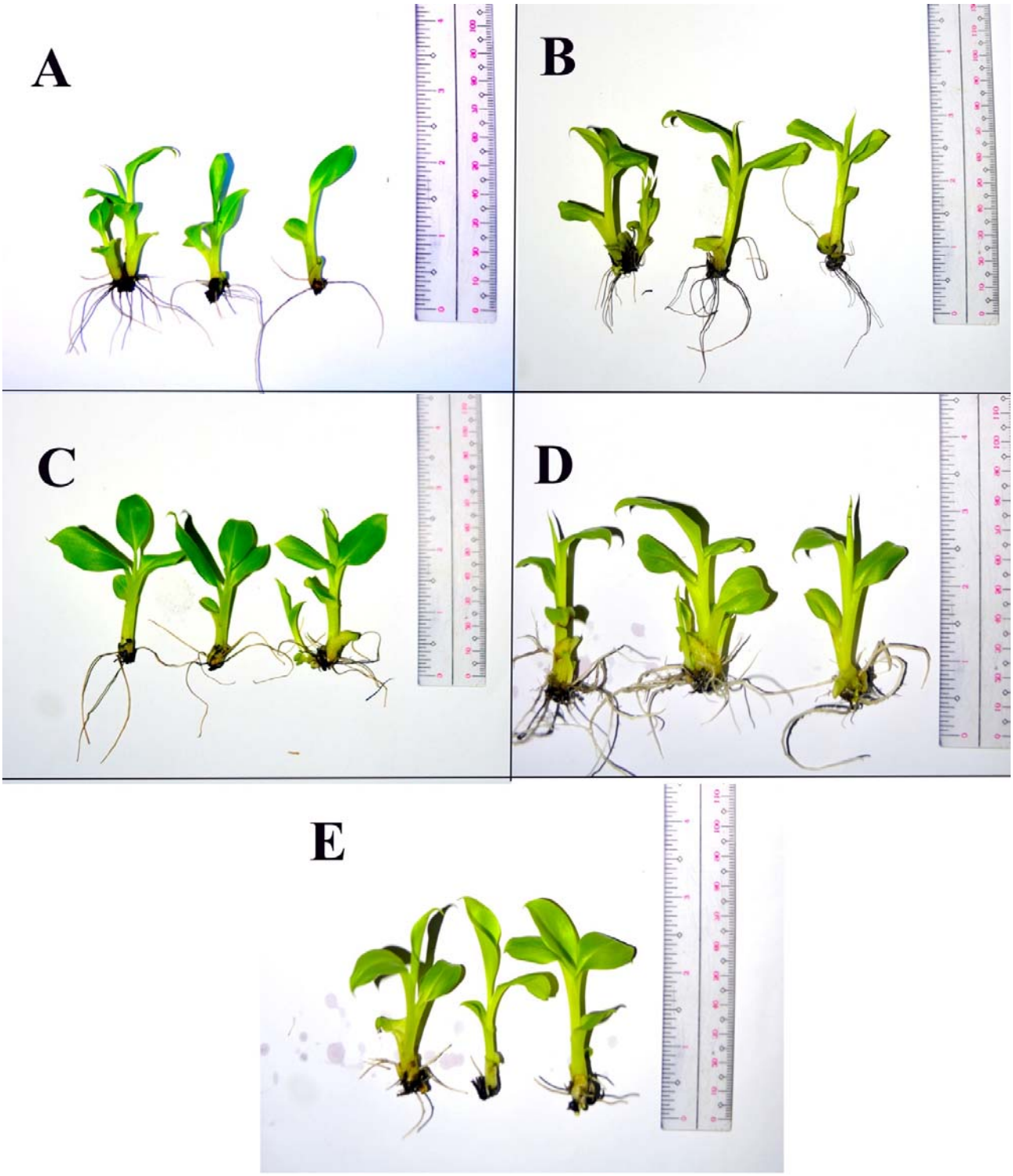

Figure-3. Representative meriplants from different treatments exhibiting significant variation in the number of roots and root length:
A-control (full strength MS Medium only); B-MS Medium + 1.5% charcoal; C- MS Medium + 3.0% charcoal; D-MS Medium and 3.0% charcoal + 2 ppm NAA; and E-MS Medium and 3.0% charcoal + 5 ppm NAA.

ACKNOWLEDGEMENTS

We would like to thank GreenlandsAgritech Ventures for kindly providing assistance in this study.

REFERENCES

Al-Amin, M.D., M.R. Karim, M.R. Amin, S. Rahman and A. N. M. Mamun. 2009. In Vitro micropropagation of Banana (Musa spp.) Bangladesh J. Agril. Res. 34(4): 645-659.

Darvari F.M., M. Sariah, M.P. Puad and M. Maziah. 2010. Micropropagation of some Malaysian banana and plantain (Musa sp.) cultivars using male flowers. African Journal of Biotechnology. 9(16): 2360-2366.

Jaisy R.C. and D. Ghai. 2011. Development of low cost methodology and optimization of multiplication and rooting hormones in the micropropagation of red banana in vitro. Plant Sciences Feed. 1(7): 84-87.

Muhammad A., I. Hussain, S.M. Saqlan Naqvi, A. Rashid. 2004. Banana plantlet production through tissue culture. Pak. J. Bot. 36(3): 617-620.

Murashige T. and Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15(3): 473-497.

Ngomuo M, E. Mneney, P. Ndakidemi. 2014. The in Vitro Propagation Techniques for producing Banana Using Shoot Tip Cultures. American Journal of Plant Sciences. 5: 1614-1622.

Philippine Statistics Authority-Bureau of Agricultural Statistics. 2014.

Qamar M., S.T. Qureshi, I.A. Khan and S. Raza. 2015. Optimization of in vitro multiplication for exotic banana (Musa spp.) in Pakistan. African Journal of Biotechnology. 14(24): 1989-1995.

Rahman S., Biswas N., Hassan M., Ahmed G., Mamun A., Islam R., *et al*. 2013. Micro propagation of banana (Musa sp.) cv. Agnishwar by In vitro shoot tip culture. International Research Journal of Biotechnology. 4 (2141-5153): 83-88.

Saad A. I. M. and A. M. Elshahed. 2012. Plant Tissue Culture Media. Recent Advances in Plant in vitro Culture. Licensee Intech http://dx.doi.org/10.5772/50569.

Shirani S., Mahdavi F. and Maziah M. 2009. Morphological abnormality among regenerated shoots of banana and plantain (Musa spp.) after in vitro multiplication with TDZ and BAP from excised shoot tips, African. J. Biotech. 8(21): 5755-5761.

Uzaribara E., V. Nachegowda H. Ansar B.N. Santhyanarayana and A. Taj. 2015. In vitro propagation of red banana (Musa acuminata).The Bioscan. 10(1): 125-129.

Workman D. October 31, 2015. World Top Exports: Bananas Exports by Country http://www.worlds-topexports.com/bananas-exports-country/336.