http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2019.201.205

Short Communication
Effect of Salinity Stress on Shoot *Musa acuminata* L. Barangan Cultivar *in vitro* Culture

[1,2]Dikayani, [1]Anas, [1]Anne Nuraini and [1]Warid Ali Qosim

[1]Faculty of Agriculture, Padjadjaran Universitas, Jl. Raya Sumedang, Jatinangor, 45363 Sumedang, Bandung, Indonesia
[2]Faculty of Technology Sciences, State Islamic University Sunan Gunung Djati, Bandung, Indonesia

Abstract

Background and Objective: Quality banana fruit is significantly related with number and type of metabolite produce. Metabolite production of *Musa acuminata* L. is effected by abiotic factors such as salinity. So the purpose of this research was to study the effect of plant response under salinity stress and also identified the chemical compound especially phenolic compound. **Materials and Methods:** Explants used in this research were shoot of *Musa acuminata* L., Murashige and Skoog medium, Benzyl amino purine, sugar, agar, sodium chloride. The research was conducted in two stages; stage (1): Developing *in vitro* culture of shoot of *Musa acuminata* L. with NaCl treatment i.e., 0, 50, 100, 150 and 200 mM concentration, stage (2): Gas Chromatography Mass Spectrometry (GCMS) analysis on the shoot of *in vitro* banana plantlets. **Results:** The findings of this study showed that compound content of shoot of banana plantlets were Amine, Ester, Propane, Keton, Alkohol, Phenol, Methyl, Acid and it was observed that Ester (27.515%) was the highest detected compound. **Conclusion:** It is concluded that the salinity had affected the plant growth. The analysis showed that the highest compound content of *Musa acuminate* L. Barangan cultivar was Ester and Phenols.

Key words: Banana fruit, salinity stress, *Musa acuminata* cv barangan, metabolite production, shoot *in vitro*

Citation: Dikayani, Anas, Anne Nuraini and Warid Ali Qosim, 2019. Effect of salinity stress on shoot *Musa acuminata* L. barangan cultivar *in vitro* culture. Pak. J. Biol. Sci., 22: 201-205.

Corresponding Author: Warid Ali Qosim, Faculty of Agriculture Padjadjaran University, Jl. Raya Sumedang, Jatinangor, 45363 Sumedang, Bandung, Indonesia Tel: +628594446008

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

Musa acuminata L. is agriculture plant which is effected by environment such as salinity. It is the second important fruit crop and approximately 22% fresh fruit banana is produce, but the salinity can cause decrease even 50% in fruit[1-3]. The plant growth and development to banana plant is affected by salinity. Plant growth and development such as seed germination, seedling growth and vigour; vegetative growth, flowering and fruit set; photosynthesis, protein and lipid metabolism are adversely affected by high salt concentration that ultimately causing decreased of plant productivity[4,5].

Response of plant to salinity such as ionic homeostatis including various osmolite like polyols, proline, betaine, trehalose, ektoin and DMSC (dimethyl sulfonium compound)[1,6]. Modern biotechnical techniques to study genetic and molecular (DNA, RNA, protein and metabolites) were applied to investigate the plant regulation and development[7]. Salinity stress and cellular processes were used *in vitro* to study the biotechnology development within micropropagation. This technique was used in banana plant with shoot as plant tissue or explant[8,9]. Tissue culture with environment which controlled such as nutrition and light can obtain plantlet. Tissue culture technique required culture medium, plant growth regulation and sodium chloride in sterile condition. Previous study of salinity on root plasma membrane and its transport to shoot tissue, nutrient content in seedling organs significantly decreased as soil salinity increased in *Ziziphus mauritiana*. Treatment of NaCl was observed to be toxic on banana micropropagation[6,10].

Metabolic plant response to stress environment by physiological defense such as secondary metabolite production, toxic to insects and micro-organism. The other case found that salinity stress affected the different changes in composition of some aromatic, essential oil and nutrition[11]. Metabolism of plant may change due to salinity effect , primer metabolism, secondary metabolism and produce of chemical compound. The research aimed to investigate the effect of response of plant under salinity. Present study was also identified the chemical compound especially phenolic compound and the next process of lignin biosynthesis to protect against environment disruption such as salinity.

MATERIALS AND METHODS

Materials: The research used explants of the shoots of banana plantlet (Fig. 1) of PT Multi Agro Kultura Pamulang Tangerang. The basic media used Murashige and Skoogs (MS) with

Fig. 1: Shoot explant of *Musa acuminate* L. cv. Barangan *in vitro*

Benzylaminopurine (BAP) 1.5 ppm and NaCl as growth regulators, sugar 30 g, jelly 8 g and sodium chloride (NaCl).

Methods: The methods with two stages performed, which were: (1) Development of the shoot culture of banana plantlet with the treatment of concentrations of NaCl 0, 50, 100, 150 and 200 mM (2) Analysis of active compounds used Gas Chromatography Mass Spectrometry (GCMS) in the roots of banana plantlet with the NaCl concentrations i.e., 0 and 100 mM. This study was conducted from January, 2013 until June, 2013 in laboratory Molecular Cell Biology School of Life Science and Technology, ITB, Indonesia.

Procedure for the making of *in vitro* banana culture: For initiation stage the explants of banana shoots were cultured *in vitro* in the Murashige and Skoog (MS) media of 4.4 g L^{-1}, sucrose 30 g, gel 8 g and an addition of growth regulator Benzylaminopurine (BAP) at a concentration of 1.5 ppm. The NaCl with the concentration of 0 and 100 mM added into the treatment media. *In vitro* culture is made in a sterile state, the medium sterilized using an autoclave.

GC-MS analysis:

Extraction and determination of chemicals compounds: Extraction of shoot was performed on 1 g sample of explant with the NaCl concentrations 0, 50, 100, 150 and 200 mM and then made finer by an addition of methanol. The result of the extraction was incubated in an ultrasonic bath for 1 h and then filtered using filter paper[12].

Gas chromatography mass spectrometry analysis: Early step the extract was injected into the GCMS system of Fisons GC 8000, GC model 8060 paired with MS Fisons MD 800 in EI (Electron Impact) with an energy electron of 70 eV and masses ranging from 25-700 MLZ. The capillarity column was that of low-bleed CP-Sil 8 CB-MS (30 m×0.32 mm, i.d) of 0.25 µm of film thickness. An injector was set at 280°C and a detector at 290°C and GC in splitless at 1 min. Temperature was set between 70-135°C with 2°C min^{-1} G1 for 10 min between 135-220°C with 4°C min G1 for 10 min between 220-270°C with 3.5°C min^{-1} G1 for 20 min. After a run of 10 min at 70°C for the next injection, the average speed was 1.9 mL min^{-1} G1. Identification of compounds was compared to the retention time with the right chemical compound and spectral data obtained from Wiley and NIST libraries. Each determination was duplicated[13].

RESULTS

Shoot *in vitro* culture: The results of multiple shoot development, growth of development and effect of different concentrations of NaCl on induction of different genotype were presented in Table 1.

The effect of different concentrations of NaCl 0, 50, 100, 150 and 200 mM on bud initiation and shoot multiplication was investigated. Multiple shoots have developed at different level of NaCl. Table 1 showed plant growth and development caused by different concentration of NaCl. Plant growth resulted that (a) Good growth and multiplication growth root. (b) The picture of plantlet growth showed that yellow leave, presence the number of multiple shoots and root didn't grow. (c) Shoot did not multiplication and root grown. (d) Shoot did not multiplication and browning on the root. (e) Browning in shoot and root, it was very bad condition of plantlet.

The result of GCMS with sodium chloride treatment 0 mM, the lowest was acid compound (0.09%) and the highest was Ester compound (27.515%) (Table 2).

The highest chemical compound treat 50, 100, 150 and 200 mM were Ester group 52.41, 32.68, 56.16 and 29.55%. Sodium chloride with treatment 0 mM (control) on shoot had chemical compound content such as Amine, Propane, Keton, Alcohol, Phenol, Methyl and Acid, while in treatment NaCl 100 Mm did not discover Alcohol and treat NaCl 150 mM didn't find Alcohol and Phenol. Analysis of GCMS result showed that due to salinity causing changed composition and quantity of compound chemical.

In treat NaCl 100 mM showed that the highest area for phenol compounds while 150 mM did not detect any Phenol compound and showed the lowest area (0.00%) (Table 3).

Table 1: Treatment of NaCl on plant growth *Musa acuminate* L. cv Barangan

Treatments	Explant
(a) MS	
(b) MS + NaCl 50 mM	
(c) MS+ NaCl 100 mM	
(d) MS+ NaCl 150 mM	
(e) MS+NaCl 200 mM	

DISCUSSION

In this study the micropropagation of banana has been achieved with shoot plant and the number of multiplication using shoot tip. The multiplication rates of banana shoot derived from sucker *in vitro* culture[14]. The effect of environment such as abiotic factor is caused by salinity to chemical compound content of *in vitro* shoot of plantlet *Musa acuminate* L. Barangan cultivar.

In GCMS analysis of each treatment (NaCl 0, 50, 100, 150 and 200 mM) particularly 83 chemicals compound were obtained and the highest compound was ester. Ester acid of *in vitro* shoot of banana plantlet was tridecanoic acid and hexadecanoic acid methyl ester[15]. Hexadecanoic acid was detected in various NaCl concentrations. Plant produce natural product in processes of primary and secondary metabolism with intermediate compound of primary carbon synthesis like phenylpropanoid, shikimate, mevalonate or MEP pathways[16]. Primary metabolism produced primary compound which are essential for plant growth such as lipid, protein, carbohydrates and secondary metabolism produced secondary metabolite like alkaloid, terpenoid and flavonoid. Plant metabolism was

Table 2: GC-MS *in vitro* shoot of banana plantlet with various concentration of NaCl

Chemical compounds	NaCl treatments				
	0 mM	50 mM	100 mM	150 mM	200 mM
Amine	1.14	0.63	1.15	0.44	0.61
Ester	27.51	52.41	32.68	56.16	29.55
Propane	0.42	0.34	0.29	0.07	1.18
Ketone	0.12	0.55	0.14	1.41	1.31
Alcohol	26.80	3.02	0.00	0.00	1.41
Phenol	1.84	4.10	12.47	0.00	0.09
Methyl	2.08	0.53	5.35	9.25	1.20
Acid	0.09	12.63	0.28	0.25	0.06

Table 3: Phenol compound on shoot of banana plantlet in various concentration of NaCl

Treatment shoots (mM)	Area (%)							
	HMDP	PCOTP	AMPP	PAP	PEA	BAH	BM	Total
0	0.00	1.84	0.07	0.00	0.00	0.00	0.00	1.90
50	0.00	4.10	0.06	0.00	0.00	0.00	0.00	4.16
100	0.00	12.20	0.09	0.27	0.00	0.00	0.00	12.56
150	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.09

HMDP: Hexahydropyridine, 1-methyl-4-[4,5 dihydroxyphenyl), PCOTP: Pyridine-3-carboxamide, oxime, N-(2-trifluoromethyl phenyl), AMPP: 2-Amino-1-(o-ethoxyphenyl)propane, PAP: 1-Propanone, 2-amino-1-phenyl, PEA: Phenyl ethyl alcohol, BAH: Benzyl alcohol, alpha-(1-aminoethyl)-m-hydroxy, BM: Benzenemethanol, alpha(1-aminoethyl))

changed due to salinity[17]. Secondary metabolite synthesis is caused by environment stress for protection against cellular and oxidative damage[18-20]. Salinity effect on plant growth and development of banana plant may disturb the balance of homeostatic of ionic and osmotic caused Na^+, beside that salinity can also effect the primary metabolism like carbohydrates, nutrition, water decrease and oxidative stress. In addition to this it can also change the level of chemical compound such as ester acid, glycerol within adaptation of salinity stress[21].

Responses of biochemical related with physiological adaptations, accumulation of soluble sugar, ester acid and proline under salt stress protect the cell by balancing the osmotic of cytosol, vacuole and external condition[5].

Phenolic compounds are most important in plant growth, it is the source of response to environmental factors such as light, pollution and saline as plant defence to disruption. Stress of abiotic have impact to antioxidant accumulation so that the plant can adapt to environment[22]. Phenols compound accumulated in vacuola that it stored in secondary cell wall as lignin[23]. Synthesized of phenol derived cinnamic acid that synthesized phenylalanine is catalyzed Phenylalanine Ammonia Lyase (PAL) that branch point between shikimate and phenylpropanoid pathway[24]. So in future this study could use for investigation of DNA and RNA molecular research.

CONCLUSION AND RECOMMENDATION

The salinity effected on plant growth and development *in vitro* shoot of plantlet *Musa acuminate* L. Barangan cultivar, it was caused homeostatic change in plant metabolism. Chemical compound group due to salinity was Amine, Ester, Propane, Keton, Alcohol, Phenol, Methyl and Acid. Composition compound in various NaCl concentration and the highest detected compound was Ester so the findings of this study recommended the further investigation of chemical compound content of shoot and root with other treatment and different analysis.

SIGNIFICANCE STATEMENT

This study discovered that obtain of metabolite of *Musa acuminata* cv. Barangan undersaline *in vitro*. The research can be beneficial for information source about banana plant and salinity. This study will help the researcher to find metabolites compound of *Musa acuminata* cv. Barangan *in vitro* under salinity. Thus a new theory that effect of salinity on *Musa acuminata* cv. Barangan *in vitro*, the plant can survive in salinity.

ACKNOWLEDGMENTS

The authors are thankful to Directorate of Highest Education Republic of Indonesia for providing financial

support (Grant Number BPPS/2013) and School of Life Science and Technology, ITB for Laboratory facilities. We are also thankful to Padjadjaran University, State Islamic University Sunan Gunung Djati, Bandung, Indonesia.

REFERENCES

1. Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol., 51: 463-499.
2. Tuteja, N., 2009. Cold, Salinity and Drought Stress. In: Plant Stress Biology: From Genomics to Systems Biology, Hirt, H. (Ed.)., Springer, Germany, pp: 137-159.
3. Bhosale, U.P., S.V. Dubhashi, N.S. Mali and H.P. Rathod, 2011. *In vitro* shoot multiplication in different species of banana. Asian J. Plant Sci. Res., 1: 23-27.
4. Rahdari, P. and S.M. Hoseini, 2011. Salinity stress: A review. Tech. J. Eng. Applied Sci., 1: 63-66.
5. Radi, A.A., F.A. Farghaly and A.M. Hamada, 2013. Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. J. Biol. Earth Sci., 3: B72-B88.
6. Bhatt, M.J., A.D. Patel, P.M. Bhatti and A.N. Pandey, 2008. Effect of soil salinity on growth, water status and nutrient accumulation in seedlings of *Ziziphus mauritiana* (Rhamnaceae). J. Fruit Ornamental Plant Res., 16: 383-401.
7. Lin, H., J. Rao, J. Shi, C. Hu and F. Cheng *et al*., 2014. Seed metabolomic study reveals significant metabolite variations and correlations among different soybean cultivars. J. Integrat. Plant Biol., 56: 826-836.
8. Perez, E.A. and C.R. Hooks, 2008. Preparing Tissue-Cultured Banana Plantlets for Field Planting. University of Hawaii, Hawaii, pp: 3.
9. Singh, H., S. Uma, R. Selvarajan and J. Karihaloo, 2011. Micropropagation for production of quality banana planting material in Asia-Pacific. Proceedings of the Asia-Pacific Consortium on Agricultural Biotechnology, May 19, 2011, New Delhi.
10. Haq, I., M. Fatima, H. Shaikh, M.U. Dahot and M.T. Rajput *et al*., 2011. Characteristics of micro-propagated banana (*Musa* spp.) cultures stressed with NaCl and polyethylene glycol. Afr. J. Biotechnol., 10: 4387-4391.
11. Badawy, E.M., E.E. Aziz, H.A.H. Ahmed and H. Fouad, 2016. Influence of selenium and boron on oil production and fatty acids composition of canola (*Brassica napus* L.) plant irrigated with saline water. Int. J. Pharmtech. Res., 9: 420-431.
12. Babu, M.A., M.A. Suriyakala and K.M. Gothandam, 2012. Varietal impact on phytochemical contents and antioxidant properties of *Musa acuminata* (Banana). J. Pharmaceut. Sci. Res., 4: 1950-1955.
13. Proestos, C., D. Sereli and M. Komaitis, 2006. Determination of phenolic compounds in aromatic plants by RP-HPLC and GC-MS. Food Chem., 95: 44-52.
14. Muhammad, A., I. Hussain, S. Navqvi and H. Rashid, 2004. Banana plantlet production through tissue culture. Pak. J. Bot., 36: 617-620.
15. Zhao, S., P.A. Tuan, X. Li, Y.B. Kim and H.R. Kim *et al*., 2013. Identification of phenylpropanoid biosynthetic genes and phenylpropanoid accumulation by transcriptome analysis of *Lycium chinense*. BMC Genomics, Vol. 14. 10.1186/1471-2164-14-802.
16. Nascimento, N.C. and A.G. Fett-Neto, 2010. Plant secondary metabolism and challenges in modifying its operation: An overview. Indian J. Biotechnol., 643: 1-3.
17. Chalker-Scott, L., 1999. Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol., 70: 1-9.
18. Winkel-Shirley, B., 2002. Biosynthesis of flavonoids and effects of stress. Curr. Opin. Plant Biol., 5: 218-233.
19. Sairam, R.K. and A. Tyagi, 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci., 86: 407-421.
20. Close, D.C. and C. McArthur, 2002. Rethinking the role of many plant phenolics-protection from photodamage not herbivores? Okios., 99: 166-172.
21. Zhang, H.X. and E. Blumwald, 2001. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nat. Biotechnol., 19: 765-768.
22. Ghasemzadeh, A., H.Z.E. Jaafar and E. Karimi, 2012. Involvement of salicylic acid on antioxidant and anticancer properties, anthocyanin production and chalcone synthase activity in ginger (*Zingiber officinale* Roscoe) varieties. Int. J. Mol. Sci., 13: 14828-14844.
23. Kefeli, V.I., M.V. Kalevitch and B. Borsari, 2003. Phenolic cycle in plants and environment. J. Cell Mol. Biol., 2: 13-18.
24. Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish J. Environ. Stud., 15: 523-530.