Biosaintifika 8 (2) (2016) 135-140

Biosaintifika
Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Growth Pattern and Copper Accumulation in Callus of *Datura metel*

✉**Yulita Nurchayati[1,5], Santosa[2], Laurentius Hartanto Nugroho[3], Ari Indrianto[4]**

DOI: 10.15294/biosaintifika.v8i2.5177

[1]Postgraduate Program, Faculty of Biology, Universitas Gadjah Mada, Indonesia
[2]Plant Physiology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia
[3]Plant Structure and Development Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia
[4]Biotechnology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia
[5]Plant Structure and Function Laboratory, Faculty of Science and Mathematic, Universitas Diponegoro, Indonesia

History Article

Received 25 February 2016
Approved 15 June 2016
Published 18 September 2016

Keywords:

Datura metel L. callus culture; Copper; Bioaccumulation; Phytoremediation

Abstract

This experiment was aimed to evaluate the copper accumulation using callus culture of *Datura metel* L. The culture was established from leaves onto MS contained NAA 2.5 mg/L and Kinetin 0.5 mg/L as the control. The exposure of the culture was carried out by 2 copper compounds as treatment, i.e. $CuCl_2.2H_2O$ and $Na_2CuEDTA$ at level concentration 0.; 0.1; 5; 10; 15; and 20 µM. The growth pattern of callus in control showed increasing growth rate in 36 days, whereas exponential stage was reached at 12-20th doi*. Whilst, after 10 doi, the treatment showed constant growth pattern. The absorption rate of the culture was increased by the addition of the $CuCl_2.2H_2O$ at 5-15 µM of level concentration but declined at 20µM. The maximum rate of accumulation of Cu (0,1519 mg g^{-1}) was obtained at 15 µM. Instead, the addition of $Na_2CuEDTA$ at 5-20µM of level concentration showed the significant increment while the maximum accumulation was obtained at 20µM (0,1420 mg g^{-1}). The existence of chelator in copper compound reduced the rate of toxicity while all tolerance index values were between 66,24 and 97,28 %.The results suggested the role of callus of *D. metel* as that fairly absorbed and accumulated Cu^{2+}. Exposure with $CuCl_2.2H_2O$ indicated higher accumulation than $Na_2CuEDTA$.

How to Cite

Nurchayati, Y., Santosa, S., Nugroho, L. H., & Indrianto, A. (2016). Growth Pattern and Copper Accumulation in Callus of *Datura metel*. *Biosaintifika: Journal of Biology & Biology Education*, 8(2), 135-140.

✉ Correspondence Author:
Bulaksumur, Yogyakarta 55281
E-mail: ariindri@ugm.ac.id

p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Heavy metals (HMs) are natural components that in many ecosystems the concentration has reached toxic level due to anthropogenic activities (Kanoun-Boule et al., 2008; Sharma, 2011). Several plants species have been investigated for their capability to reduce HMs toxicity. Member of Brassicaceae (Quartacci et al., 2003, Sharma & Dietz, 2006), *Zea mays*, (Ali et al., 2002), and aquatic plant i.e. water hyacinth (*Eichhornia crassipes)* or duckweed *(Lemna minor* L.*)* showed their role as phytoremediator towards Zn and Pb (Prassad & Freitas, 2003). A microalga *Chlamydomonas* sp. was reported as soil and water remediator that contaminated by Arsenic (As) with accumulation rate more than 2 g As kg-1 DW. *Datura metel* is a herb that widely distributed in the tropical region, with many roles in pharmaceutical field as the antibacterial agent and potential to be exploited as the local anaesthetic (Bruce, 1999; Facchini, 2001. This plant has not been reported yet as a tolerant species for copper or another HMs. Copper is one of heavy metal and has a significant role in the plant for the normal growth and development (Hall, 2002; Yruela, 2005). The copper is an important component of soil micronutrient for growth enhancement. This metal ion is also the essential factor for metalloprotein function (Prassad, 2004). Cu^{2+} is also required as the cofactor of the enzymes involved in secondary metabolism (Yruela, 2005. Instead of many roles of copper for plants, the high concentration of this essential metal can be harmful to growth and shows toxicity symptoms (Marschner, 1995; Hall, 2002; Prassad, 2004).

The adaptive responses of plants towards HMs-contaminated environments are efficient processes affected by physiological, ecological, and genetic traits. The application of plants to remediate environmental pollution is called phytoremediation to remove, transform and/ or stabilize HMs. This method is relatively cheap and easy to be applied (Pillon-Smits, 2005). To obtain genetic traits of new species with copper tolerance, *Datura metel* is planted by tissue culture systems. *In vitro* culture is a modern tool in plant science, as plant tissue or isolated cells can be propagated under controlled condition (pH, humidity, light etc.) and exposed to defined concentration of stress. Many researches showed that plant regeneration by tissue culture and application of hormones in this method can induce somaclonal variation. The somaclonal variation with its qualitatively character indicated a chance to obtain a more tolerant-plant (Rahayu & Sudarsono, 2009), include to heavy metal tolerance. In this study, the callus formed by *in vitro* culture was indicated the capability of absorbing Cu^{2+} from the medium.

METHODS

Plant Material

The seeds of *D. metel* as plant material were collected from Central Java Indonesia in 2012 and were identified as the specimen in Departement of Biology, Faculty of Natural Science and Mathematics, Universitas Diponegoro, Semarang, Indonesia. The seeds were germinated in pot after soaking with tap water for 3 days. The seedlings were merged after 2 weeks of germination in the greenhouse, and it showed a good growth after 2 months, with 6 – 8 leaves. The third leaves from the apical shoot were used as an explant. The surface of leaves was sterilized in 30% of sodium hypochlorite for 5 min and then 70% ethanol for 30 sec. The explants were then washed with sterile distilled water for three times.

Callus Induction and Growth Pattern

The sterile leaves were cut into the small size and were cultured on the Murashige & Skoog (MS) as the basal medium with the addition of 3% (w/v) sucrose and it was solidified with 0.2% (w/v) gellan gum. This basal medium also was added to 2.5 mg/L NAA and 0.5 mg/L Kinetin as initiation medium. The pH was adjusted to 5.8, and then it was sterilized with the autoclave (121°C, at 1.5 atm for 15 min). Callus cultures were incubated in a growth chamber at 25 °C temperature and 16 h photoperiod of tube lamp. The cultures were subcultured by transferring callus into fresh medium each 12 days. At the end of the third subculture (after 36 days), the callus were harvested as biomass and then was measured to obtain the growth curve.

One gram of callus from 36-days-old was transferred into fresh MS medium supplemented by NAA 2.5 mg/L and Kinetin 0.5 mg/L . Then, the callus was harvested each 4 days until 36 days. The fresh callus was cleaned from agar residue by filter paper, then were dried by the oven at 60°C. This dry weight of callus represented the biomass.

Callus Treatment

The step of callus bioassay was initiated by transferring callus into MS medium supplemented by the hormones with the different combination of concentration levels and copper compound i.e. $CuCl_2.2H_2O$ and $Na_2CuEDTA$ (0, 0.1,

5, 10, 15, and 20 μM). The pH of the medium was adjusted to 5,8.

Sample Harvesting

Ten-days-old calluses in all treatment were harvested separately. The fresh calluses were cleaned from agar residue by filter paper, then were dried by the oven at 60 °C and subsequently be stored for further analysis.

Determination of Cu Content

Dried-callus of *D. metel* were digested using 4:1 of HNO_3/HCl at 150 °C until the digest solution became clear in color. The digested residue was filled up with distilled water up to 10 mL. The solution samples were analyzed for Cu content by atomic absorbance spectroscopy (AAS-Annalitik Jenna USA).

Tolerance Index

Dried-biomass measurement was used to determine tolerance index (TI) following the formula:

The ratio between a measured variable in treated plants and measured variable in control plants expressed as a percentage, as Equation(1), considering biomass dry weight, already defined:

$$TI = \frac{\text{Biomass with Cu}}{\text{Biomass without HMs}} \times 100\%$$

Statistical Analysis

The experiments were carried out in five replication. The mean values with standard deviation (SD) were shown in the table and figures in the next section. The results were derived from statistical analysis using analysis of variance (ANOVA). Levels of significance were indicated by Duncan multiple range test at $P<0.05$. The coefficients of correlation were expressed using r-values.

RESULTS AND DISCUSSION

Callus Growth in MS Medium

The observation of growth pattern was carried out for 36 days. The callus was harvested each 4 days until the growth pattern showed the deceleration stage. The growth of the callus increased significantly with the exponential stage were gained between 12^{th}-20^{th} days of incubation. Furthermore, the growth was declined and was reached the stationary stage (Fig. 1).

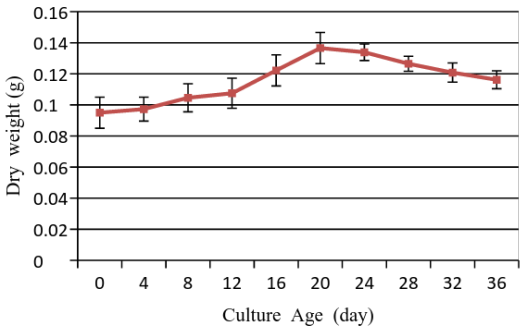

Figure 1. Growth curve of *D. metel* callus in MS basal medium supplemented with 2,5 mg/L NAA and 0.5 mg/L Kinetin. Data showed means ± deviation standard of five replication of measurements

Leaves are commonly more convenient to be induced into callus *in vitro* by application of growth regulator than another plant organ. Leaves cutting were more easily to be callus in *Andrographis paniculata* (Habibah, 2009), and in *Hibiscus sabdariffa* than the petiols or *calyx* (Noviati, et al., 2014). This phenomenon was caused by the thick structure of leaves and less complexity of anatomical structure compared to either stem or root. This dedifferentiation of Datura leaves occurred on MS basal medium added by NAA 2.5 mg/L and Kinetin 0.5 mg/L. All parts of the explant enlarged and wrinkled in 7 days after initiation. Primary callus was emerged from each side of cuttings, followed by the middle part of explant after 10 days of incubation. Callus proliferation occurred after the callus was subcultured into fresh medium. Some endogenous factor encouraged callus formation. *Datura* leaves are herbaceous in structure, arranged with parenchymatous mesophyll and trichomes-rich on adaxial surface. Besides, the bicolateral vascular bundle of the stele of Datura was rich of cambium to stimulate cells division. Many plant tissue culture were showed the positive response to callus induction by 2,4 dichloro phenoxy acetic acid (2,4 D) as auxin source i.e. callus of *Tridax procumbens* (Wani, et al., 2010) or *Jatropha curcas* (Antonio, et al., 2014). Some other species, *Hibiscus sabdariffa* (Nurchayati & Rahmah, 2010; Noviati et al., 2014); and *Catharanthus roseus* responded to NAA instead of 2,4 D (Darsini, 2011) to induce callogenesis. It suggested that effect of hormones was planted species-dependent. Morphologically, the callus was friable and white-yellowish in color (Fig 2A). This character was suitable to be prepared as starting material to further step, potential examination in Cu^{2+} accumulation.

Copper Accumulation in Callus

Accumulation of copper ion was observed in *D. metel* callus, on both different kinds of copper compounds. The callus showed capability in absorbing copper ion and the ion then be stored in their vacuoles. Based on Fig. 3, the callus showed the high activity of Cu^{2+} absorption at 15 µM of level concentration, followed by lowering its activity at 20 µM significantly. The cells allowed this Cu^{2+} to pass across the plasma membrane and occupy its capacity without any leakage. Further, the highest concentration Cu^{2+} caused membrane disorder and the cells were lost its capability to accumulate the ion. This level indicated the critical point of callus to accumulate the ion.

The different result was observed in treatment by second Cu species, an organic copper. Absorption of $Na_2CuEDTA$ into cells of callus showed the increasing pattern (Fig 3.). The callus showed the increasing activity even in the highest level of Cu. In 20 µM of $Na_2CuEDTA$ concentration, yet the Cu absorption was observed 0,1266 mg g^{-1} higher than the control equal to 822,08%. It was suggested that copper was absorbed in chelating form (CuEDTA). Copper was bound by EDTA and then would neutralize the charge of the molecule. By chelating form, this copper may pass through into cells. This evidence became the main reason that the application of highest level of copper will also correlate to the increasing of copper accumulation. Several research-

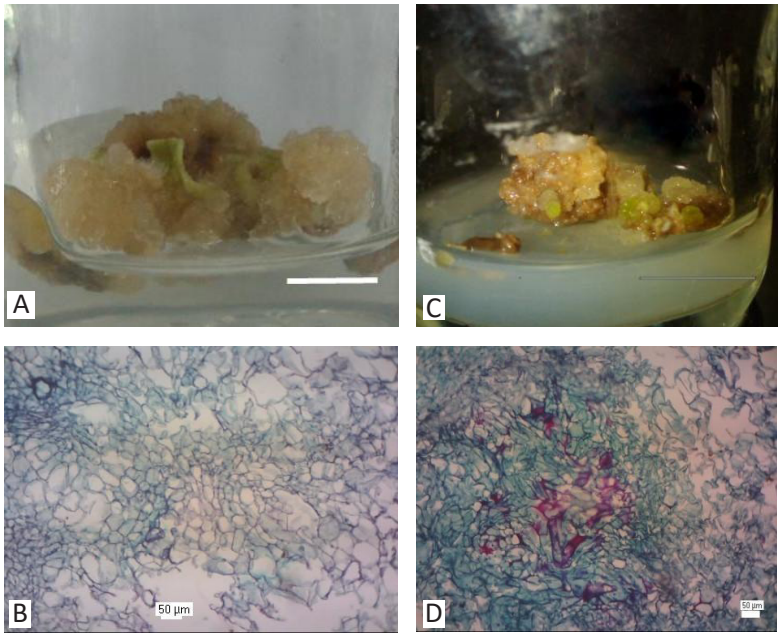

Figure 2. Callus of *D. metel* in (A-B) MS+NAA+Kinand in (C-D) MS+NAA+Kin+Cu10µM bar (A,C) = 1 cm

Table 1. Biomass (g) and tolerance index (%) of callus after 10-days treatment by different type of copper compound and different level of concentration

Treatment	Biomass (mg)		Tolerance Index (%)	
Cu (µM)	$CuCl_2.2H_2O$	$Na_2CuEDTA$	$CuCl_2.2H_2O$	$Na_2CuEDTA$
0.0	1.252^{cd}	1.252^{cd}	87.70	84.30
0.1	1.424^{a}	1.485^{a}	100.00	100.00
5.0	1.338^{b}	1.218^{d}	94.00	97.28
10.0	1.283^{c}	1.162^{de}	86.70	75.08
15.0	1.232^{d}	1.088^{e}	86.38	73.00
20.0	1.078^{e}	0.999^{f}	75.70	66.26

Note: Means followed by the same letter are not significantly different at a probability as determined by Duncan's Multiple Range Test (P=0.03)

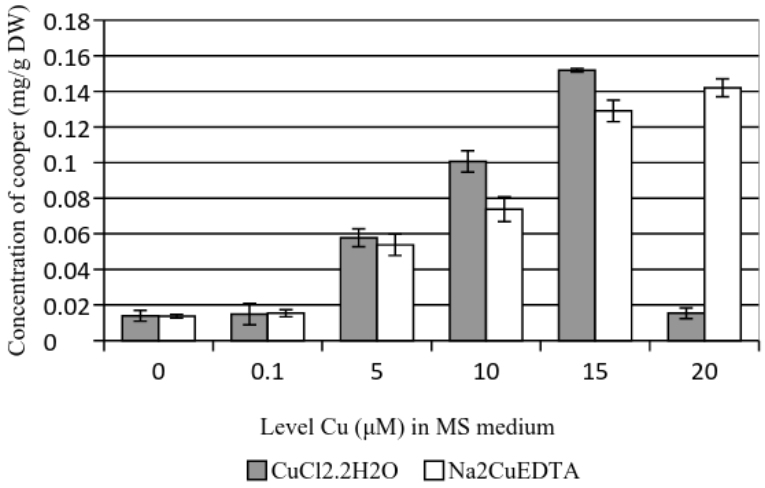

Figure 3. Accumulation of Cu^{2+} in *D. metel* callus by two different types of copper compound in MS medium supplemented by NAA and Kinetin.

ers reported that chelation reduced heavy metals toxicity (Hall, 2002; Prassad, 2004; Sharma & Dietz, 2006). Examination of chelation form was applied to a metallophyte *Thlaspi caerulescens* with ZnEDTA compared with $ZnSO_4$. This hyperaccumulator plant tolerated and accumulated $ZnSO_4$ under stress condition. Application of ZnEDTA decreased Zn uptake on both agar or liquid medium (Gerstmann et al., 2010).

The capability of Datura callus as Cu^{2+} bioaccumulator suggested that the cells contained phytochelatin, such protein analog with metallothionein. This protein was composed by glutamate and cysteine, that bound HMs ion and store them in vacuoles. Therefore, it reduces HMs toxicity as natural mechanisms occurred in the cell (Cobbett, 2000). This phytochelatin was also detected in *Rauwolfia serpentina* cell culture, which acts as marker towards Zn^{2+} and Cu^{2+} toxicity (Grill, et al., 1997). The culture showed Cu^{2+} absorption into cells (served in $CuCl_2.2H_2O$) until 15 µM of level concentration. The biomass tended to decrease in correlation with the increment of the copper level. Moreover, this callus had no capability to uptake this ion at 20 µM and this indicated the toxicity level. It suggested that this ion may cause growth inhibition. Hyperaccumulator plants will show the satisfied growth pattern even though they were exposed under stress condition (Sharma, 2011). According to Prassad (2004), tolerant plants species can adapt to the high level of Cu by changing the ion form to non-ionic protein complexes. Probably, metabolic changes may occur to permit enzymes to act normally in a high concentration of metal, e.g. cell wall-bound phosphatase in the roots area. Moreover, the high level of Cu^{2+} was toxic for the non-accumulator plant, include *D. metel*, so that the biomass showed the lower mass than control (Yruela, 2005). The cellular copper toxicity was affecting the binding of sulfhydryl protein and inhibits enzyme function (Marschner, 1995). In this case, callus culture of Datura had no copper translocation mechanism into other parts, because of uniform-tissue-system. Nevertheless, the callus had the capability to uptake and accumulate them into their cells.

Based on Fig. 2C-D, callus section at $CuCl_2.2H_2O$ 10 µM or 100-fold from normal level (0,1 µM) showed the thickness of cell wall. Secondary cell wall composed of lignin has become the early response to recognizing the contaminated–environment. This parts indicated first response or defense stage to toxic materials existed surrounding the cell.

CONCLUSION

The results concluded that the callus culture of *D. metel* showed excellent growth in MS supplemented by NAA and Kinetin. This culture system showed the capability to absorb and accumulate both species of copper. All treatments were significantly increased the Cu accumulation compared with the control, except for the 20µM of $CuCl_2$ treatments. Examination of Cu^{2+} species ($CuCl_2.2H_2O$ form) showed more convenient to be absorbed than chelating form though it showed inhibition of callus growth. The results indicated the concentration-dependent characteristics ($r = 0.951$) for the second compound of copper.

In further studies, this callus culture system would be regenerated into plantlets system and subsequently be used as a model to explore

the mechanism for tolerance and accumulation of copper. Moreover, this *in vitro*-generated callus can be exploited for secondary metabolites observation.

ACKNOWLEDGEMENT

The author would like to thank the Directorate General of Higher Education of the Republic Indonesia for its BPPDN Scholarship of the doctoral programme for 2011-2014.

REFERENCES

Ali, N. A., Bernal, M. P., & Ater, M. (2002). Tolerance and bioaccumulation of copper in Phragmites australis and Zea mays. *Plant and Soil*, 239(1), 103-111.

Antonio, A. B., Álvarez, L., Buendía-González, L., Maldonado-Magaña, A., & Cruz-Sosa, F. (2015). Accumulation and tolerance of Cr and Pb using a cell suspension culture system of Jatropha curcas. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 120(1), 221-228.

Bruce, N. C. (1999). *Alkaloid*. Cambridge,UK. www.wiley-vch.de/books/pdf/v08a_alk.pdf

Cobbett, C. S. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology*, 123(3), 825-832.

Darsini, N. N. (2011). The Development of Laticifer on Callus Culture Catharanthus Roseus (L) G Don which were Induced by Plant Growth Regulator Combination Kinetin+ NAA. *Journal Biology*, 15, 34-38.

Facchini, P. J. (2001). Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation, and metabolic engineering applications. *Annual Review of Plant Biology*, 52(1), 29-66.

Gerstmann, S., Adlassnig, W., Puschenreiter, M., Steinhauser, G., & Lichtscheid, I. (2010). *In vitro* Culture of Metallophyte *Thlaspi caerulescens* : culture media and Application of Toxic Heavy Metals. Poster in Universitat Wien.

Grill, E., Winnacker, E. L., & Zenk, M. H. (1987). Phytochelatins, a class of heavy-metal-binding peptides from plants, are functionally analogous to metallothioneins. *Proceedings of the National Academy of Sciences*, 84(2), 439-443.

Habibah, N. (2009). Effectiveness of Jasmonic Acid Elicitor Addition for Andrographolide Synthesis Induction of Sambiloto Culture. *Biosaintifika: Journal of Biology & Biology Education,* 1(1), 11-18.

Hall, J. L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany*, 53(366), 1-11.

Kanoun-Boulé, M., De Albuquerque, M. B., Nabais, C., & Freitas, H. (2008). Copper as an environmental contaminant: phytotoxicity and human health implications. *Trace Elements as Contaminants and Nutrients: Consequences in Ecosystems and Human Health*, 653-678.

Marschner, H. (1995). *Mineral Nutrition of Higher Plants, second edition*. London: Academic Press.

Nurchayati, Y. & Rahmah, F. A. (2010). Ascorbic acid Content on *Hibiscus sabdariffa* L. Callus at various Concentration of Sucrose in MS media. *Majalah Obat Tradisional,* 15(2), 71-74.

Noviati, A., Nurchayati, Y., & Setiari, N. (2015). Respon Pertumbuhan dan Produksi Senyawa Antioksidan pada Kalus Hibiscus sabdariffa L. dari Eksplan yang Berbeda secara in vitro. *Jurnal Sains dan Matematika*, 22(1), 25-29.

Pilon-Smits, E. (2005). Phytoremediation. *Annual Review of Plant Biology*, 56, 15-39.

Prasad, M. N. V., & Freitas, H. D. O. (2003). Metal hyperaccumulation in plants—biodiversity prospecting forphytoremediation technology. *Electronic Journal of Biotechnology*, 6(3), 110-146.

Quartacci, M. F., Cosi, E., Meneguzzo, S., Sgherri, C., & Navari-Izzo, F. (2003). Uptake and translocation of copper in Brassicaceae. *Journal of Plant Nutrition*, 26(5), 1065-1083.

Rahayu, S. E., & Sudarso. (2009). Qualitative Variants of Peanut Plants Obtained from in-Vitro Culture and in-Vitro Selection. *Biosaintifika: Journal of Biology & Biology Education*, 1(1), 33-40.

Sharma, H. (2011). Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. *Journal of Environmental Science and Technology*, 4(2), 118-138.

Sharma, S. S., & Dietz, K. J. (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany*, 57(4), 711-726.

Sheldon, A. R., & Menzies, N. W. (2005). The effect of copper toxicity on the growth and root morphology of Rhodes grass (Chloris gayana Knuth.) in resin buffered solution culture. *Plant and Soil*, 278(1-2), 341-349.

Wani, M., Pande, S., & More, N. (2010). Callus induction studies in Tridax procumbens L. *International Journal of Biotechnology Applications*, 2(1), 11-14.

Xu, X. Y., Shi, G. X., Wang, J., & Kang, Y. N. (2011). Copper-induced oxidative stress in Alternanthera philoxeroides callus. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 106(2), 243-251.

Yruela, I. (2005). Copper in plants. *Brazilian Journal of Plant Physiology*, 17(1), 145-156.