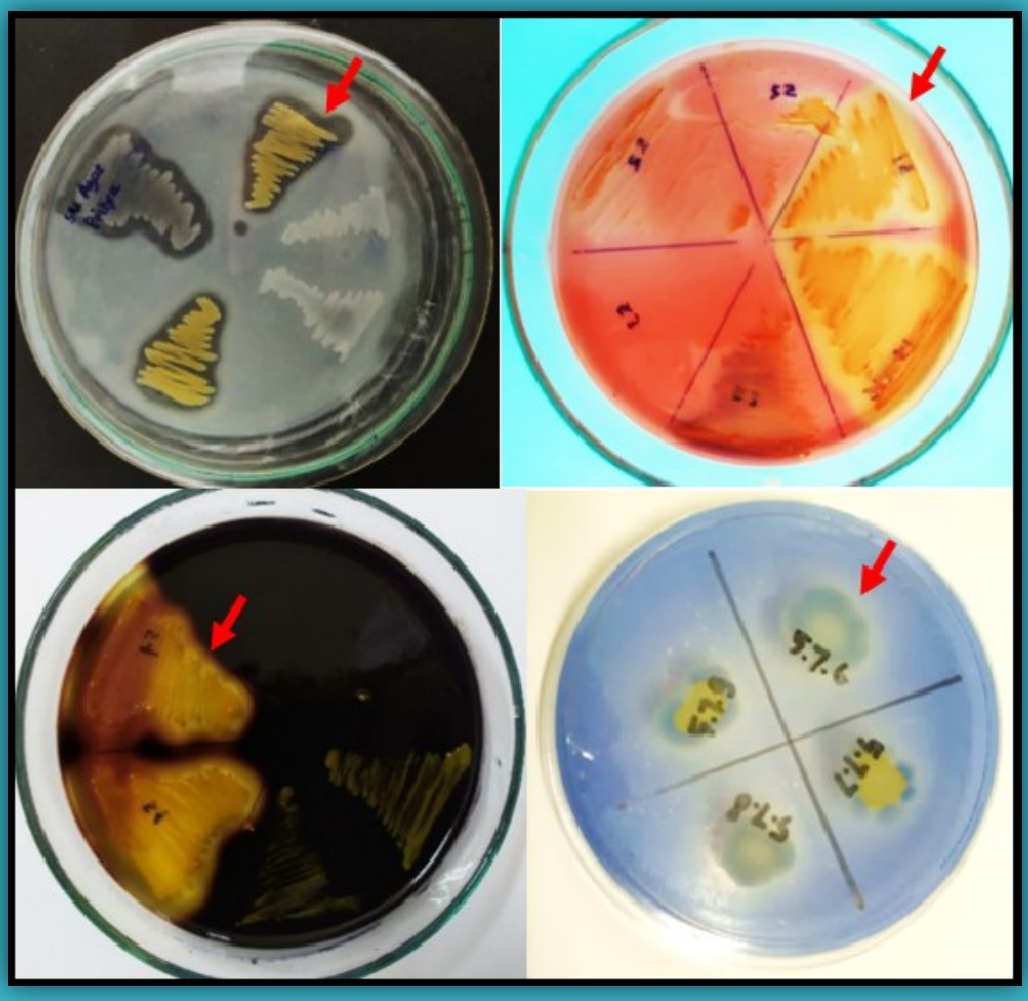
P-ISSN 0126-1754
E-ISSN 2337-8751
Terakreditasi Peringkat 2
21/E/KPT/2018
Volume 19 Nomor 2, Agustus 2020

Berita Biologi

Jurnal Ilmu-ilmu Hayati



Berita Biologi	Vol. 19	No. 2	Hlm. 127– 244	Bogor, Agustus 2020	ISSN 0126-1754

Pusat Penelitian Biologi - LIPI

BERITA BIOLOGI

Vol. 19 No. 2 Agustus 2020
Terakreditasi Berdasarkan Keputusan Direktur Jendral Penguatan Riset dan Pengembangan, Kemenristekdikti RI
No. 21/E/KPT/2018

Alamat (*Address*)
Pusat Penelitian Biologi-LIPI
Kompleks Cibinong Science Center (CSC-LIPI)
Jalan Raya Jakarta-Bogor KM 46,
Cibinong 16911, Bogor-Indonesia
Telepon (021) 8765066 - 8765067
Faksimili (021) 8765059
Email: berita.biologi@mail.lipi.go.id
jurnalberitabiologi@yahoo.co.id
jurnalberitabiologi@gmail.com

Keterangan foto cover depan: Seleksi bakteri pada media selektif, sesuai dengan halaman 151
(*Notes of cover picture*): (*Bacterial selection on selective medium, as in page 151)*

P-ISSN 0126-1754
E-ISSN 2337-8751
Terakreditasi Peringkat 2
21/E/KPT/2018
Volume 19 Nomor 2, Agustus 2020

Berita Biologi

Jurnal Ilmu-ilmu Hayati

Berita Biologi	Vol. 19	No. 2	Hlm. 127 – 230	Bogor, Agustus 2020	ISSN 0126-1754

Pusat Penelitian Biologi - LIPI

Ucapan terima kasih kepada
Mitra Bebestari nomor ini
19(2) – Agustus 2020

Dr. Haryono, M.Si.
(Ekologi dan Budidya ikan, Pusat Penelitian Biologi-LIPI)

Dr. Nisa Rachmania Mubarik
Mikrobiologi, Departemen Biologi, FMIPA, IPB

Tri Haryoko, S.Pt., M.Si.
(Pusat Penelitian Biologi-LIPI)

Ir. Eka Sugiyarta, MS.
(Genetika dan Pemuliaan, Pusat Penelitian Perkebunan Gula Indonesia)

Indra Bachtiar, Ph.D.
(Stem Cell & Cancer Institute, Kalbe Farma Tbk.)

Eka Fatmawati Tihurua S.Si., M.Si.
(Anatomi/Histologi Tumbuhan, Pusat Penelitian Biologi-LIPI)

Dr. Djunijanti Peggie
(Sistematika dan konservasi kupu-kupu, Pusat Penelitian Biologi-LIPI)

Kartika Dyah Palupi S. Farm.
(Fitokimia, Pusat Penelitian Kimia-LIPI)

Dr. Yuzammi
Taksonomi Tumbuhan, PKT Kebun Raya Bogor, LIPI

Dr. Nurainas
(Taksonomi Tumbuhan, FMIPA-Universitas Andalas)

Aninda Retno Utami Wibowo, S.Si.
(Taksonomi Tumbuhan, BKT Kebun Raya “Eka Karya” Bali – LIPI)

Dr. Laode Alhamd
(Ekologi Tumbuhan, Pusat Penelitian Biologi-LIPI)

Dr. Ir. Praptiwi, M.Agr.
(Fitokimia, Pusat Penelitian Kimia– LIPI)

Dr. Sc. Agr. Agung Karuniawan, Ir., Msc. Agr.
(Pemuliaan Tanaman, Fakultas Pertanian, Universitas Padjadjaran)

Dr. Sudarmadi Purnama
(Pemuliaan dan Genetika Tanaman, Balai Pengkajian Teknologi Pertanian Jawa Timur)

Berita Biologi
LIPI
Jurnal Ilmu-ilmu Hayati

DOI: 10.14203/beritabiologi.v19i2.3851
P-ISSN 0126-1754
E-ISSN 2337-8751

CHROMOSOME COUNT ON YOUNG ANTHER OF BANANA MALE BUD USING EZYMATIC MACERATION AND DAPI STAINING IN SLIDE PREPARATION

[Penghitungan Jumlah Kromosom Pisang dari Jaringan Anther Muda Menggunakan Metode Maserasi Enzimatik dan Pewarnaan DAPI Pada Persiapan Preparat Mikroskop]

Fajarudin Ahmad*✉ and Yuyu Suryasari Poerba

Botany Division, Research Center for Biology-LIPI
Jl. Raya Jakarta-Bogor Km. 46, Cibinong, Bogor 16911
email: kangfajar@yahoo.com

ABSTRACT

Chromosome counting is the basis in describing the chromosomes number of organism that might useful for genetic study and classification. In banana studies, the root tip with a combination of non-fluorescent staining methods such as carmine or orcein and squash is the most common material for chromosome counting. In this study, we presented the usefulness of young anther of banana male bud with enzymatic maceration method for cell spreading and 4,6-diamino-2-phenyl-indole (DAPI) for staining agent to get a satisfying chromosomes image at metaphase for mitotic study of diploid and tetraploid bananas. The principle of this study is fixation using ethanol:acetic acid (3:1), enzymatic digestion, maceration and staining using DAPI. Our result showed that this method can provide well spread cells with intensely contrast of chromosomes images that satisfying for chromosome counting.

Keywords: Diploid, Fluorescent, Metaphase, Mitotic, Staining, Tetraploid

ABSTRAK

Penghitungan kromosom merupakan dasar dari penentuan jumlah kromosom dari suatu organisme. Selanjutnya, hasil dari penghitungan kromosom ini bermanfaat untuk studi genetika dan klasifikasi. Dalam pengamatan kromosom pisang, kombinasi ujung akar dan metode *squash* dengan pewarnaan menggunakan senyawa *carmine* dan *orcein* merupakan metode yang paling umum digunakan. Dalam penelitian ini, kami memperlihatkan kegunaan anther muda dari jantung pisang yang dikombinasikan dengan metode maserasi secara enzimatik dan pewarnaan kromosom secara *fluorescent* menggunakan 4,6-diamino-2-phenyl-indole (DAPI) untuk mendapatkan citra kromosom yang tajam pada pengamatan kromosom saat metafase pembelahan mitosis dari pisang diploid dan tetraploid. Prinsip utama dalam metode yang digunakan dalam penelitian ini adalah fiksasi menggunakan ethanol:asam asetat (3:1), perlakuan enzimatik, maserasi dan pewarnaan menggunakan DAPI. Metode ini menghasilkan sel-sel dari anther dapat tersebar tanpa saling tumpang tindih dengan citra kromosom yang tajam, sehingga kromosom dapat diamati dengan mudah.

Kata kunci: Diploid, *Fluorescent,* Metafase, Mitosis, Pewarnaan, Tetraploid

INTRODUCTION

Cytogenetic study of banana in the centre of diversity, *i.e.* Indonesia, is still minimal and needs more explorations to understand the genetic and the diversity. In the exploration, as many as characters of samples need to be recorded and any possible samples need to be collected for further observation. Morphological characters can be recorded on the spot or stored as herbarium and photos. Ploidy confirmation only possible to be studied later in the laboratory using flowcytometry or chromosome counting. Flowcytometry is a convenience method in ploidy estimation by comparing the relative genome content of the sample and the reference that easily analysed from plant tissue, such as young leaf (Dolezel *et al*., 1997; Roux *et al*., 2003). However, chromosome counting is essential for the ploidy determination. Chromosome counting helps geneticist and taxonomist to get better understanding of the ploidy diversity and the classification of genus *Musa* (Cheesman, 1947; Argent, 1976).

The most common way in determining banana ploidy is by counting the chromosomes from root tip, which abundant of active growing cells (Bakry and Shepherd, 2014). This method requires health root tip that can be obtained from plants that grown under optimal condition (Bakry and Sheperd 2008). However, during an exploration, collecting young root tip and immediately chemical treatment, such as in 8-hydroxyquinoline treatment and fixation (Doležel *et al*., 1998; Bakry and Shepherd, 2014) is not practical, because researchers spent most of the time for collecting, morphological characterization and moving around in forest and villages. Another part of plant with abundant active cell division is shoot or apical meristem (Crang *et al*., 2018). Hence, this part is potentially useful for chromosome counting or karyotyping. Thus, in banana, young

*Kontributor Utama
*Diterima: 28 April 2020 - Diperbaiki: 3 Juni 2020 - Disetujui: 7 Juli 2020

anther in the male bud could be an alternative material that not necessarily on the spot chemical treatment, because the young anther protected by bract layers, so the fixation of the young anther can be done later.

An important stage in cytogenetic study is cell spreading on the slide, in order to get a well spread cells to avoid overlapping cells. Squash method is typically practiced by researchers to spread the cells (Bakry and Shepherd, 2014; Osuji *et al*., 2006, 1996). Some other researchers used a mixture of enzymes, *i.e.* pectolyase, cellulose, and cytohelicase, to digest the cell wall and followed by a maceration method to disperse the cells (Kantama *et al*., 2017; Adeleke *et al*., 2002). The enzymatic method is offer more complexity, but provide a better cell spreading.

The classical staining method for chromosome counting is done by using orcein (Osuji *et al*., 2006, 1996; Bakry and Shepherd, 2014) or aceto-carmine (Rekha and Hiremath, 2008). Alternatively, involving fluorescent agent as staining substitution, such as 4,6-diamino-2-phenyl-indole(DAPI) (Kanchanapoom and Koarapatchaikol 2012). DAPI is a fluorescent agent that effectively useful for observation of chromosome during pachytene and metaphae I of meiosis in banana (Kantama *et al*., 2017). Hence, DAPI might offer a better resolution for microscopy of metaphase during mitotic division.

In this study, we presented the usefulness of banana apical meristem, the young anther of male bud, with a combination of enzymatic digestion and DAPI to get a sharp chromosomes image for chromosome counting or mitotic study.

MATERIALS AND METHODS

Materials

In this study we used a wild diploid *Musa acuminata* ssp. *malaccensis* accession II 17B#4 and tetraploid 'Pisang Rejang' accession I 05A#2 (Poerba *et al.,* 2017). We selected these two types of ploidy in order to see whether the ploidy level affects in the clarity of the chromosome counting using a technique presented in this study. The plants were obtained from Research Center for Biology-LIPI banana growing in Cibinong, Bogor, Indonesia. The preparation for the chromosome microscopy was that adopted from Kantama *et al*. (2017) that consisted of four procedures, *i.e.* fixation, enzyme digestion, slide preparation and microscopy.

Fixation

Male buds were collected in late morning approximately 9 to 11 am. The young part of the male buds, approximately 5 cm to the tip, were fixed in fresh ethanol-acetic acid (3:1) for 30 minutes to 1 hour in the room temperature. The volume of the fixation solution should be 10–20 times of the material volume. Subsequently, the male buds were rinsed with 70% ethanol for three times. Afterward, keep them in 70% and stored in fridge until used.

Enzyme digestion

We collected young anthers from fixed male buds and washed in water for 1 minute. The washing repeated three times. Later, the anthers were put in the 2 ml tube with 100–300 ul enzyme mixture or until the samples completely soaked. The enzyme mixture contains 0.2% pectolyase from *Aspergillus japonica* (Sigma-Aldrich®), cellulase from *Trichoderma viride* (Sigma-Aldrich®) and cytohelicase from *Helix pomatia* (Sigma-Aldrich®) in 0.1 M citrate buffer (Doležel *et al*., 1998). Subsequently, it incubated in 37 °C for two hours. After the digestion, the enzyme solution was removed and the materials were washed using water three times, a minute each, and then added 500 µl fresh ethanol-acetic acid (3:1). The samples were stores in the 4 °C until slide preparation.

Slide preparation

A 1–2 mm part of anther was put on a glass slide, to avoid dried in a short time, we added a drop of water. Afterwards, the part of anther was macerated using fine needle and removed the hard material or un-macerated part. A drop of methanol-acetic acid (3:1) (50–60 µl) was added on the macerated samples and immediately passed on a Bunsen or lighter flame and let the fire put it out by itself and air dried in room temperature. Subsequently, 10–12 µl (for 22 x 22 mm cover slip) DAPI (100 mg/ml) were added in Vectashield® antifade mounting medium (Vector laboratories) and mounted with the coverslip.

Microscopy and imaging

The slides were observed under a fluorescent microscope with DAPI filter (Olympus BX-53). In order to the get a sharp image and detail, such as chromosome satellite, the images were improved by setting the optimum level of bright and contrast using Adobe Photoshop CC. Then, the chromosomes length was measured using ImageJ (Schneider *et al*., 2012).

RESULTS

In this study, we found that the cells were well spread and DAPI staining was effectively complement with the chromosome to provide a contrast chromosome images with minor cytoplasm background (Figure 1). In our observation, we found that most of the cells were in prophase that not useful for chromosome counting and few cells in metaphase that satisfying for chromosome counting. Here, we presented two cells of diploid *M. acuminata* ssp. *malaccensis* and a cell of tetraploid 'Pisang Rejang' with a contrast chromosome images of metaphase and sufficient for chromosome counting. In these cells, 22 chromosomes were observed from the diploid banana (Figure 1A-B) and 44 chromosomes were observed from the tetraploid banana (Figure 1C). Chromosomes in diploid were dispersed, no overlapping between chromosomes arm. In contrast, most of the chromosomes of tetraploid were overlapped each other that might diffuse the detail. In this study, we can noticed the present of secondary constriction that form satellite in *M. acuminata* ssp. *malaccensis* cells (pointed by arrows in Figure 1A-B). Satellite is a part of the tip of chromosome that separated by a secondary constriction, which the primary constriction is the centromere (Ferguson-Smith, 2001). In contrast, we could not observed such satellite in tetraploid that might because of the chromosome overlapping that diffused the presence of this structure.

Our finding showed that the average lengths of chromosome in metaphase of two observed cells of diploid ssp. *malaccensis* in this study were 2.31 µm with range 1.36–3.23 µm in cell A (Figure 1A, Figure 2A) and 1.63 µm with range 1.08–2.99 µm in cell B (Figure 1B, Figure 2B). We hardly measured the length of the tetraploid banana's chromosomes, because the chromosomes were overlapping, so the tips of chromosomes were not clear and not possible to be measured precisely.

DISCUSSION

In this study, a combination of young anther, enzymatic maceration and DAPI provided a satisfying chromosome image for chromosome counting. Usually, young anther is used for pollen mother cells meiotic study (Shepherd, 1999; Adeleke *et al*., 2002; Kantama *et al*., 2017). However, since the young anther is rich of cells in growth and division cycle (Crang *et al*., 2018), thus this part is potential for mitotic study. Typically, the young part root tip is used for chromosome counting and karyotyping in banana study (Doležel *et al*., 1998; Rekha and Hiremath, 2008; Kanchanapoom and Koarapatchaikul, 2012; Bakry and Shepherd, 2014). Leaf meristem is another material for mitotic study. Sapre and Barve (1983) reported the usefulness of intercalary leaf meristem of grasses, *i.e. Zea mays*, *Trilobachne cookie,* and *Coix aquatica*, for high quality karyotype. Recently, Anamthawat-Jónsson (2014) provided that the quality of the chromosome images from leaf meristem and root tip of birch (*Betula* L.) was equal. Callus is another material that useful for cytogenetic study (Kanchanapoom and Koarapatchaikul, 2012), but it needs in-vitro tissue culture to propagate the cells. Here, we showed that young anther could be a good material for mitotic study.

Ploidy level might influence the appearance of chromosome formation during metaphase. In previous study, Kanchanapoom & Koarapatchaikul (2012) showed that chromosomes of diploid were more dispersed than the tetraploid, a similar appearance as in this study. The dispersed chromosomes in diploid make the length measurement could be done precisely. In this study, we able to measure the chromosome length of the diploid banana. In average, we found one cell (Figure 2A) was a slight longer than the other one (Figure 2B). It might happen-, because of chromatin condensation level in each cell is different (Antonin and Neumann, 2016), and the maximum compaction of chromosome is at the late anaphase (Mora-Bermúdez *et al*., 2007).

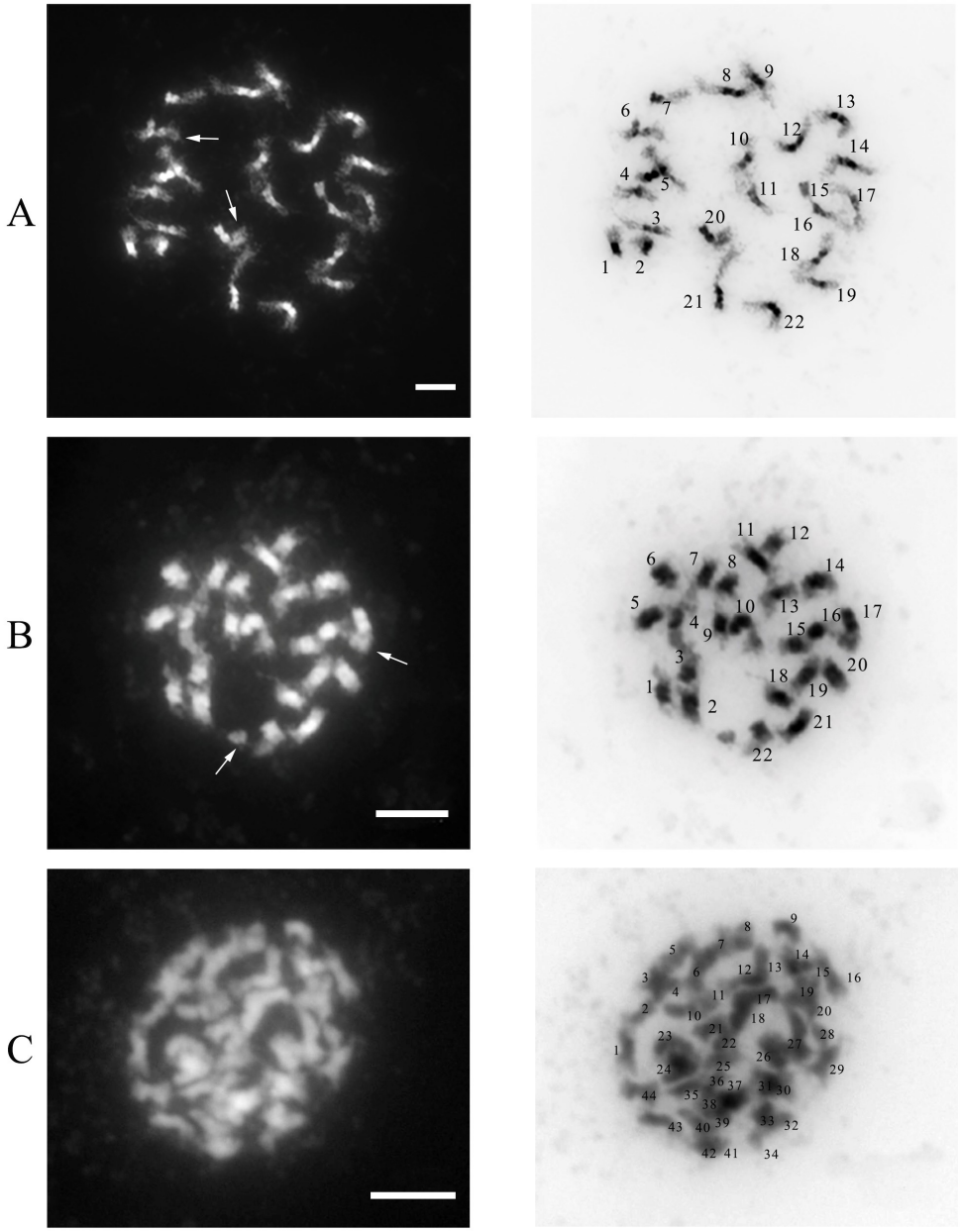


Figure 1. Miotic methaphase of male bud with DAPI staining of diploid (2x=22) *Musa acuminata* ssp. *malaccensis* (A, B) and tetraploid (2x=44) 'Pisang Rejang' (C). At early metaphase the chromosomes are more relaxed (A) than at metaphase (B). Secondary contractions as indication of the present of satellite are observed in this phase (arrow). Bar = 5 µm *(Sel diploid (2x=22) Musa acuminata* ssp. malaccensis *(A, B) dan tetraploid (2x=44) 'Pisang Rejang' di tahap metafase pada pembelahan mitosis. Pada saat metafase awal, lengan kromosom terlihat lebih panjang (A) dari pada saat metafase (B). Keberadaan satelit kromosom terindikasi dengan keberadaan 'secondary contraction' (tanda panah). Tanda garis = 5 µm)*

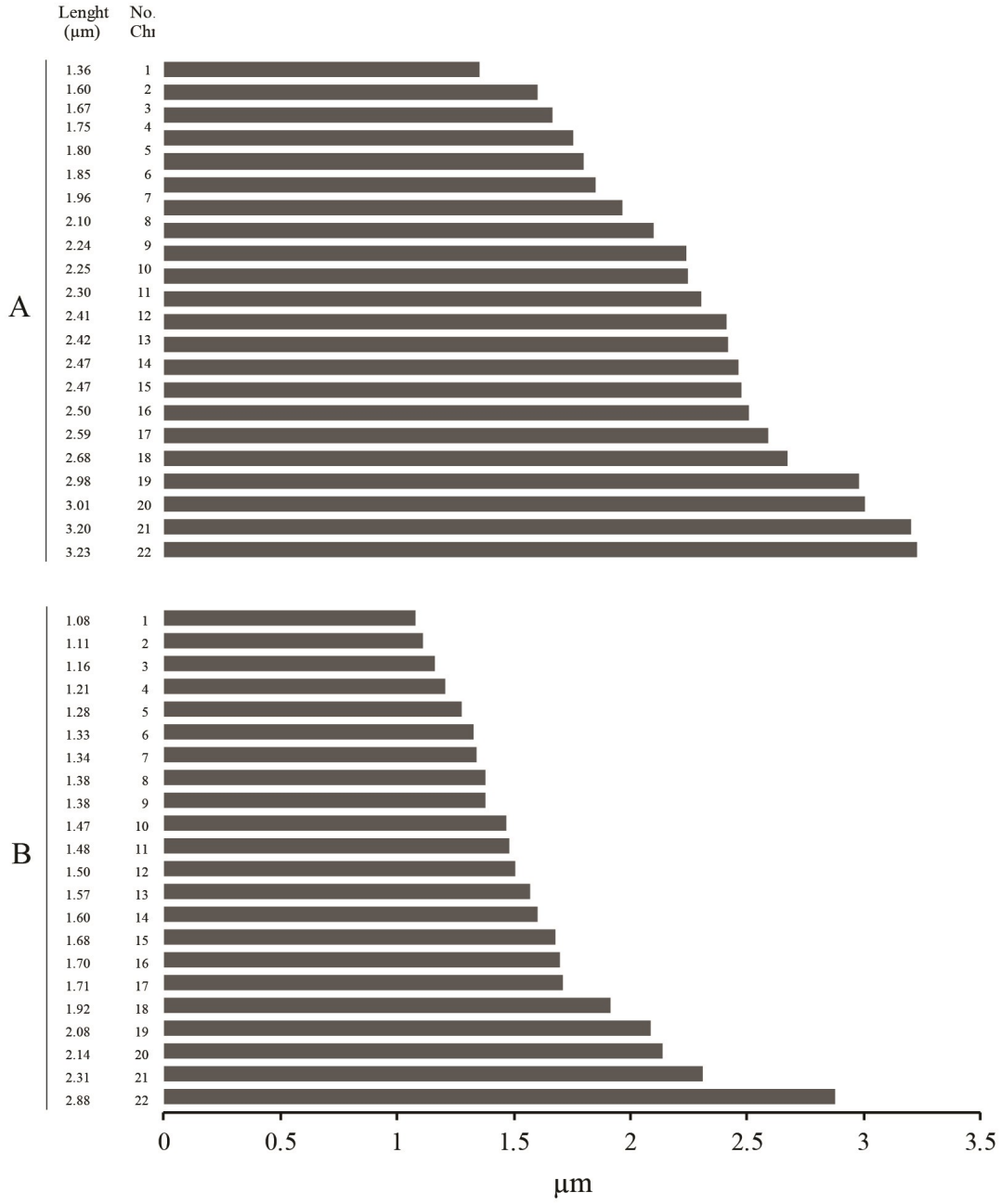


Figure 2. Chromosomes length of two cells (A and B) during metaphase of *Musa acuminata* ssp. *malaccensis*. *(Panjang kromosom dari dua sel (A dan B) pisang Musa acuminata* ssp. malaccensis *yang berada pada tahap metaphase)*

Banana chromosome is relatively small, approximately 1–3 µm in length (Doležel *et al*., 1998; Ortiz, 2000). However, a detail such as satellite can be observed. Bakry and Shepherd (2008) reported the appearance of a satellite in a chromosome and Rekha and Hiremath (2008) described that in a set of chromosome of cultivars 'Robusta', 'Dwarf Cavendish', 'Gros Michel', 'Grand Naine' and 'Red Banana' at least have one chromosome with a satellite. As in the previous studies mentioned above, our study also showed the presence of satellites. Satellite may useful in plant characterization, *i.e.* the satellite appearances were useful to distinguish between *Brachypodium stacei* and *B. distachyon* (Lusinska *et al.,* 2018).

The application of enzymatic digestion before the slide preparation provided a good spreading of cells. The enzyme was used to break the cell wall and result protoplast. Later, on the glass slide the protoplast will wide side to side and make the chromosome spread (Doležel *et al*., 1998). Enzymatic treatment was not frequent to be applied in banana mitotic study, but common in meiotic study (Adeleke *et al*., 2002; Kantama *et al.*, 2017). In mitotic study, squash, a non-enzymatic cell spreading technique is more common in slide preparation. This method is usually combined with aceto-carmin and -orcein as staining agent, and sufficient for chromosome counting (Rekha and Hiremath, 2008; Bakry and Shepherd, 2014).

The using of fluorescent DAPI staining in this study provided a high contrast and detailed of chromosomes. DAPI has been used in previous banana mitotic study in ploidy conformation of tetraploid banana 'Kluai Lap Mu Nang' (Kanchanapoom and Koarapatchaikul, 2012). DAPI staining could provide a better detail compared to orcein or carmin staining method in the presentation of heterochromatin along chromosome arms (Sheikh and Kondo, 1995). Another advantage of DAPI is as background staining in fluorescent in-situ hybridization (FISH). With combinations of other fluorescents agent and probe, FISH is useful to differentiate *M. acuminata* and *M. balbisiana* chromosomes and to confirm the present of interspecific hybridizations (Heslop-Harrison *et al*., 1998; D'Hont *et al*., 2000).

CONCLUSIONS

The availability of material and a good quality chromosome image is essential in cytogenetic study. Our study provided the combination of young anther in the banana male bud as an alternative material, enzymatic digestion prior cell maceration and DAPI staining to get a satisfactory chromosome images for mitotic study.

ACKNOWLEDGEMENTS

We acknowledge to the Research Center for Biology - Indonesian Institute of Sciences as the provider for the banana collections and supplies. We also thanks to Herlina for her technical helps and supplies.

REFERENCES

Adeleke, M.T .V, Pillay, M. and Okoli, B.E., 2002. An improved method for examining meiotic chromosomes in *Musa* L. *HortScience*, *37*(6), pp. 959–961.

Anamthawat-Jónsson, K., 2014. Preparation of chromosomes from plant leaf meristems for karyotype analysis and Preparation of chromosomes from plant leaf meristems for karyotype analysis and in situ hybridization. *Method in Cell Science*, *25*, pp. 91–95.

Antonin, W. and Neumann, H., 2016. Chromosome condensation and decondensation during mitosis. *Current Opinion in Cell Biology*, *40*, pp. 15–22.

Bakry, F. and Shepherd, K., 2014. Banana protocol chromosome count on banana root tip squashes. *Fruits*, *63*, pp. 179–181.

Cheesman, E.E., 1947. Classification of the bananas: the genus *Musa* L. *Kew Bulletin*, *2*, pp. 106–117.

Crang, R., Lyons-sobaski, S. and Wise, R., 2018. *Plant Anatomy: A Concept-Based Approach to the Structure of Seed Plants*. Springer Nature. Cham. pp. 123–154.

D'Hont, A., Paget-Goy, A., Escoute, J. and Carreel, F., 2000. The interspecific genome structure of cultivated banana, *Musa* spp. revealed by genomic DNA in situ hybridization. *Theoretical and Applied Genetics*, *100*, pp. 177–183.

Doležel, J., Doleželová, M., Roux, N. and Van den Houwe, I., 1998. A novel method to prepare slides for high resolution chromosome studies in *Musa* spp. *Infomusa*, *7*, pp. 3–4.

Doležel, J., Lysak, M.A., van Den Houwe, I., Dolezelova, M. and Roux, N., 1997. Use of flow cytometry for rapid ploidy determination in *Musa* species. *Infomusa*, *6*, pp. 6–9.

Ferguson-Smith, M.A., 2002. Satellited Chromosome. In: Brener, S. and Miller, J.H. eds. *Ensiclopedia of Genetics*. pp. 1773–1774. Academic Press.

Heslop-Harrison, J., Osuji, J. and Hull, R., 1998. Fluorescent in situ hybridization of plant chromosomes: illuminating the Musa genome. In: INIBAP: Networking Banana and Plaintain: INIBAP Annual Report 1998. pp. 26–29. International Network for the Improvement of Banana and Plaintain, Montpellier, France.

Kanchanapoom, K. and Koarapatchaikul, K., 2012. In vitro induction of tetraploid plants from callus cultures of diploid bananas (*Musa acuminata*, AA group) "Kluai Leb Mu Nang" and "Kluai Sa". *Euphytica*, *183*, pp. 111–117.

Kantama, L., Wijnker, E. and de Jong, H., 2017. Optimization of Cell Spreading and Image Quality for the Study of Chromosomes in Plant Tissues. In: Schmidt, A. ed. *Plant Germline Development: Methods and Protocols*. pp. 141–158. Humana Press. New York.

Lusinska, J., Majk, J., Betekhtin, A., Susek, K., Wolny, E. and Hasterk, R., 2018. Chromosome identification and reconstruction of evolutionary rearrangements in *Brachypodium distachyon*, *B. stacei* and *B. hybridum*. *Annals of Botany*, *122*, pp. 445–459.

Mora-Bermúdez, F., Gerlich, D. and Ellenberg, J., 2007. Maximal chromosome compaction occurs by axial shortening in anaphase and depends on Aurora kinase. *Nature Cell Biology*, *9*, pp. 822–831.

Ortiz, R., 2000. Understanding the Musa genome: An update. *Acta Horticulturae*, *340*, pp. 157–168.

Osuji, J., Okoli, B.E. and Ortiz, R., 1996. An improved procedure for mitotic studies of of the Eumusa section of the genus *Musa* L. (Musaceae). *Infomusa*, 5, pp. 12–14.

Osuji, J., Okoli, B.E. and Edeoga, H.O., 2006. Karyotypes of the A and B Genomes of *Musa* L. *Cytologia*, *71*, pp. 21–24.

Poerba, Y.S., Handayani, T. and Witjaksono, 2017. Characterization of tetraploid Pisang Rejang induced by oryzalin. *Berita Biologi*, *16*, pp. 85–93.

Rekha, A. and Hiremath, S.C., 2008. Chromosome studies and karyotype analysis of some triploid banana (*Musa* species) cultivars of AAA genomic group. *Journal of Horticultal Science*, *3*, pp. 30–34.

Roux, N., Toloza, A., Radecki, Z., Zapata-Arias, F.J. and Dolezel, J., 2003. Rapid detection of aneuploidy in *Musa* using flow cytometry. *Plant Cell Reports*, *21*, pp. 483–490.

Sapre, A.B. and Barve, S., 1983. Somatic chromosomes from intercalary. *Cytologia*, *48*, 539–541.

Sheikh, S. and Kondo, K., 1995. Differential Staining with Orcein, Giemsa, CMA and DAPI for Comparative Chromosome Study of 12 Species of Australian *Drosera* (Droseraceae). *American Journal of Botany*, *82*, pp. 1278–1286.

Shepherd, K., 1999. *Cytogenetics of the genus Musa*. International Network for the Improvement of Banana and Plantain. Montpellier, France. pp. 9–14.

Schneider, C.A., Rasband, W.S. and Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), pp. 671–675.

Wong, C., Kiew, R., Argent, G., Set, O., Lee, S.K. and Gan, Y.Y., 2002. Assessment of the validity of the sections in *Musa* (Musaceae) using AFLP. *Annals of Botany*, *90,* pp. 231–238.

Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput harus menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up to date*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan atau baru, agar dapat segera diketahui oleh umum. Hasil dan pembahasan dapat digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah Bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul diberikan dalam bahasa Indonesia dan inggris. Judul ditulis dalam huruf tegak kecuali untuk nama ilmiah yang menggunakan bahasa latin, Judul harus singkat, jelas dan mencerminkan isi naskah dengan diikuti oleh nama serta alamat surat menyurat penulis dan alamat email. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*). Jika penulis lebih dari satu orang bagi pejabat fungsional penelitian, pengembangan agar menentukan status sebagai kontributor utama melalui penandaan simbol dan keterangan sebagai kontributor utama dicatatan kaki di halaman pertama artikel.
3. **Abstrak**
   Abstrak dibuat dalam dua bahasa, bahasa Indonesia dan Inggris. Abstrak memuat secara singkat tentang latar belakang, tujuan, metode, hasil yang signifikan, kesimpulan dan implikasi hasil penelitian. Abstrak berisi maksimum 200 kata, spasi tunggal. Di bawah abstrak dicantumkan kata kunci yang terdiri atas maksimum enam kata, dimana kata pertama adalah yang terpenting. Abstrak dalam Bahasa Inggris merupakan terjemahan dari Bahasa Indonesia. Editor berhak untuk mengedit abstrak demi alasan kejelasan isi abstrak.
4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Perlu disebutkan juga studi terdahulu yang pernah dilakukan terkait dengan penelitian yang dilakukan.
5. **Bahan dan cara kerja**
   Bahan dan cara kerja berisi informasi mengenai metode yang digunakan dalam penelitian. Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasinya dan apabila ada modifikasi maka harus dituliskan dengan jelas bagian mana dan hal apa yang dimodifikasi.
6. **Hasil**
   Hasil memuat data ataupun informasi utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada suatu tabel/ grafik/diagram atau gambar, maka hasil yang terdapat pada bagian tersebut dapat diuraikan dengan jelas dengan tidak menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata- rata maka harus menyertakan pula standar deviasinya.
7. **Pembahasan**
   Pembahasan bukan merupakan pengulangan dari hasil. Pembahasan mengungkap alasan didapatkannya hasil dan arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, hasil penelitian ini dapat dibandingkan dengan studi terdahulu.
8. **Kesimpulan**
   Kesimpulan berisi infomasi yang menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, implikasi dari hasil penelitian dan penelitian berikutnya yang bisa dilakukan.
9. **Ucapan terima kasih**
   Bagian ini berisi ucapan terima kasih kepada suatu instansi jika penelitian ini didanai atau didukungan oleh instansi tersebut, ataupun kepada pihak yang membantu langsung penelitian atau penulisan artikel ini.
10. **Daftar pustaka**
    Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses *peer review*. Apabila harus menyitir dari "laporan" atau "komunikasi personal" dituliskan '*unpublished*' dan tidak perlu ditampilkan di daftar pustaka. Daftar pustaka harus berisi informasi yang *up to date* yang sebagian besar berasal dari *original papers* dan penulisan terbitan berkala ilmiah (nama jurnal) tidak disingkat.

Format naskah

1. Naskah diketik dengan menggunakan program Microsoft Word, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak spasi tunggal. Batas kiri-kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan Bahasa Indonesia, angka desimal ditulis dengan menggunakan koma (,) dan ditulis dengan menggunakan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5 cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan *international system of units*.
4. Nama takson dan kategori taksonomi ditulis dengan merujuk kepada aturan standar yang diakui. Untuk tumbuhan menggunakan *International Code of Botanical Nomenclature* (ICBN), untuk hewan menggunakan International *Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae*, *Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
5. Tata nama di bidang genetika dan kimia merujuk kepada aturan baku terbaru yang berlaku.
6. Untuk range angka menggunakan en dash (–), contohnya pp.1565–1569, jumlah anakan berkisar 7–8 ekor. Untuk penggabungan kata menggunakan hyphen (-), contohnya: masing-masing.
7. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
8. Tabel
   Tabel diberi judul yang singkat dan jelas, spasi tunggal dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah.

8. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul gambar ditulis secara singkat dan jelas, spasi tunggal. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi, untuk *line drawing* minimal 600dpi.
9. Daftar Pustaka
   Sitasi dalam naskah adalah nama penulis dan tahun. Bila penulis lebih dari satu menggunakan kata 'dan' atau *et al*. Contoh: (Kramer, 1983), (Hamzah dan Yusuf, 1995), (Premachandra *et al*., 1992). Bila naskah ditulis dalam bahasa Inggris yang menggunakan sitasi 2 orang penulis maka digunakan kata 'and'. Contoh: (Hamzah and Yusuf, 1995). Jika sitasi beruntun maka dimulai dari tahun yang paling tua, jika tahun sama maka dari nama penulis sesuai urutan abjad. Contoh: (Anderson, 2000; Agusta *et al*., 2005; Danar, 2005). Penulisan daftar pustaka, sebagai berikut:

   a. **Jurnal**
      Nama jurnal ditulis lengkap.
      Agusta, A., Maehara, S., Ohashi, K., Simanjuntak, P. and Shibuya, H., 2005. Stereoselective oxidation at C-4 of flavans by the endophytic fungus *Diaporthe* sp. isolated from a tea plant. *Chemical and Pharmaceutical Bulletin*, *53*(12), pp.1565–1569.
   b. **Buku**
      Anderson, R.C. 2000. *Nematode Parasites of Vertebrates, Their Development and Tramsmission*. 2nd ed. CABI Publishing. New York. pp. 650.
   c. **Prosiding atau hasil Simposium/Seminar/Lokakarya.**
      Kurata, H., El-Samad, H., Yi, T.M., Khammash, M. and Doyle, J., 2001. Feedback Regulation of the Heat Shock Response in *Eschericia coli*. *Proceedings of the 40th IEEE Conference on Decision and Control*. Orlando, USA. pp. 837–842.
   d. **Makalah sebagai bagian dari buku**
      Sausan, D., 2014. Keanekaragaman Jamur di Hutan Kabungolor, Tau Lumbis Kabupaten Nunukan, Kalimanan Utara. Dalam: Irham, M. & Dewi, K. eds. *Keanekaraman Hayati di Beranda Negeri*. pp. 47–58. PT. Eaststar Adhi Citra. Jakarta.
   e. **Thesis, skripsi dan disertasi**
      Sundari, S., 2012. Soil Respiration and Dissolved Organic Carbon Efflux in Tropical Peatlands. *Dissertation*. Graduate School of Agriculture. Hokkaido University. Sapporo. Japan.
   f. **Artikel online.**
      Artikel yang diunduh secara online ditulis dengan mengikuti format yang berlaku untuk jurnal, buku ataupun thesis dengan dilengkapi alamat situs dan waktu mengunduh. Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses peer review misalnya laporan perjalanan maupun artikel dari laman web yang tidak bisa dipertangung jawabkan kebenarannya seperti wikipedia.
      Himman, L.M., 2002. A Moral Change: Business Ethics After Enron. San Diego University Publication. *http:ethics.sandiego.edu/LMH/oped/Enron/index.asp*. (accessed 27 Januari 2008) bila naskah ditulis dalam bahasa inggris atau (diakses 27 Januari 2008) bila naskah ditulis dalam bahasa indonesia

Formulir persetujuan hak alih terbit dan keaslian naskah
Setiap penulis yang mengajukan naskahnya ke redaksi Berita Biologi akan diminta untuk menandatangani lembar persetujuan yang berisi hak alih terbit naskah termasuk hak untuk memperbanyak artikel dalam berbagai bentuk kepada penerbit Berita Biologi. Sedangkan penulis tetap berhak untuk menyebarkan edisi cetak dan elektronik untuk kepentingan penelitian dan pendidikan. Formulir itu juga berisi pernyataan keaslian naskah yang menyebutkan bahwa naskah adalah hasil penelitian asli, belum pernah dan tidak sedang diterbitkan di tempat lain serta bebas dari konflik kepentingan.

Penelitian yang melibatkan hewan dan manusia
Setiap naskah yang penelitiannya melibatkan hewan (terutama mamalia) dan manusia sebagai obyek percobaan/penelitian, wajib menyertakan *'ethical clearance approval*' yang dikeluarkan oleh badan atau pihak berwenang.

Lembar ilustrasi sampul
Gambar ilustrasi yang terdapat di sampul jurnal Berita Biologi berasal dari salah satu naskah yang dipublikasi pada edisi tersebut. Oleh karena itu, setiap naskah yang ada ilustrasinya diharapkan dapat mengirimkan ilustrasi atau foto dengan kualitas gambar yang baik dengan disertai keterangan singkat ilustrasi atau foto dan nama pembuat ilustrasi atau pembuat foto.

Proofs
Naskah *proofs* akan dikirim ke penulis dan penulis diwajibkan untuk membaca dan memeriksa kembali isi naskah dengan teliti. Naskah proofs harus dikirim kembali ke redaksi dalam waktu tiga hari kerja.

Pengiriman naskah
Naskah dikirim secara online ke website berita biologi: *http://e-journal.biologi.lipi.go.id/index.php/berita_biologi*

Alamat kontak
Redaksi Jurnal Berita Biologi, Pusat Penelitian Biologi-LIPI
Cibinong Science Centre, Jl. Raya Bogor Km. 46 Cibinong 16911
Telp: +61-21-8765067, Fax: +62-21-87907612, 8765063, 8765066,
Email: berita.biologi@mail.lipi.go.id
jurnalberitabiologi@yahoo.co.id atau
jurnalberitabiologi@gmail.com

BERITA BIOLOGI

Vol. 19(2) **Isi (*Content*)** Agustus 2020

P-ISSN 0126-1754
E-ISSN 2337-8751

MAKALAH HASIL RISET (*ORIGINAL PAPERS*)

KOMUNIKASI PENDEK (*SHORT COMMUNICATION*)