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Floribunda merupakan organ resmi Penggalang Taksonomi Tumbuhan Indonesia, diterbitkan dua kali setahun dan menerbitkan makalah dalam bahasa Indonesia dan Inggris mengenai pelbagai gatra sistematika keanekaragaman flora Malesia pada umumnya dan Indonesia pada khususnya yang berasal dari hasil penelitian, pengamatan lapangan, pengalaman pribadi, telaahan bergagasan, dan tinjauan kritis.

Sidang Penyunting

Ketua Penyunting

Tutie Djarwaningsih (BO)

Penyunting

Bayu Adjie (KREKB)

Ida Haerida (BO)

Abdulrokhman Kartonegoro (BO)

Deden Girmansyah (BO)

Priyanti (UIN)

Dewi Susan (BO)

Penyunting Pelaksana

Wita Wardani (BO)

Tata Letak

Andi Hapid (BO)

Petunjuk kepada pengarang

Jenis tulisan

Makalah lengkap memuat hasil penelitian floristik, revisi, atau monografi unsur-unsur flora Malesia. Komunikasi pendek mencakup laporan kemajuan kegiatan penelitian, pengembangan dan rekayasa keanekaragaman flora Malesia yang perlu segera dikomunikasikan.

Tulisan lain meliputi obituari tokoh keanekaragaman flora, tinjauan kritis bergagasan, telaahan serta pembahasan persoalan aktual seputar kegiatan penelitian, pengembangan dan rekayasa tetumbuhan Indonesia, serta timbangan buku akan dimuat berdasarkan undangan.

Rujukan pembakuan

Pemakaian Bahasa Indonesia sepenuhnya mengikuti *Pedoman Umum Ejaan yang Disempurnakan*, *Pedoman Umum Pembentukan Istilah*, *Kamus Besar Bahasa Indonesia*, serta kamus-kamus istilah yang dikeluarkan Pusat Bahasa. Bahasa Inggris yang dipakai adalah the Queen English dengan berpedoman pada *Oxford Dictionary of*

the English Language. Ketentuan-ketentuan yang dimuat dalam *Pegangan Gaya Penulisan, Penyuntingan, dan Penerbitan Karya Ilmiah Indonesia*, serta *Scientific Style and Format: CBE Manuals for Author, Editor, and Publishers*, dan buku-buku pegangan pembakuan lain akan sangat diperhatikan. Kepatuhan penuh pada *International Code of Botanical Nomenclature* bersifat mutlak.

Gaya penulisan

Penulisan naskah yang akan diajukan supaya disesuaikan dengan gaya penulisan yang terdapat dalam nomor terakhir terbitan *Floribunda*.

Abstrak informatif supaya diberikan dalam bahasa Indonesia dan Inggris yang masing-masing tidak melebihi 200 kata. Sediakan sekitar 7 kata kunci untuk keperluan pengindeksan dan pemindaian.

Bilamana diperlukan ucapan terima kasih dan bentuk persantunan lain dapat dicantumkan sesudah tubuh teks tetapi sebelum daftar pustaka.

Pengacuan pada pustaka hendaklah dilakukan dengan sistem nama-tahun. Daftar pustaka supaya disusun berdasarkan alfabet nama pengarang dengan memakai sistem Harvard.

Gambar dan tabel merupakan pendukung teks sehingga perlu disusun secara logis dalam bentuk teks atau tabel atau sebagai gambar, tetapi tidak dalam bentuk ketiganya sekaligus. Siapkan gambar yang lebarnya dua kolom cetak.

Penyumbangan naskah

Naskah dikirimkan secara *online* atau melalui *e-mail*. Naskah yang ingin diterbitkan dalam *Floribunda* akan dipertimbangkan pemuatannya *hanya* jika pengirimannya disertai pernyataan tertulis dari 2 (dua) orang mitra bestari yang dipilih sendiri oleh penulisnya (akan lebih diutamakan bila mitra bestari dipilihkan dari luar lingkungan kerja penulis), yang menyatakan bahwa secara ilmiah keorisinalan dan makna sumbangan naskah tersebut memang layak diterbitkan. Makalah yang dimuat dikenai biaya Rp. 450.000,00 untuk anggota PTTI dan Rp. 500.000,00 untuk non anggota.

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MITOTIC AND KARYOTYPE OF INDIGOFERA SUFFRUTICOSA MILL. IN CENTRAL JAVA

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Wahyu Kusumawardani, Muzzazinah & Murni Ramli. 2021. Mitosis dan Kariotipe *Indigofera suffruticosa* Mill. di Jawa Tengah. *Floribunda* 6(6): 213–219 . — *Indigofera suffruticosa* merupakan salah satu spesies dari genus *Indigofera* yang banyak dimanfaatkan oleh masyarakat Indonesia di berbagai bidang, namun masih terbatas informasi sitologinya. Penelitian ini bertujuan untuk mengetahui fase mitosis dan struktur kromosom *I. suffruticosa*. Analisis jumlah kromosom dilakukan pada *I. suffruticosa* dari individu yang berasal di wilayah Magelang, Jawa Tengah. Pengamatan fase mitosis dan struktur kromosom dilakukan dengan mengamati kromosom somatik ujung akar menggunakan metode squash. Metode pembuatan sampel kromosom menggunakan metode *squash*. Struktur morfologi kromosom dianalisis menggunakan peta kariotipe dengan bantuan Image Raster, Corel Draw, dan Microsoft Excel. Hasil pengamatan menunjukkan fase mitosis dari profase, prometafase, metafase, anafase, dan telofase. Kromosom somatik yang diamati dan dianalisis kariotipenya pada fase prometafase. *I. suffruticosa* memiliki jumlah kromosom diploid yaitu $2n=16$. *I. suffruticosa* memiliki panjang rata-rata lengan kromosom 2,08 μm dengan tipe kromosom metasentris dan sub metasentris. Data struktur kromosom *I. suffruticosa* dari Indonesia ini dapat melengkapi kebaruan dokumentasi informasi klasifikasi taksonomi *Indigofera* peneliti sebelumnya.

Kata kunci: Sitologi, *Indigofera*, marka genetik, evolusi.

Wahyu Kusumawardani, Muzzazinah & Murni Ramli. 2021. Mitotic and Karyotype of *Indigofera suffruticosa* Mill. in Central Java. *Floribunda* 6(6): 213–219 . — *Indigofera suffruticosa* has been widely used by Indonesians, but its cytological information is still limited. This study was aimed to observe the mitotic phases and chromosome structures of *I. suffruticosa*. The analysis was performed on *I. suffruticosa* sampled from Magelang, Central Java. Mitotic phases and chromosomes' structures were observed using root tips prepared with the squash method. The chromosome morphological structure was analyzed with karyotype maps using Image Raster, Corel Draw, and Microsoft Excel. The observation showed that *I. suffruticosa* were at various mitosis phases starting from prophase, pro-metaphase, metaphase, anaphase, and telophase. The chromosome in the pro-metaphase was analyzed for its karyotype morphology. The species has some diploid chromosomes ($2n = 16$). *I. suffruticosa*'s chromosomes have an average chromosome' length of 2.08 μm with submetacentric and metacentric types. Data about the chromosome structure of *I. suffruticosa* from Indonesia could complement the novelty of the taxonomic classification information obtained by previous researchers.

Keywords: Cytology, *Indigofera*, genetic markers, evolution.

Indigofera L. is a legume genus belonging to the tribe *Indigoferaeae*, subfamily *Papilionoideae* (LPWG 2017; Schrire 2005). The *Indigofera* has over 700 species as members and distributed throughout the tropics and subtropics in Africa, Asia, and America (Jansen & Cardon 2005). *Indi-*

gofera consist of herbs, shrubs and small trees with simple or unifoliolate, trifoliolate or imparipinnate leaves and small flowers in axillary racemes (Adema 2011). The species has various usages, especially for medicine and industry such as as a medicinal plant (Luiz-Ferreira *et al.* 2011; Rahman

et al. 2018; Samant & Pant 2006), and natural dyes (Khan *et al.* 2003; Muzzazinah *et al.* 2016; Yingying *et al.* 2016). Two *Indigofera* species have been identified as the main producer for natural indigo dyes worldwide, those were *I. suffruticosa*, and *I. tinctoria* (Jansen & Cardon 2005; Jahan *et al.* 2013).

A recent study by Muzzazinah (2016) showed that *I. suffruticosa* was found in Java, Sumatra, Sulawesi, Kalimantan (Borneo), Lombok, Bali, Madura, Flores, Sumbawa, Timor, Sumba, Tanimbar, Kangean, Roti and Sawu. A study on the genetic diversity of Indonesian *I. tinctoria* has been carried out using samples obtained from 33 locations across Java, Madura, Cirebon, Sumenep, and Flores (Muzzazinah *et al.* 2019). A recent study showed a high level of genetic diversity in *I. tinctoria* species from Cirebon, while the diversities from Waioti and Wairleber were low. Studies over the past few years have revealed that cytology has become an important part of taxonomy and forming the new scientific discipline of cytotaxonomy (Venkatesh *et al.* 2015). The cytological information about the *Indigofera* chromosomes has not yet included the species from Indonesia. *I. suffruticosa* and *I. tinctoria* also found in tropical regions of Indonesia and used as coloring agents. Although they were commonly used as a coloring agent for traditional textile products, research about *I. tinctoria* found in Java, Madura, and Flores showed the different morphological characters affecting the level of indigo color hues of the fabric (Muzzazinah *et al.* 2018).

Previous studies revealed variations in chromosome character among the *Indigofera* (Sanjappa & Bhatt 1984). The difference in structure, shape, and size of chromosomes affects the phenotypic characters' differences between species. (Singh 2012; Soltis & Doulgas 2012) have reported the variation in the numbers of chromosomes in *I. suffruticosa* ($2n = 32$ and $2n = 16$). With this information, then we sought to find out the number of chromosomes and mitotic phase of Indonesian *I. suffruticosa*. Analysis of cytological characters such as chromosome numbers and the karyotype have contributions towards the study of taxonomic and evolutionary relationships (Ahirwar & Verma 2014). Karyotypes described the character of chromosomes, including the number of chromosomes, the length of the chromosomes' short arm and long arm, the absolute length, the centromere index, the size and position of the satellites (if any), and the karyotype formula

(Sivarajan 1984). This study was aimed to complement the latest cytotaxonomic data documentation about mitotic and chromosome structures of *I. suffruticosa* in Indonesia. Results from this study can be considered as an initial exploration of genetic markers that further used to improve the quality of *Indigofera* plant products.

MATERIALS AND METHODS

Sample preparation

Samples of *I. suffruticosa* seeds were harvested between October and November 2018 from Karangpandan, Karanganyar, Central Java, with an average height of 511 meters above sea level and a tropical climate with temperatures of 22–31°C. The seeds were germinated in 3–5 days on the Petri containers with wet flannels in the Central Laboratory Unit, Sebelas Maret University, Surakarta. The root tips were used as the material for the preparation using a modification of the squash method (Okada 1981).

Root tips were cut about 0.3–0.5 cm in length in the morning between 7.30–10.15 AM (UTC+7). Root tips were fixed in a 45% solution of glacial acetic acid for 15 minutes at 4 °C. The sample then macerated using 1N hydrochloric acid solution for eight minutes at 62 °C. Next, the sample was stained using 1% Acetoorcein for 24 hours at room temperature. After being colored, the samples were placed on a glass object and dropped with glycerin, then covered with degrees glass and crushed using a pencil tip.

Chromosome observation was carried out at the Laboratory of Genetics and Plant Breeding, Gadjah Mada University, Yogyakarta using an Olympus BX 41 microscope with 1000x magnifications. The cytological analysis was conducted from December 2018 to October 2019.

Karyotype Analysis

The analysis was carried out on 200 *I. suffruticosa* cells. Chromosomes were measured five times in the pro-metaphase cells selected for each species. Data were tabulated into tables, and chromosome sizes were visualized into the ideograms. Homologous chromosomes were cut and arranged using Corel Draw X5. The length of the chromosomes' arms was calculated using Image Raster 3.0, and Microsoft Office Excel was used to calculate the values related to the chromosomes' arms.

The obtained chromosome morphological data were:

- a. Short Arm Length (SA), μm .
- b. Long Arm Length (LA), μm .
- c. The Whole Chromosome Length (TL), μm .
TL = Short Arm (SA) + Long Arm (LA), μm .
- d. Centromery Index (CI), μm .

$$\text{CI} = \frac{\text{Short arms (SA)}}{\text{the whole chromosome (TL)}}$$

- e. Arm Ratio (AR), μm .

$$\text{AR} = \frac{\text{ShortArm (SA)}}{\text{LongArm}}$$

- f. Chromosome Type (CT).

Chromosome types were analyzed and determined based on AR (Arm Ratio) values. For the metacentric (M), the value was 1.00–1.67 μm , sub metacentric (BC) was 1.68–3.00 μm , acrocentric (A) was 3.01–7.00 μm , and telocentric (T) was >7.00 μm (Levan *et al.* 1964).

RESULT AND DISCUSSION

The phases of mitosis in *I. suffruticosa* were presented in Figure 1 A-E, the somatic chromosomes of *I. suffruticosa* were presented in Figure 1B, and the ideograms in Figure 2.

Mitotic

The mitotic phases occurred continuously in several stages including the prophase, pro-metaphase, metaphase, anaphase, and telophase (Campbell *et al.* 2008). Mitotic produces two identical daughter cells with the same chromosome pair through successive nuclear divisions. At the prophase, the chromatin condenses, the nuclear membrane began to disappear, and the mitotic spindle began to form (Fig. 1A). The cell nucleus looks rounded, enlarged, and dark. The chromatin threads are disappearing. The chromatin threads are getting shorter and thicker, and the chromosomes are formed. At the pro-metaphase, the chromosomes were condensed, shortened, and thickened (Fig. 1B). In the pro-metaphase stage, the chromosomes are more spread out and their structures are clearer because they have not been arranged in the equatorial plane. In the metaphase (Fig. 1C), the chromosomes were scattered in the cell's equator (the metaphase plate) (Snustad *et al.* 1997). Each chromosome with a pair of chromatids moves towards and assembles in the cleavage plane (equatorial plane), pulled by spindle fibers

from the centromere. At the anaphase (Fig. 1D), the sister chromatids of each chromosome were separated and move toward the cell's poles when kinetochore microtubules shortened (Campbell *et al.* 2008). The centromere divides and the two chromatids separate towards the opposite poles of the cell. Each cleavage chromatid is a new chromosome and has the same hereditary traits. The telophase occurred when the chromatids at the poles turn back into chromatin strings and the new nucleus and nuclear membrane began to form (Fig. 1E). In the early telophase, the dividing cell wall is formed but incomplete, resulting in imperfect insulation. But signs of cytokinesis have appeared. At the end of telophase, the wall is more separated. This is indicated by thick chromosomes appeared in the middle of the newly formed cells.

Karyotype Analysis

Complete mitotic phase analysis was demonstrated in the *I. suffruticosa*, and the pro-metaphase phase selected for karyotype analysis. The pro-metaphase stage is the best stage for the characterization of chromosomes (Suryo 1995) because at this phase, the chromosomes are scattered and not overlapping. Each chromosome has a distinct cylindroid shape with four arms because it has two twin chromatids (Kurata & Omura 1978).

The results showed *I. suffruticosa* has the chromosome diploid of $2n = 16$ (Figure 1B). The results supported the research by Gupta & Agarwal (1982) and Singh (2012). The karyotype formula for *I. suffruticosa* was $2n= 16 = 14M + 2SM$. The length of the chromosomes was varied (Table 1). The longest was 3.61 μm and the shortest one was 1.27 μm , with the arm ratio between 1.12 μm to 2.18 μm and the CI values between 31.45%–47.15%. *I. suffruticosa* has an average length for short chromosome arms of 0.93 μm . *I. suffruticosa* has an average length for long chromosomes arms of 1.16 μm .

According to Singh (2012) the diploid chromosome number ($2n$) *I. suffruticosa* is 16 pairs or 32 chromosomes. The *I. suffruticosa* samples examined by Singh (2012) came from Florida, Louisiana, Mississippi, Texas, Mexico, Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Anguilla, Antigua, Barbuda, Bahamas, Barbados, Cayman Islands, Cuba, Dominica, Vincent, Grenadines, British Virgin Islands, US Virgin Islands, French Guiana, Guyana, Suriname, Venezuela, Brazil, Bolivia, Colombia, Ecuador, Peru, Argentina, Paraguay. Whereas in this study, *I. suffruticosa* in Indonesia, especially the

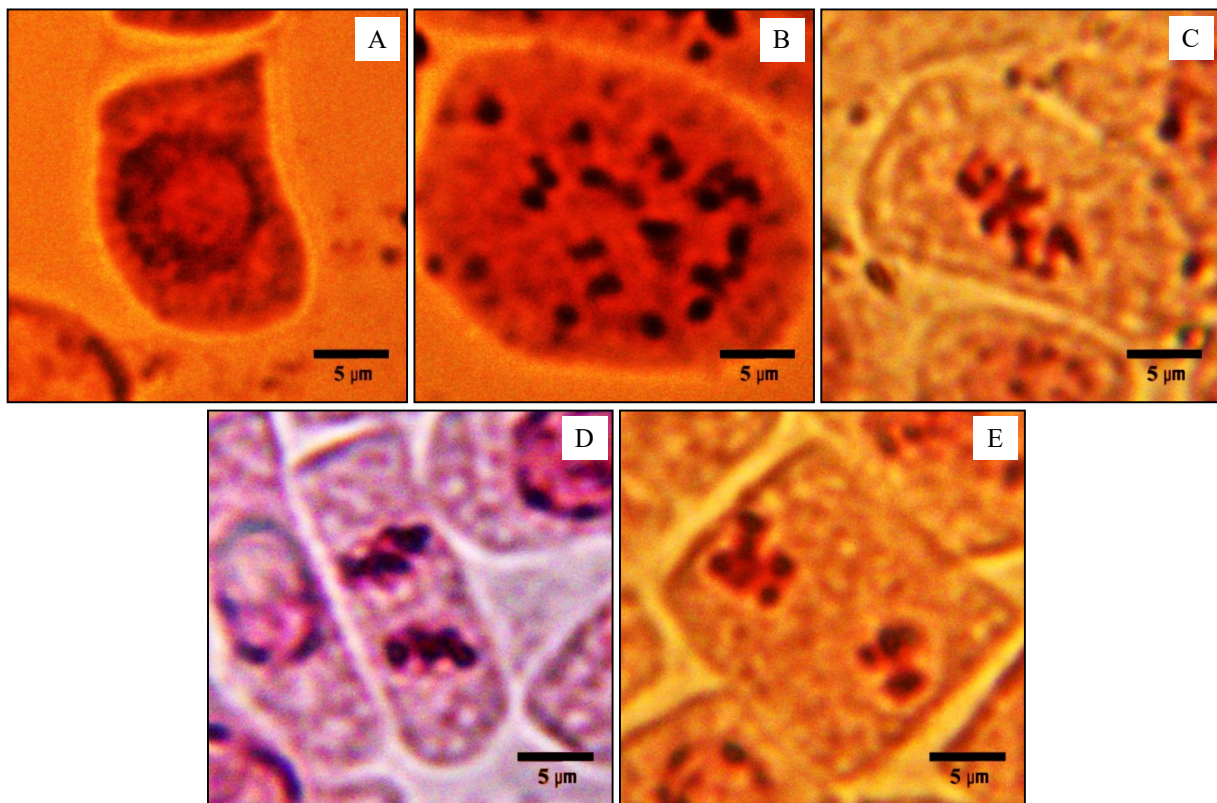


Figure 1A-E. Phases of mitotic on *I. suffruticosa*; A. prophase; B. pro-methaphase; C. metaphase; D. ana-phase; E. telophase. Bar 5µm

sample population from Central Java, showed the number of diploid chromosomes ($2n$) was 16 pairs. The samples came from 37 locations in Java and Madura, the same as previously researched by Muzzazinah *et al.* (2016). The number, sizes, and shapes of chromosomes can characterize the plant karyotypes which are useful in taxonomic classification (Soliman 2002). It also gives the evi-

dence of changes in chromosomes' structures during the course of evolution (Ahirwar & Verma 2014). Chromosome evolution tended to increase the number of chromosomes and development towards the terminal centromeric chromosome, whereas the centromeric or metacentric are considered primitive (Imai *et al.* 1988).

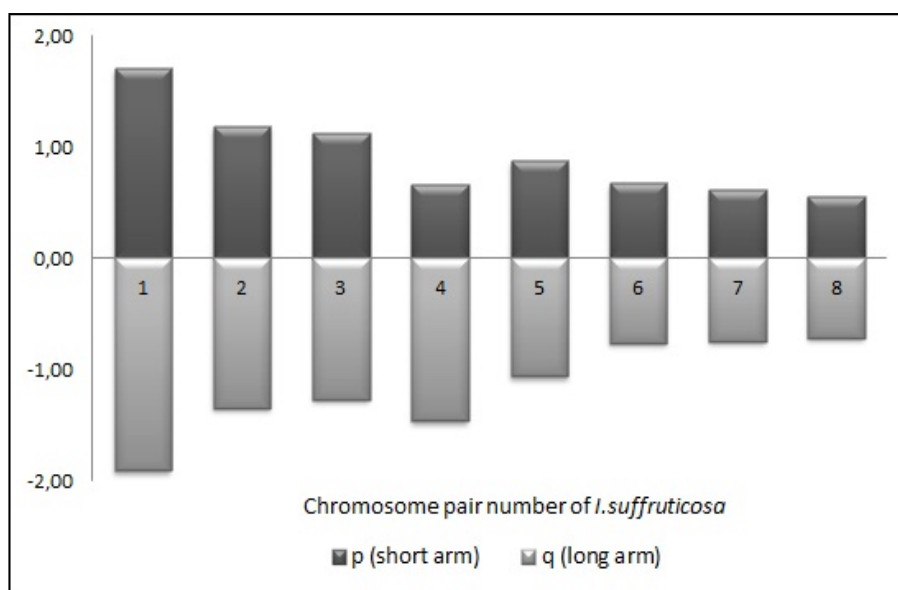


Figure 2. Ideogram of *I. suffruticosa*

Table 1. Morphology of somatic chromosome of *I. suffruticosa*

No	Short Arm (p) μm	Long Arm (q) μm	Total Chromosome Length (TL) μm	Centromere Index (CI)%	Arm Ration (AR) μm	Type
1	1.70	1.91	3.61	47.20	1.12	M
2	1.18	1.34	2.52	46.87	1.13	M
3	1.13	1.26	2.39	47.15	1.12	M
4	0.67	1.46	2.13	31.45	2.18	SM
5	0.87	1.06	1.93	45.26	1.21	M
6	0.68	0.76	1.44	47.01	1.13	M
7	0.61	0.75	1.36	45.10	1.22	M
8	0.56	0.71	1.27	44.03	1.27	M

I. suffruticosa has up to seven metacentric chromosomes (Ch1, Ch2, Ch3, Ch5, Ch6, Ch7, Ch8) and one submetacentric chromosome (Ch4) (Fig. 2). The length of a chromosome is an important indicator (other than DNA) for selecting an individual, sample, population or species (Kiran 2018). The variation in chromatin length and differences in karyotype formulas indicates the chromosomal structural changes and chromatin remapping such as removal or addition (Kashyap & Mehra 1983). The consistency of the karyotype indicated the adaptability to environmental conditions (Jha & Sen 1983). The evolution of karyotypes generally tends toward increasing the numbers and the progression toward terminal centromeric chromosomes, while the centromeric or metacentric type were the primitive ones (Imai *et al.* 1988). Cytological analysis, including the number of chromosomes and karyotypes, is a reliable method for studying taxonomic and evolutionary relationships (Ahirwar & Verma 2014). In addition, chromosome mapping can be used as a reference to analyze genetic diversity, and the selection of genetic markers in the initial process of plant breeding.

CONCLUSION

The number of chromosomes *I. suffruticosa* shows a difference in some countries, some have the formula $2n=32$, while another $2n=16$. In Indonesia showed $2n=16$. Chromosome cytology studies are important in completing the cytota-xonomic information of a plant. The results can support and complete the taxonomic classification information from previous research, Muzzazinah *et al.* (2016)

has already mentioned about its distribution in Indonesia. Karyotypes analysis of *I. suffruticosa* provides valuable data to overcome the problem of phenetic relationships. This research needs to be refined by expanding the samples to include *I. suffruticosa* from various regions in Indonesia. The information on the genetic composition of chromosomes in this study can be used for plant breeding through cytogenetic techniques.

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