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Research Article
Cytogenetic Analysis of Seven Local Corn Cultivars from Kisar Island-southwest Maluku, Indonesia

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Abstract

Background and Objective: Cytogenetic and kinship of seven local corn cultivars from Kisar Island, have different chromosome characteristics. The research was aimed to analyze the cytogenetic and kinship of seven local corn cultivars from Kisar Island, Southwest Maluku based on the chromosome characteristics. **Materials and Methods:** The samples of the study were the seed of seven local corn cultivars. Chromosome analysis was conducted with the squashing method. Cytogenetic traits were observed namely chromosome number, size and shape using Micromeasure 3.3 software and Karyotype Smart Express and the identification of the corn cultivars kinship was performed using cluster analysis. **Results:** Seven Kisar corn cultivars have the same chromosome number (2n = 20). The smallest size of *merah delima tongkol coklat* (69.49 µm), the largest *Kuning genjah* (120.12 µm). The composition of the corn cultivar karyotype is similar, except for *merah darah* (chromosomes 14 and 15). The closest relationship between *pulut* and *putih* (c.i 0.004) while the farthest between *kuning genjah* and *merah delima tongkol coklat* (c.i 3.214). **Conclusion:** Also, corn has the potential to be developed into higher quality plants and needs to be supported by cytogenetic information, namely chromosome analysis. It is expected that karyotype research on the seven Kisar corn cultivars can provide information for the community to develop breeding efforts for the seven Kisar corn cultivars.

Key words: Cytogenetic analysis, kinship, Kisar Island, local corn cultivars, chromosome analysis, breeding efforts

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant cytogenetics continues to evolve and make important contributions to genomic research by drawing marker sequences and revealing genome rearrangements[1]. Ferguson-Smith[2], suggests that in cytogenetics the shape and structure of chromosomes are studied, the behavior of chromosomes, changes in the structure and number of chromosomes and the evolution of chromosomes. Cytogenetic studies provide information about the number of chromosomes, length, type, ploidy and chromosome distribution of specific sequences that are important to explain the genome structure and constitution of plant species. This information can be useful in identifying species, tracking phylogenetic relationships between related species and breeding and developing plant species[3]. Maize is the most thoroughly researched genetic system. Several attributes of the maize plant, including a vast collection of mutant stocks, large heterochromatic chromosomes, extensive nucleotide diversity and genic collinearity within related grasses, have positioned this species as a centerpiece for genetic, cytogenetic and genomic research[4].

Chromosomes have a very important role for the survival of a living creature because chromosomes are a means of transporting genes that will be transferred from a parent cell to their daughter cells, from one generation to another generation[5]. Observing chromosome behavior is as important as studying the structure of chromosomes. The behavior or activity of chromosomes can be seen in the cell cycle, including cell division[6]. Organized and different genomes at the chromosome level are fundamental to understanding the dynamics of chromosome structure and karyotypes[7]. Traditionally, the numbers of chromosomes, genome size and number of nucleoli regulators have been used as a basis for studying the organization of chromosomes[8].

Chromosome analysis which includes the morphological characteristics of chromosomes (shape and size) can produce information on the formation of chromosomes called karyotypes[9]. Kinship was based on phenotypic analysis of several appearances on the phenotype of an organism. The phylogenetic relationship between two individuals or populations can be measured by the number of characters in common with the assumption that different characters are caused by differences in genetic makeup[10]. Microscopic observations, including phase, polarization and fluorescence optics, provide descriptive information about mitotic events and also allow important experiments on the function of mitoses, such as spindle fiber dynamics and the forces generated for chromosome movement[11]. The karyotype analysis has been used to characterize cultivars, to integrate genetic and physical maps and for the integration of relationships between species[12]. According to Her and Jiang[12], karyotypes are usually hampered by a lack of chromosome markers, which successfully collect individual chromosomes. McCouch[13], states that important food ingredients such as corn have been developed as plant breeding products. The cereal breeding business was carried out with tissue culture techniques, cross-breeding between cultivars and chromosomal analysis[14].

Research on chromosomal analysis in maize has been carried out for a long time in various cultivars[15-24]. These studies prove that corn at different cultivar levels has a diversity of chromosome numbers. The number of corn chromosomes, in general, is 2n = 20 except Zea perennis with 2n = 40[25,26]. Corn (*Zea mays*) is one of the most widely grown Gramineae plants for various purposes both in the food, feed or bioenergy industry[27]. Corn can maintain environmental conditions and quickly produce homozygous populations.

In Maluku, maize development is directed at dry land agroecosystems because maize is easily cultivated and grows in various types of soil. Corn has a broad land adaptation but low productivity. According to Ivakdalam[28], an obstacle often faced in increasing corn production is low soil fertility, where around 89% of corn plants in Indonesia are produced on dry land with low fertility rates. Also, other abiotic constraints such as low availability of nutrients in the soil and disturbance caused by plant-disturbing organisms. Potential land for agricultural food crops, including corn, in Maluku is 903 thousand ha, consisting of 718 thousand ha (80%) dry land and 55.6 thousand ha (6%) wetlands. In dry soils, superior varieties remain superior varieties because they are tolerant or resistant to biotic and abiotic stress[29].

Morphologically, the seven cultivars differ in vegetative and generative organs in local corn cultivars from Kisar Island. Sinay and Karuwal[30] stated that there are several variations on the color of the stem, the shape of the ligule edge, the presence of feathers on the midrib, the leaf strands and the number of leaves, the number of colors of male and female flowers, the fruit especially the color of the seeds, the shape and size fruit. Also, local maize cultivars from Kisar Island are tolerant of drought[31]. This is caused by conditions of growth with limited water in this area. The results of another study conducted by Sinay *et al.*[32], by studying the morphological appearance of corn cultivars in Southwest Maluku, concluded that maize corn showed different morphologies based on differences in cultivars. Seven cultivars found in Southwest Maluku, among others *merah delima tongkol coklat*, *merah delima tongkol Putih*, *merah darah*, *lokal putih*, *pulut*, *kuning genjah* and *kuning dalam*.

The study of the cytogenetic corn cultivar of Kisar has not been carried out to obtain a database in the development of plant breeding, especially superior varieties of corn Kisar. Other than that, on the other hand, the maize has the potential to be developed into higher quality plants. This effort was supported by cytogenetic information namely chromosome analysis. For this reason, the purpose of this study was to analyze seven local cytogenetic corn cultivars from Kisar Island, Southwest Maluku and the kinship analysis of seven local corn cultivars based on variations in chromosome characteristics.

MATERIALS AND METHODS

Study area: Observation of chromosomes through the identification of cytogenetic properties (number, size and shape of chromosomes) was carried out in the General Biology Laboratory, Biology Education Program, Faculty of Teacher Training and Education, Pattimura University, Ambon. This research was conducted for 2 months, starting on May 1-June 31, 2019.

Plant materials: Seed of seven local corn cultivars from Kisar island, Southwest Maluku were used as the samples of the current study i.e., *kuning dalam, kuning genjah, merah delima tongkol coklat, merah delima tongkol putih, merah darah, pulut* and *putih* (Fig. 1).

Procedures: The seeds of the seven local corn cultivars were germinated on moist cotton in Petri dishes. This process was intended to let the roots grow. The roots tips would be used as the preparation materials because these meristematic organs are always splitting to move and look for nutrients. The roots would be cut at the optimum time which had been determined earlier for mitosis. Pre-treatment was conducted by soaking the materials in distilled water for 24 h at 5-8°C. Pre-treatment executed in cold water at 5-8°C for 24 h would generate microscopic preparations with scattered chromosomes[33,34].

Samples were planted until 8 weeks old, then the root tips were taken. The root tip was cut (0.5 cm) and then put in a 45% acetic acid fixative solution and placed at -20°C for 15 min. For long-term storage of the sample, the sample is

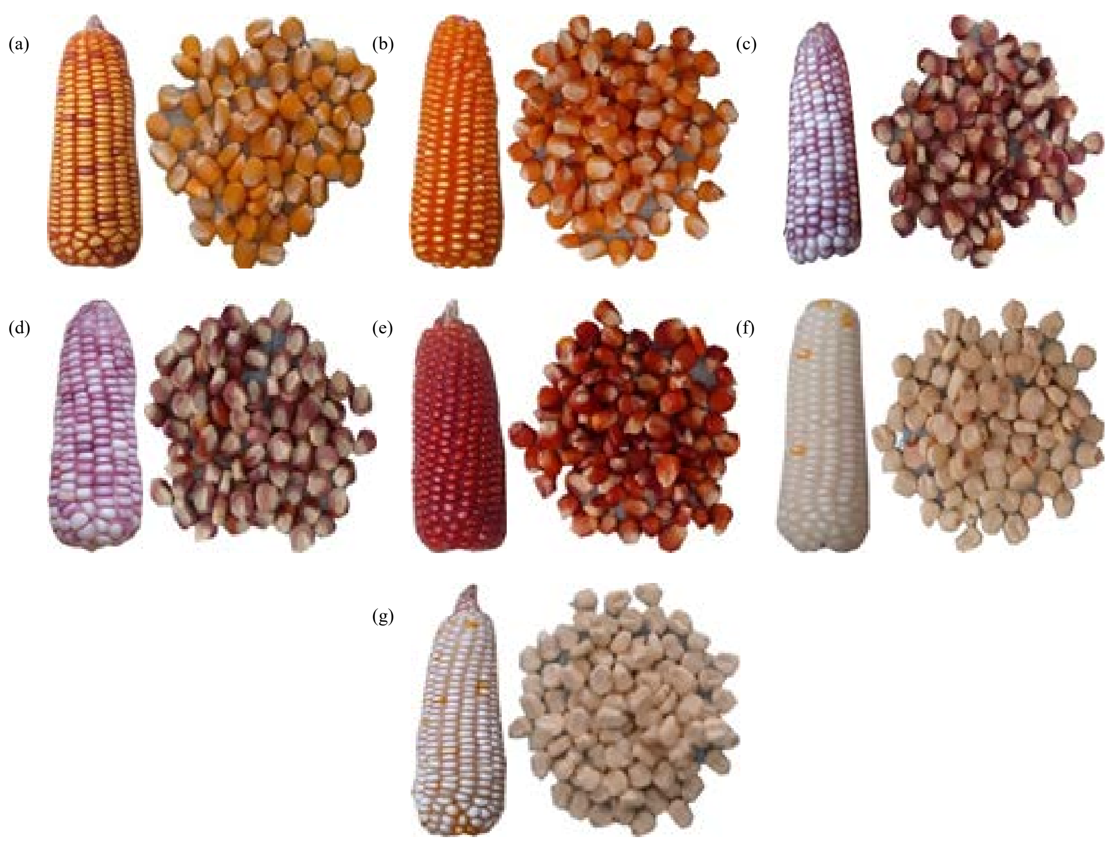

Fig. 1(a-g): Samples of the current study, (a) *Kuning dalam*, (b) *Kuning genjah*, (c) *Merah delima tongkol coklat*, (d) *Merah delima tongkol putih*, (e) *Merah darah*, (f) *Pulut* and (g) *Putih* transferred to a 25% acetic acid fixative solution and stored at 4°C. Hydrolysis is done by immersing the sample in a 10% HCl solution and placed in a water bath at 60°C for 30 min. The next process is rehydration. Rehydration is done by immersing the sample in graded alcohol (90, 70, 50%) each for 2 min followed by a coloring process. Staining is done by soaking the sample in aceto-orcein and placed in a water bath at 50° for 30 min, then the root tip samples are squash, observed and documented[35]. Finally, the measurement of the chromosome arm uses the scion-image application and determines the shape of the chromosome based on the arm (r) ratio. Based on the arm ratio (r = q/p) there are 4 chromosome shapes, namely metacentric: 1.0 μm <r £ 1.7 μm; submatrices: 1.7 μm <r £ 3.0 μm; acrocentric: 3.0 μm <r £ 7.0 μm and telocentric> 7.0 μm[36-38].

Data analysis: The observations were analyzed descriptively to identify the cytogenetic properties (number, size and shape of chromosomes) of seven kisar corn cultivars using Micromeasure 3.3 software and Smart express Karyotype. The results of the seven corn kisars cultivar karyotype were then used to identify the kinship between the seven kisar corn cultivars using cluster analysis.

RESULTS AND DISCUSSION

Distribution and karyotype of local corn cultivars: The findings suggest that there are some differences found in the distribution, size and shape of the cultivars chromosomes; however, the seven cultivars were reported to have the same number of chromosomes, 2n = 20. It has been suggested byAristya[39] that the in plants that are in the same species have the same number of basic chromosomes, it is because one of the characters that states an organism in the same species other than the morphological character of flowers as a means of reproduction is the number of basic chromosomes.

The Distribution and karyotype on each cultivar in Fig. 2 showed that there were some similarities and differences found in each observed cultivar.

In Fig. 2a performed that *Kuning dalam* cultivar has a big number of chromosomes that seemed to be overlapping while in Fig. 2b, chromosomal images which were arranged in a karyotype showed that the chromosome number determined was 2n = 20. In addition, the result of the microscopic photograph of the *Kuning genjah* cultivar at a magnification of 1000x such as suggested in Fig. 2c showed a considerable amount of chromosomes that lied over each other. Chromosomal images which were arranged in a karyotype in Fig. 2d indicated that the chromosome number determined similar to *Kuning dalam*.

Chromosome number is an observable and stable chromosome characteristic. Iqbal *et al.*[40] have found that corn commonly has 2n = 20 chromosomes, except for *Zea perennis* which has 2n = 40. The difference in the chromosome number results from the aneuploidy process which allows the addition or reduction of one chromosome or some chromosomes in one ploidy. This process can occur due to the deviation of chromosomal segregation during meiosis in metaphase. This segregation failure may happen at meiosis I, where two homologous chromosomes move to the same polar or at meiosis II, where two chromosome siblings in one chromatid do not move separately to different polar[41].

On *Merah darah* and *merah delima tongkol coklat* cultivar suggested a big amount of chromosomes that were also overlapping. Chromosomal images which were arranged in a karyotype showed that had 2n = 20 chromosomes (Fig. 2e-h). Unlike *merah delima tongkol coklat,* the result of the microscopic photograph of *merah delima tongkol puith* at a magnification of 1000x such as suggested in Fig. 2i indicated a considerable amount of chromosomes that lied over each other. Chromosomal images which were arranged in a karyotype in Fig. 2j indicated that the chromosome number determined was 2n = 20.

This finding shows that there are genetic differences found in the seven cultivated cultivars of Kisar corn. The different chromosome lengths are caused by mitosis division. Points out that change in chromosome length may occur during mitosis, especially at the prophase. As a result, plants that belong to the same species but different cultivars may have varied chromosome lengths because the chromosome is measured from dissimilar cells and cell division time. It confirms the statement by Piskadlo and Oliveiraa[42], who suggest that chromosome condensation level may affect the chromosome length variations. Based on its size, the chromosome of all seven cultivars can be categorized as a large chromosome (>1 μm) and a small chromosome (<1 μm)[43].

Similar to *Merah darah* cultivar, *pulut* and *putih* cultivars also have the microscopic photograph at 1000x magnification suggested a big amount of chromosomes which were overlapping and chromosomal images which were arranged in a karyotype had 2n = 20 chromosomes (Fig. 2k-n). In general, all cultivars have the chromosome number many as 2n = 20.

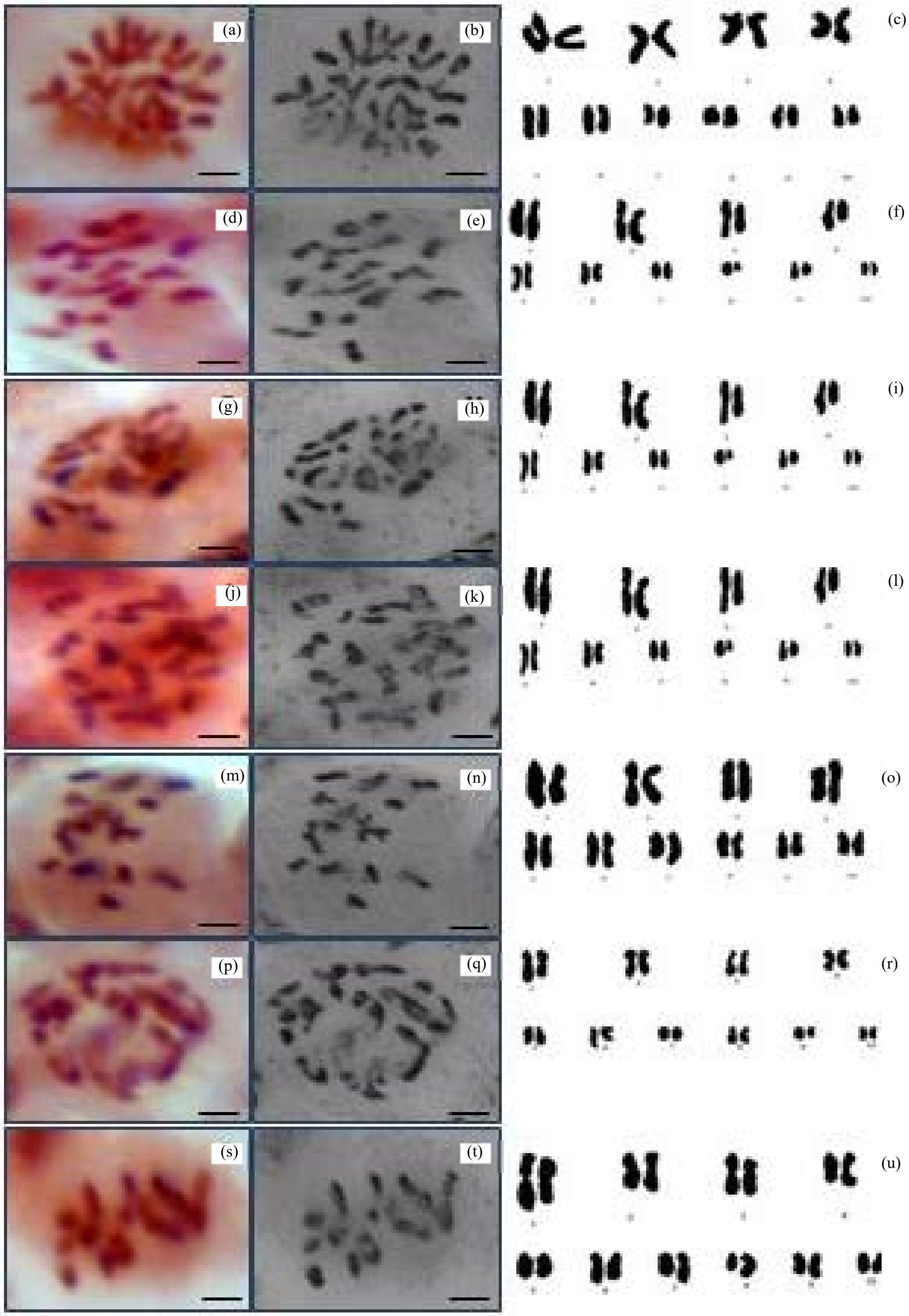

Fig. 2(a-u): Distribution of the chromosome and karyotype of the local corn cultivars from Kisar Island, (a) Color, (b) Gray, (c) *Kuning dalam*, (d) color, (e) Gray, (f) *Kuning genjah*, (g) Color, (h) Gray, (i) *Merah darah*, (j) Color, (k) Gray, (l) *Merah delima tongkol coklat*, (m) Color, (n) Gray, (o) *Merah delima tongkol putih*, (p) Color, (q) Gray, (r) Pulut, (s) Color, (t) Gray, (u) *Putih*

Scale bars = 5 µm

In addition, the centromere location suggests that the seven cultivars have some karyotype compositions as follows: *Kuning dalam* cultivar is composed of 15 M + 5 SM, *Kuning genjah* is 19 M+1 SM, *merah darah* is 10 M+8 SM+2 T, *merah delima tongkol coklat* is 19 M+1 SM, *merah delima tongkol putih* is 19 M+1 SM, *pulut* is 20 M, *putih* is 20 M. The differences in the karyotype compositions and shapes also result from the chromosome length variations. The chromosome type of the seven cultivars differs significantly. Among all, only *Merah darah* cultivar reported an acrocentric chromosome which was found in chromosome number 14 and 15. On the other hand, other cultivars only reported metacentric and submetacentric chromosomes. These shapes cannot be found in all cultivated cultivars of Kisar corn. Some corn cultivars only have metacentric chromosome *pulut* and *putih* cultivars. Even so, it still can be said that the seven cultivars have a close kinship relationship despite a few differences found in the karyotype composition and chromosome length. According to Cuacos *et al.*[44] metacentric chromosomes are generally found in plants.

Size and shape chromosome of local corn cultivars: Cytogenetic traits consist of the length of the arm, total length, arm ratio and the shape of the chromosome observed in all cultivars are different (Table 1).

On the Table 1, size and shape of the chromosome observed in *Kuning dalam* varied greatly. The smallest chromosome total length was 2.01 µm and the highest chromosome total length was 8.75 µm. These Fig. 2a suggested that the cultivar chromosome was categorized as a large chromosome. *Kuning dalam* cultivar gave two apparent chromosome shapes i.e., metacentric and submetacentric. There were 15 metacentric and 5 submetacentric chromosomes observed in this cultivar. The submetacentric chromosomes were coded for chromosome numbers 9, 13, 14, 15 and 20 while the rest was metacentric. Thus, the karyotype formula for the *Kuning dalam* cultivated cultivar of Kisar corn can be defined as 2n = 15 metacentric (m)+5 submetacentric (sm).

Kuning genjah cultivar, the smallest total length of the chromosome was 0.73 µm and the highest total length of the chromosome was 10.57 µm. These numbers suggested that the chromosomes could be categorized into two, large and small chromosome. There were two chromosome shapes reported by the *Kuning genjah* cultivar, metacentric and submetacentric. There were 19 metacentric and 1 submetacentric chromosome (chromosome number 20) observed in this Kisar corn cultivated cultivar. Therefore, karyotype for *Kuning genjah* cultivated cultivar of Kisar corn can be formulated as 2n = 19 m+1 sm.

Merah darah, the smallest total length of the chromosome was 1.35 µm and the longest was 5.67 µm. These numbers categorized the chromosomes into the large type of chromosome. There were three chromosome shapes observed in *Merah darah* cultivar, metacentric, submetacentric and acrocentric. There were 10 metacentric, 8 submetacentric and 2 acrocentric chromosomes. The metacentric chromosomes were coded for chromosome number 1, 2, 6, 8, 10, 11, 12, 13, 18 and 20 while submetacentric chromosomes were found in chromosome number 2, 4, 5, 7, 9, 16, 17, 19 and acrocentric chromosomes were reported by chromosome number 14 and 15. Thus, the karyotype formula can be defined as 2n = 10 m+8 sm+2 t.

Merah delima tongkol coklat, the smallest chromosome total length was 0.25 µm and the highest chromosome total length was 9.83 µm. These Figs suggested that the cultivar chromosome could be categorized as a large chromosome. It gave two apparent chromosome shapes, metacentric and submetacentric. The chromosomes were divided into 19 metacentric and 1 submetacentric. The submetacentric chromosome was found in chromosome number 15 while the rest was metacentric with the karyotype formula can be defined as 2n = 19 m+1 sm.

Merah delima tongkol putih, the smallest total length of the chromosome was 0.54 µm and the highest total length of the chromosome was 8.37 µm. These Figs suggested that the chromosomes could be categorized into two, large chromosomes and a small chromosome. There were two chromosome shapes reported by *merah delima tongkol putih*, metacentric and submetacentric. There were 19 metacentric and 1 submetacentric chromosome (chromosome number 20) observed in this Kisar corn cultivated cultivar. Therefore, karyotype for this cultivar can be formulated as 2n = 19 m+1 sm.

Pulut, the smallest total length of the chromosome was 0.42 µm and the longest was 10.65 µm. These numbers categorized the chromosomes into two, large and small type of chromosome. *Pulut* cultivar gave one chromosome shape that is metacentric. Thus, the karyotype formula could be 2n = 20 m and this is unique.

Putih, the smallest chromosome total length was 0.32 µm and the highest chromosome total length was 10.41 µm. These Figs suggested that the cultivar chromosome could be categorized into two, large and small chromosomes. *Putih*

Table 1: Size and shape of the chromosome observed in seven local corn cultivars

		LA (µm)							LA (µm)				
Cultivars	NC	q	p	TL (µm)	AR	SC	Cultivars	NC	q	p	TL (µm)	AR	SC
Kuning dalam	1	5.23	3.10	8.33	1.69	M	*Merah delima tongkol coklat*	1	4.97	4.86	9.83	1.02	M
	2	5.51	3.24	8.75	1.70	M		2	4.75	4.54	9.29	1.05	M
	3	3.32	2.10	5.42	1.58	M		3	4.32	4.21	8.53	1.03	M
	4	3.83	3.59	7.42	1.07	M		4	4.00	3.73	7.73	1.07	M
	5	3.23	2.78	6.01	1.16	M		5	3.52	3.21	6.73	1.10	M
	6	3.97	2.94	6.91	1.35	M		6	3.00	2.74	5.74	1.09	M
	7	2.62	2.41	5.03	1.09	M		7	2.55	2.36	4.91	1.08	M
	8	2.73	1.78	4.51	1.53	M		8	2.17	1.78	3.95	1.22	M
	9	3.15	1.26	4.41	2.50	SM		9	1.59	1.32	2.91	1.20	M
	10	2.24	1.53	3.77	1.46	M		10	1.21	1.00	2.21	1.21	M
	11	2.52	1.51	4.03	1.67	M		11	0.87	0.78	1.65	1.12	M
	12	2.29	1.80	4.09	1.27	M		12	0.65	0.64	1.29	1.02	M
	13	1.97	0.76	2.73	2.59	SM		13	0.53	0.42	0.95	1.26	M
	14	1.62	0.63	2.25	2.57	SM		14	0.42	0.31	0.73	1.35	M
	15	2.41	1.12	3.53	2.15	SM		15	0.43	0.24	0.67	1.79	SM
	16	1.25	0.76	2.01	1.64	M		16	0.35	0.36	0.71	0.97	M
	17	2.24	1.55	3.79	1.45	M		17	0.37	0.29	0.66	1.28	M
	18	1.33	1.22	2.55	1.09	M		18	0.21	0.22	0.43	0.95	M
	19	1.51	1.39	2.90	1.09	M		19	0.15	0.17	0.32	0.88	M
	20	1.89	0.77	2.66	2.45	SM		20	0.13	0.12	0.25	1.08	M
Kuning genjah	1	5.34	5.23	10.57	1.02	M	*Merah delima tongkol putih*	1	4.23	4.14	8.37	1.02	M
	2	5.12	4.91	10.03	1.04	M		2	4.00	3.75	7.75	1.07	M
	3	5.58	4.77	10.35	1.17	M		3	3.86	3.67	7.53	1.05	M
	4	5.26	4.55	9.81	1.16	M		4	3.32	3.00	6.32	1.11	M
	5	4.94	4.73	9.67	1.04	M		5	3.21	3.13	6.34	1.03	M
	6	4.52	3.91	8.43	1.16	M		6	3.14	2.75	5.89	1.14	M
	7	4.33	3.83	8.16	1.13	M		7	2.27	2.58	4.85	0.88	M
	8	4.00	3.54	7.54	1.13	M		8	2.49	2.37	4.86	1.05	M
	9	3.93	3.82	7.75	1.03	M		9	2.26	2.15	4.41	1.05	M
	10	3.75	3.56	7.31	1.05	M		10	2.00	1.74	3.74	1.15	M
	11	3.00	2.87	5.87	1.05	M		11	1.93	1.51	3.44	1.28	M
	12	2.78	2.59	5.37	1.07	M		12	1.82	1.35	3.17	1.35	M
	13	2.31	2.00	4.31	1.15	M		13	1.66	1.27	2.93	1.31	M
	14	2.00	1.92	3.92	1.04	M		14	1.38	1.00	2.38	1.38	M
	15	1.73	1.55	3.28	1.12	M		15	1.25	0.96	2.21	1.30	M
	16	1.47	1.28	2.75	1.15	M		16	1.00	0.74	1.74	1.35	M
	17	1.00	0.93	1.93	1.08	M		17	0.83	0.51	1.34	1.63	M
	18	0.74	0.65	1.39	1.14	M		18	0.72	0.45	1.17	1.60	M
	19	0.57	0.38	0.95	1.50	M		19	0.36	0.28	0.64	1.29	M
	20	0.52	0.21	0.73	2.48	SM		20	0.39	0.15	0.54	2.60	SM
Merah darah	1	3.00	1.92	4.92	1.56	M	*Pulut*	1	5.38	5.27	10.65	1.02	M
	2	3.93	1.74	5.67	2.26	SM		2	5.35	5.16	10.51	1.04	M
	3	3.35	2.26	5.61	1.48	M		3	4.84	4.73	9.57	1.02	M
	4	3.28	1.37	4.65	2.39	SM		4	4.32	4.24	8.56	1.02	M
	5	3.26	1.13	4.39	2.88	SM		5	4.15	3.76	7.91	1.10	M
	6	2.54	2.21	4.75	1.15	M		6	3.97	3.56	7.53	1.12	M
	7	3.12	1.58	4.70	1.97	SM		7	3.64	3.23	6.87	1.13	M
	8	2.19	1.76	3.95	1.24	M		8	3.12	3.00	6.12	1.04	M
	9	3.27	1.84	5.11	1.78	SM		9	2.71	2.55	5.26	1.06	M
	10	2.75	1.92	4.67	1.43	M		10	2.37	2.19	4.56	1.08	M
	11	2.23	1.61	3.84	1.39	M		11	2.15	1.56	3.71	1.38	M
	12	2.00	1.85	3.85	1.08	M		12	1.74	1.53	3.27	1.14	M
	13	1.95	1.27	3.22	1.54	M		13	1.62	1.48	3.10	1.09	M
	14	1.78	0.53	2.31	3.36	T		14	1.27	1.00	2.27	1.27	M
	15	1.24	0.31	1.55	4.00	T		15	1.16	0.75	1.91	1.55	M
	16	1.52	0.78	2.30	1.95	SM		16	0.84	0.63	1.47	1.33	M
	17	1.39	0.47	1.86	2.96	SM		17	0.72	0.51	1.23	1.41	M
	18	1.26	0.97	2.23	1.29	M		18	0.62	0.44	1.06	1.41	M
	19	1.38	0.74	2.12	1.86	SM		19	0.43	0.31	0.74	1.39	M
	20	0.85	0.50	1.35	1.70	M		20	0.25	0.17	0.42	1.47	M

Table 1: Continue

		LA (µm)				
Cultivars	NC	q	p	TL (µm)	AR	SC
Putih	1	5.25	5.16	10.41	1.02	M
	2	4.87	4.69	9.56	1.04	M
	3	4.72	4.53	9.25	1.04	M
	4	4.35	4.00	8.35	1.09	M
	5	3.94	3.73	7.67	1.06	M
	6	3.82	3.61	7.43	1.06	M
	7	3.57	3.38	6.95	1.06	M
	8	3.29	3.26	6.55	1.01	M
	9	3.15	3.00	6.15	1.05	M
	10	2.72	2.53	5.25	1.08	M
	11	2.34	2.25	4.59	1.04	M
	12	1.78	1.69	3.47	1.05	M
	13	1.51	1.32	2.83	1.14	M
	14	1.33	1.15	2.48	1.16	M
	15	1.00	0.94	1.94	1.06	M
	16	0.72	0.53	1.25	1.36	M
	17	0.51	0.42	0.93	1.21	M
	18	0.35	0.34	0.69	1.03	M
	19	0.28	0.17	0.45	1.65	M
	20	0.19	0.13	0.32	1.46	M

NC: Chromosome number, LA: Length of the arm, q: Long arm, p: Short arm, TL: Total length, AR: Arm ratio, SC: Shape of the chromosome, M: Metacentric, SM: Sub metacentric, T: Telocentric

Table 2: Proximity matrix of local corn cultivars

	Squared euclidean distance					
Cultivars	*Kuning dalam*	*Kuning genjah*	*Merah darah*	*Merah delima tongkol coklat*	*Merah delima tongkol putih*	*Pulut*
Kuning genjah	1.263					
Merah darah	0.408	3.010				
Merah delima tongkol coklat	0.893	3.214	0.362			
Merah delima tongkol putih	0.410	2.052	0.332	0.132		
Pulut	0.286	0.686	0.980	0.930	0.366	
Putih	0.347	0.706	1.036	0.913	0.361	0.004

Table 3: Average linkage (between group)

	Cluster combined			
Stage	1	2	Coefficients	Next stage
1	6	7	0.004	3
2	4	5	0.132	4
3	1	6	0.317	5
4	3	4	0.347	5
5	1	3	0.700	6
6	1	2	1.822	0

cultivar gave one chromosome shape that is metacentric. Thus, the karyotype formula of the *puith* cultivar could be defined as 2n = 20 m where, m = metacentric chromosome.

Based on the karyotype results from the seven cultivars, some similarities and differences could be identified in each cultivar. *Pulut* and *putih* cultivars were reported to have a similar shape for all chromosomes that is metacentric. Meanwhile, an obvious difference was found in *merah darah* cultivar of which two chromosomes were acrocentric. The biggest total length of the chromosome was reported by *kuning genjah* cultivar while the smallest was observed *merah delima tongkol coklat*.

Kinship of seven local corn cultivars : Based on the character's chromosome (size and shape) is obtained the kinship on the seven local corn cultivars from Kisar Island, Southwest Maluku. The results of the cluster statistical analysis showed that the seven local corn cultivars established a relationship. Findings on the cluster test are presented in Table 2 and provide information that the biggest squared euclidean distance value was found in between the *kuning*

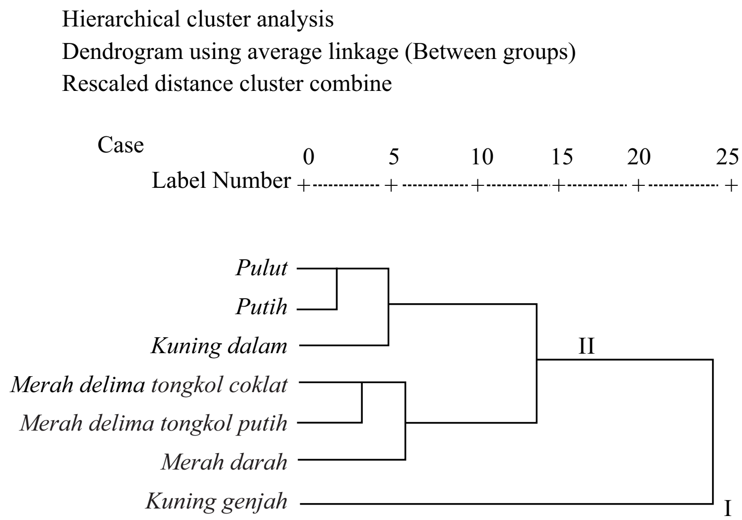

Fig. 3: Dendrogram on local corn cultivars from Kisar Island based the chromosome characters using cluster analysis

genjah and *merah delima tongkol coklat* cultivar (3.214) while the smallest was reported by *pulut* and *putih* cultivar (0.004). The cross groups cluster analysis which shown on cluster 1 and cluster 2 can be seen in Table 3 that suggests that the closest kinship is established between *pulut* and *putih* cultivar (0.004), followed by the k*uning dalam* and *pulut* cultivar (0.317), *Kuning dalam* and *Merah darah* cultivar (0.700) and *kuning dalam* and *kuning genjah* cultivar (1.822). Cluster hierarchical analysis explains the kinship through the following dendrogram.

Cluster analysis has proven that the closest kinship is established between pulut and putih cultivar (0.004). This close kin relationship is also indicated by the total length of the chromosome which does not vary greatly in the cultivars. The total length of the chromosome observed in pulut is 96.72 and the total length of the chromosome reported by putih cultivar is 96.52. Both cultivars only have a metacentric chromosome. In terms of the chromosome length, *kuning genjah* has the furthest kinship with other cultivated cultivars but is closest to *kuning dalam* cultivar (1.822). Xianran *et al.*[45], asserts that the further the relationship, the greater variations can be found in chromosome number, shape and order. *Kuning genjah* cultivar, in this case, has a different chromosome size and shape. The length of the chromosome observed in kuning genjah cultivar is 120.12 µm.

The dendrogram in Fig. 3 shows the formation of two clusters. The first cluster consists of the *pulut*, *putih*, *kuning dalam* and *kuning genjah* cultivars. *Kuning genjah* cultivar has been categorized into the first cluster due to its squared Euclidean distance value which is closer to those of the *pulut*, *putih* and *kuning dalam* cultivars than to those of other cultivars in the second cluster. Meanwhile, the other cultivars, *merah delima tongkol coklat*, *merah delima tongkol putih* and *merah darah* cultivars form the second cluster. The dendrogram obviously suggests that *pulut* and *putih* cultivar has the closest kinship (0.004), followed by *merah delima tongkol coklat* and *merah delima tongkol putih* (0.132), then *kuning dalam*, *pulut* and *putih* cultivars (0.317) and *merah darah*, *merah delima tongkol coklat* and *merah delima tongkol putih* (0.347). *Kuning dalam, putih* and *pulut* cultivars establish a kinship with *merah darah, merah delima tongkol putih* and *merah delima tongkol coklat* (0.700). *Kunig genjah* cultivar has a kinship relationship with *kuning dalam*, *pulut*, *putih*, *merah darah*, *merah delima tongkol coklat* and *merah delima tongkol putih* (1.822).

In other hand, chromosome size is one of the criteria to identify chromosome morphology. Chromosome size may vary from one species to another and usually ranges from 0.2-50 µm[46]. However, Silva *et al.*[47], reported that corn has a chromosome size of 1.11-2.22-1.11-2.22 µm. The chromosome size observation usually covers an observation on the total length of the chromosome (q+p), the length of the long arm (q) and the length of the short arm (p). Finding of the current study suggest that the longest total length of the chromosome can be observed in *kuning genjah* cultivar (120.12 µm), followed by the *pulut* cultivar (96.72 µm), *putih* cultivar (96.52 µm), *kuning dalam* cultivar (91.1 µm), *merah delima tongkol putih* (79.61 µm), *merah darah* (73.05 µm), *merah delima tongkol coklat* (69.49 µm).

Some extraneous factors may affect the results of the seven cultivars karyotype, such as bias factor. The bias factor could be indicated by the preparation technique performed in the laboratory. This study used squashing as the preparation technique. In squashing, the most important stages are fixation and coloring. Very quick fixation can result in poor result. The chromosome of some cultivars such as of the sticky cultivar and the white cultivar (Fig. 2) is not very transparent due to short and adjacent fixation time. In addition, in the observation process, chromosomes are overlapping because solution used in the pre-treatment is not 8-Hydroxyquinoline 0,002 can help improve the visibility of the chromosome. 8-hydroxyquinoline can be very useful in pre-treatment observation of chromosome distribution during the metaphase[48].

The results of the identification of these chromosomes can be used in detecting plant diversity and efforts to assemble new superior Kisar corn cultivars, so it is necessary to carry out research on chromosome Kisar corn using chromosome mapping techniques (chromosome banding) to identify homologous chromosomes individually and more accurately.

CONCLUSION

The results of the study can be concluded that all seven cultivars have the same number of chromosome that is 2n = 20. The smallest total length of the chromosome was observed in the red ruby corn with a brown cob (69.49 μm) and the biggest was reported by the yellow ripe corn variety (120.12 μm). The karyotype arrangement for each corn cultivar was the same, except for the blood-red corn variety of which chromosome was acrocentric (chromosome number 14 and 15). The kinship of the seven corn cultivars was established in two clusters. The closest kinship was observed in the waxy and the white corn variety (coefficient index of 0.004) while the furthest kinship was established between the yellow ripe corn and the red ruby corn with a brown cob (coefficient index of 3.214).

SIGNIFICANCE STATEMENT

Seven cultivars of maize range are almost the same except for the *merah darah* cultivars which have an acrocentric chromosome shape on chromosomes number 14 and 15. In addition, the kinship relationship of the seven corn cultivars consists of two clusters with the closest kinship found in the *pulut* with *putih* cultivars with coefficient index 0.004 and the farthest kinship was found in *kuning genjah* cultivars with *merah delima tongkol coklat* cultivars with a coefficient index of 3,214. This study will help researchers to uncover critical areas of the question how the karyotypes of the seven corn maize cultivars and how the kinship between the seven maize corn cultivars is based on their chromosome characteristics, these results which cannot be explored by many researchers. Thus, a new theory about the information on the characteristics of chromosomes can be analyzed the kinship of seven cultivars of maize corn as an effort to improve the genetic quality of the species of corn, can be achieved.

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