The comparison of morphological characters and capsaicinoid contents of the 4th generation chili pepper genotypes G1/01 and G7/01 (Capsicum frutescens L.)

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Abstract. The study aims to identify the morphological and physiological variation of the 4th generation of C. frutescens L. G1/01 and G7/01. The morphological data measured based on IPGRI, AVRDC and CATIE descriptors, and capsaicinoid content measured using spectrophotometry were used to build Jaccard similarity-based cladogram. The results showed that 40% of the G1/01 and 100% of the G7/01 plant height were categorized into very high category (> 85 cm). One hundred percent of the G1/01 and G7/01 were categorized into very early and late flowering, respectively. Eighty-five percent of the G1/01 and 100% of the G7/01 fruit length were categorized into normal (1-5 cm). Ninety percent of the G1/01 and 56% of the G7/01 fruit diameters were categorized into normal (> 0.5-1 cm). Eighty-nine percent of G1/01 and 100% of G7/01 fruit weight were categorized into moderate (0.1-2 g). Eighty percent of the G1/01 and 56% of the number of seeds were categorized into moderate (> 50-200 seeds) and small (<50 seeds), respectively. The capsaicinoid content of G1/01 and G7/01 was significantly lower than the original type. Mutant T39 (G7/01) had the highest capsaicinoid content (19.35 mg/g). In the dendrogram, genotypes G1/01 and G7/01 were separated based on the character of the node color, petal color, peel surface, leaf color, and flowering time. G1/01 formed five clusters while G7/01 formed two clusters. G1/01 is more varied than G7/01.

Keywords: Morphology, Capsaicinoid, C. frutescens, mutation, EMS.

1. Introduction
Chili (Capsicum spp.) is a species of the Solanaceae family originated from tropical areas in Central America and South America [1]. Chili (Capsicum spp.) has spread to other areas such as Europe, Africa and Asia [2]. There are five varieties of chili that have been cultivated such as Capsicum chinense Jacq., C. annuum L., C. frutescens L., C. pubescens, and C. baccatum [3]. Chili pepper (C. frutescens. L.) is one of the favorite vegetables and spices due to its spicy and nutritional content [4]. Chili pepper contains capsaicinoid, carotenoid, ascorbic acid, tocopherols, and flavonoids [5]. Capsaicinoid belongs to the alkaloid group that is synthesized by the Capsicum genus in fruit placenta tissue [6]. Capsaicinoids provide a hot or spicy sensation so that they become an attraction for consumers.

In Indonesia, chili pepper has been used as the basic spice for almost all dishes. The consumption of chili pepper relatively high and increasing on special day or religious celebrations. According to the Ministry of Agriculture the consumption of chili pepper in Indonesia in the period of 2002-2018...
fluctuates but tends to increase. In 2002, consumption was around 1.126 kg/capita then increased by 1.835 kg/capita in 2018 or increased by an average of 7.53% [7]. The high of chili pepper consumption needs to be balanced with an increase in production. Therefore, it is necessary to develop high-quality chili cultivar as an effort to optimize chili productivity. Genetic diversity is important in the production of superior plants [4]. The existence of diversity in a population makes it easy to select plants based on the desired traits [8]. Generally desired properties are those associated with high productivity, pets, and environment resistance. Genetic diversity can be done by induction of mutations. Induction of mutations in chili pepper collected from several places in Indonesia has been carried out in previous studies [9,10,11].

Mutation altered DNA and RNA sequences that have an impact on changes in gene composition, nitrogen bases, and even chromosomes, resulting in genetic variations [12,13]. Ethyl Methane Sulfonate (EMS) as a mutation agent caused variations in the genotypes of chili G1/01 and G7/01 [11,14,15]. Selection on G1/01 and G7/01 has been carried out until the 3rd generation, resulted in better characters such as higher plants, more branching numbers, earlier flowering times, and drought resistance (specifically G7/01). Plant height has the potential to reduce the risk of fungal infection or soil pests because it is far from the fruit, the number of branches has the potential to produce a lot of twigs and flowers, and earlier flowering times character can help farmers harvest faster and increase productivity. These characters began to stabilize in the 3rd generation, but segregation was still detected. Based on the above background, analysis the genetic variation of the 4th generation G1/01 and G7/01 genotypes was carried out based on morphology and physiology characters (capsaicinoid content).

2. Material and Method

Fifty seeds of chili pepper G1/01 and G7/01 and their original types (n = 10) were grown on soil and compost media (1: 1) in 25X30 cm3 polybag. Morphology analysis includes identification of vegetative organs such as habitus, leaf color, and plant height at 16 weeks after seedling. Identification of generative organs including flowering time and petal color were observed when the flowers were in full bloom. Fruit color and peel surface were observed at 20 days after anthesis (DAA), while fruit length, fruit width, fruit weight, and number of seeds were measured at 55 DAA. Morphology character descriptions were carried out based on the Capsicum spp. IPGRI, AVRDC, & CATIE, 1975 [16].

Capsaicinoid content was measured from 20-25 DAA fruit. The placenta and pericarp of chilies weighed as much as 0.5 g, then crushed with a mortar and pestle to form a paste. Five milliliters of 96% ethanol are added. Furthermore, the solution is filtered using filter paper that has been placed on the flacon. The extract was diluted 10 times with absolute ethanol. The absorbance value of the extract was measured using a UV-Vis spectrophotometer at a wavelength of 280 nm. The absorbance value obtained was used to calculate capsaicinoid content based on the linear equation between the absorbance value and the known standard solution concentration. The data obtained were analyzed statistically using the T-independent test (normal data) or the Mann-Whitney test (abnormal data) to determine differences with the original type.

The binary data format is used to determine the similarity and groups with the same character using the unweighted pair group method with arithmetic mean (UPGMA) based on the Jaccard coefficient and the numerical taxonomy and multivariate analysis system2.1 (NTSYS) software.

3. Results and Discussion

3.1 Qualitative character analysis
The G1/01 and G7/01 genotypes have differences on node color, leaf color, petal color, and peel surface. G1/01 genotype has purple node, dark green leaf, white petal, and smooth peel (Fig. 1). The characteristics of the G7/01 genotype were green node, light green leaf, greenish-white petals, and semi-wrinkled peel (Fig. 1). Previous research asserted that the characteristics of G1/01 are more
similar to those of *C. annuum* than *C. frutescens* [9]. *C. annuum, C. chinense, and C. frutescens* originated from the same gene pool parents called the “annuum-chinense-frutescens complex” making it difficult to separate overlapping morphological characters [17,18]. However, G1/01 genotype is a member of *C. frutescens* as characters from G1/01 were also found in accessions of *C. frutescens* from Brazil, such as CNPH3716 and CNPH4304 [19]. *C. frutescens* is an upright plant with small fruit, upright fruit type, white-greenish petal, elongated fruit shape, ovale or ovoid-shaped leaves, and absent a fruit tip protrusions [20]. The original type of G1/01 is Cabai Rawit Cakra Hijau, while the origin of G7/01 is local chili pepper obtained from Lombok Island, NTB.

### Figure 1. Differences between G1/01 and G7/01 based on the color of the nodes, leaf color, petal color and fruit peel.

*Capsicum* spp. has three types of habitus, namely prostrate, compact, and erect [16]. Most of the G1/01 and G7/01 mutants had erect and prostrate habitus. However, some of the G1/01 mutants had a branchless habitus which became unique (Fig. 2). The branchless habitus produced a single stem. Leaves and flowers grow but not as much as the prostrate and erect habitus. Plant habitus is determined by branching, and branching is very sensitive to environmental factors [21]. However, the research environment was prepared equally in terms of both the distances and treatments of plants. EMS 0.01% mutation caused allele changed that caused chili plants to grow without branching. According to Arumingtyas, the presence of new alleles due to mutations can cause distinctive and unique characters [13].
There are three types of branching in this study, namely monopodial, dichotome, and trichotome. Monopodial is a branching with one main stem, this branching is only found in the G1/01 mutant. Dichotome branching produced two stems of the same size. Dichotome branching was found in many G1/01 and G7/01 genotypes and the original type. Trichotomes formed three branches on the main stem. Trichotomes were found in several G1/01 and G7/01 mutants (Fig. 3c). Another study found trichotome branching in the Arabidopsis mutant plant [22]. Branches are very important for plants because these are growing points for leaves, flowers, and fruit [23]. Plants with trichotome branching have the potential to produce more leaves, flowers, and fruits.

Generally, the fruit color in G1/01 is dark green. However, in this study, light green and white color were also observed. Meanwhile, the G7/01 has light green fruit color only. G1/01 has more variation in fruit color than G7/01 (Fig. 4). The color of the fruit was dominated by dark green and light green. According to Wahyuni the dark green and light green colors are commonly found in C. frutescens fruit [24]. Previously study reported that the fruit color of C. frutescens is generally green[19]. The white fruit is very different from the others which probably the effect of 0.01% EMS mutation. White fruit is
an expression of recessive genes while the dominant gene controlled green [25]. Segregation occurs in the G1/01 mutants due to the EMS mutation. According to Srivastava & Jitendra, segregation cause changes in physiology properties and chemical processes of plants [26].

![Figure 4. Color and shape fruit. Color of G1/01 mutants: Dark green, light green, and white; Color of G7/01 mutants: Light green. Shape of G1/01 mutants: Hornshaped (Dominantly) and Moderately triangular (yellow triangular); Shape of G7/01: Hornshaped.](image)

The results of the qualitative character analysis showed that G1/01 has more variations than G7/01. Qualitative characters are controlled by simple genes or do not involve many genes and can be influenced by environmental factors [27]. The environmental conditions in this study have been arranged the same. Thus, the phenotypic differences in qualitative characters were caused by the induction of 0.01% EMS mutation.

### 3.2 Quantitative character analysis

G1/01 and G7/01 genotypes had mean heights of $79.24 \pm 17.5$ cm and $128 \pm 18.5$ cm respectively and did not differ significantly from their original type ($\alpha < 0.05$). Forty percent of the G1/01 were categorized into very high (> 85 cm), 26% were into high category (66-85 cm), and 33% were into the normal category (46-65cm). The plant height of the original type of G1/01 was $77.6 \pm 14.6$ cm, it was into the height category. The G1/01 plant height showed variation, on the other hand, the G7/01 had a similar height. One hundred percent of the G7/01 were into very high as well as the original type (Table 2.). There were 50% of G7/01 genotype higher than the original types. The highest mutants (T3, G7/01) reached 160 cm. Other investigation had noted that the maximum height of *C. frutescens* was 120 cm [19]. The presence of mutants reaching 160 cm in height indicates that EMS 0.01% affected
the height of the G7/01 mutant. EMS 0.01% was also reported to cause an increase in the height of chili plants [28] and Coriandrum sativum by [29]. Plant height has been used as an important indicator in determining the effect of mutation induction using EMS [30]. The advantage of tall plants over short plants is that it reduced the risk of developing fruit by various pests from the soil. The long-distance between the fruit and the soil reduces fungal infections that are carried by staining water when watering or raining [31,32].

The G1/01 and G7/01 genotypes had a mean flowering time of 64 ± 4.4 days after planting (DAP) and 115 DAP, respectively. The genotypes flowering slightly faster than their original type (Table 1). Mutant T45 (G1/01) was the fastest (57 DAP) and T19 and T30 mutants were the latest (69 DAP). Flowering time under 77 DAP is into the early category, thus the harvest period can be done around 115 DAP [33]. Therefore, 100% of the G1/01 were into the early flowering category and 100% of the G7/01 were into the late flowering category. The early flowering was related to the time of the harvest. Therefore, it can help farmers save on maintenance costs [27]. Harvest time has a positive correlation with the number and weight of fruit per plant, the weight correlated with the length and diameter of the fruit [34], thus the faster the harvest will have the potential to produce more fruit. A different phenomenon was shown by the G7/01. EMS had a negative effect on the G7/01, that it was causing delays in flowering. EMS mutation was random so that the phenotypes of mutant plants produced can be different, such as in plants G1/01 and G7/01.

The fruit of the G1/01 genotype (4 ± 0.78 cm) was significantly longer than the original type (3.64 ± 0.4 cm) (table 2). Eighty-five percent of the G1/01 were into the normal category (1-5 cm) and 8% were into the long category (>5-10 cm). The longest fruit, T45 was 6.77 ± 0.5 cm was and the shortest fruit was 2.5 ± 0.12 cm (T43). In contrast, the fruit length of G7/01 was more similar, all mutants were categorized into normal with an average length of 4.4 ± 0.2 cm. The mean fruit diameter of G1/01 was 0.84 ± 0.15 cm and showed a significant difference from the original type (0.72 ± 0.05 cm) (Table 1). Eighty-eight percent of the G1/01 were into normal category (>0.5-1 cm) and the remaining 11% were into wide category (>1-2 cm). The diameter of the G7/01 had an average of 0.6 ± 0.15.10% of the G7/01 were - very wide (>10 cm), 56% were - normal, and 33% were - narrow (>0.1-1 cm). Therefore, it could be concluded that half of the G7/01 was into normal diameter. Fruit diameter and fruit length correlated with fruit weight. G1/01 fruit weight has a significant difference with the original type. The mean fruit weights of G1/01 and G7/01 were 1.46 ± 0.5 g and 1.17 ± 0.08 g, respectively (Table 2). The heaviest fruit was 2.60 g in the T45 and the lightest fruit was 0.73 g in T38 and T29 (G1/01). Meanwhile, the heaviest and lightest fruits of genotype G7/01 were T18 (1.5 g) and T22 (1.1 g), respectively. Almost all of the G1/01 (88%) and G7/01 (100%) were into mild category (0.1–2 g) and 11% of the G1/01 were in the normal category.

The mean number of seeds in G1/01 and G7/01 was 66 ± 17.2 and 31 ± 3, respectively (Table 1). The number of seeds of these mutant plants did not differ significantly from their original type. The number of seeds in the G1/01 mutant plants more varied than the G7/01. Eighty-six percent of G7/01 was into medium category (>50-200 seeds) and 13% were in the small category (<50 seeds). However, all G7/01 mutants (100%) were into small category.

| Table 1. Descriptive values for examined morphology characters of G1/01 and G7/01 |
|---------------------------------|---------------|----------------|----------------|
| Category                        | Average       | Average        | Average        |
|                                 | Control       | G1/01          | Control        | G7/01          |
| Plant height                    | 77.6 ± 14     | 79 ± 17        | 131 ± 7.6      | 128.6 ± 18     |
| Flower Time (days)              | 67.2±0.6      | 64.2 ± 3*      | -              | 115            |
| Fruit Length (cm)               | 3.64±0.4      | 4.02 ± 0.8*    | -              | 4.24 ± 0.24    |
| Fruit Diameter (cm)             | 0.72±0.05     | 0.84 ± 0.2*    | -              | 0.6 ± 0.15     |
| Fruit Weight (g)                | 1.06±0.2      | 1.46 ± 0.5*    | -              | 1.17 ± 0.1     |
| N. of Seed (seeds)              | 56.2±9.8      | 66.2 ± 17      | -              | 31.7 ± 3       |

* indicate significant differences with control at the 0.05 by Mann-Whitney. – : indicate that the data missing.
The G1/01 genotype was faster in flowering time, higher fruit diameter and fruit weight. Meanwhile, the G7/01 genotype was similar in habitus and higher than G1/01. The results of this study indicated that mutation induction by EMS 0.01% caused variation between plants. Mutations also gave rise to positive and negative characters such as delayed flowering in the G7/01 mutant. The advantages and disadvantages of induction of EMS mutations were due to its random mutation and the types of mutations that generally produced point mutations [35].

**Table 2. Quantitative analysis on various morphology characters and capsaicinoid content**

| No | Traits                  | G1/01               | G7/01               |
|----|-------------------------|---------------------|---------------------|
|    |                         | Min (cm) | Max (cm) | Average (cm) | Min (cm) | Max (cm) | Average (cm) | Category (%) | Min (cm) | Max (cm) | Average (cm) | Category (%) |
| 1  | Plant length            | 46        | 113      | 79±17        | 90        | 161      | 128±18        | very high    | 100       |
|    |                         |           |          |             |           |          |             | high (26)    |           |
|    |                         |           |          |             |           |          |             | normal (33)  |           |
| 2  | Fruit length            | 2,23      | 6,77     | 4±0,8*      | 3,8       | 4,7      | 4,2±0,2      | long (8)     | 100       |
|    |                         |           |          |             |           |          |             | normal (85)  |           |
| 3  | Fruit diameter          | 0,57      | 1,37     | 0,8±0,1*    | 0,2       | 0,7      | 0,6±0,15     | Wide (11)    | 100       |
|    |                         |           |          |             |           |          |             | Normal (88)  |           |
| 4  | Fruit weight (g)        | 0,58      | 3        | 1,5±0,5*    | 1,1       | 1,54     | 1,2          | normal (11)  | 100       |
|    |                         |           |          |             |           |          |             | mild (88)    |           |
| 5  | Flowering time (DAA)    | 57        | 69       | 64±3*       | 115       | 120      | -            | very early    | 100       |
|    |                         |           |          |             |           |          |             | (100)        |           |
| 6  | Pungency (SHU)          | 27638     | 89209    | 68539±20609* | 51875     | 309120   | 99419±75699* | very hot     | 40        |
|    |                         |           |          |             |           |          |             | (60)         |           |
|    |                         |           |          |             |           |          |             | hot (40)     |           |

*) significant difference with the original type with a different degree (α <0.005)

### 3.3 Capsaicinoid content analysis

The mean capsaicinoid content of the G1/01 and G7/01 genotypes were 4.3 ± 1.2 mg/g and 6.2 ± 18 mg/g, which were significantly lower than the original types (5.8 ± 0.7 and 15.76 ± 2, respectively) (Fig. 5). Ten mutants were selected from the total mutant population G1/01 and G7/01 based on their unique characteristics. Sixty percent of the G1/01 and G7/01 and their original types were into very hot category, while 40% of the G1/01 and G7/01 are into hot category. The level of spiciness was stated by the Scoville Heat Units (SHU), and it has been grouped into 5 categories; not spicy (0-700 SHU), mild (700-3000 SHU), moderately (3000-25000 SHU), hot (25000-70000 SHU), and very hot (>80000 SHU) [36,37]. The G1/01 and G7/01 have not a spiciness value lower than 25,000 SHU. According to Ribeiro, *C. frutescens* is a hot pepper [20]. The T39 (G7/01) had a high capsaicinoid (19.32 ± 0.56 mg/g). The spiciness value was higher than the original type and about four times higher than the other mutants. The high level of spiciness related to the capsaicin content, the capsaicin content in the total capsaicinoid is around 71% [38]. The potential for high capsaicin content is important in the pharmaceutical industry [39].
3.4 Cluster Analysis

The results of cluster analysis based on morphology characters and capsaicinoid contents separated the two chili pepper genotypes G1/01 and G7/01. G1/01 and G7/01 formed clusters and sub-clusters. The nodes color nodes, the petal color, the leaves color, and the peel surface were apomorphy between the two genotypes. Apomorphy is a special character that belongs to one group or clade only. All G1/01 members had purple node color, white petal color, smooth peel surface, dark leaf color, and early flowering. G7/01 members have green nodes, greenish-white petals, lighter leaves, wrinkled peel surface, and late flowering times. Different mutants in the same cluster have a high degree of similarity. The total clusters formed from the two genotypes were seven clusters with a similarity index (0.89) (Fig. 6.).

The G1/01 formed five clusters. Cluster I consisted of one original line only. It was different from mutant lines in the plant height category. Cluster II consisted of T8, T10, T18, T19, and T30 mutants. The cluster had characteristics of very tall, normal fruit diameter, and very hot. Cluster III consisted of plants with normal height characteristics. T29 plants belonging to cluster III with white fruits form a specialization in cluster III. Cluster IV consisted of plants that have unique characteristics, namely large diameter but low number of seeds. Cluster V consisted of mutants that have the longest fruit. G1/01 members were into the category of early flowering. This showed that the character has been stable in the 4th generation.

G7/01 genotype consisted of two clusters, namely cluster VI and VII. Cluster VI was original type which has a late flowering and fruit had not appeared until the study period is over. Mutants in cluster VII have not variations in the characters of plant height, fruit length, fruit diameter, fruit weight, and fruit color. Cluster VII was separated into two sub-clusters due to its capsaicinoid content. The 4th generation G7/01 is more stable and has less variation than the G1/01 mutant.
Figure 6. Dendrogram of cluster analysis results using the UPGMA method based on the similarity coefficient by Jaccard using the scoring of the morphology character values of fruit color, node color, petal color, kalix margin, peel surface, plant height, flowering time, fruit length, fruit diameter, fruit weight, number of seeds, length of petal, and level of spiciness.

The formation of many clusters in the 4th generation of G1/01 provided an evidence that EMS mutation induction was effective in producing variations, thus researchers can efficiently select plants according to what is needed for human welfare [8]. However, the variation observed also showed that segregation was still occurred. On the other, mutants G7/01 which concentrated into one group only (Cluster VII) proved that the mutant has reached stability.

4. Conclusion
In the 4th generation, the G1/01 mutants were more varied than the G7/01 mutants. Several characters that varied in G1/01 were plant height, habitus, branching, fruit color and shape, fruit length, and diameter. G1/01’s superior characters, such as early flowering, has become stable in the 4th generation. Meanwhile, G7/01 showed higher stability than G1/01 and showed slight variations in the branching and capsaicinoid content.

Acknowledgements
The authors thank to all current and previous members of the Capsicum group for providing capsaicinoid data control and figures. This project was financially supported by Hibah Professor Scheme, Brawijaya University.

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