Genetic Analysis of G1/01 Chili pepper Based on Morphological, Physiological and Molecular Characteristics

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Abstract. The G1/01 mutant line is a chili pepper variant resulting from a mutation with Ethyl Methane Sulfonate to the genotype 1 (G1) which is a descendant of a commercial chili pepper varieties Cakra Hijau. In this study the G1/01 line was compared with Capsicum frutescens varieties, Cakra Hijau and Genie and large chili Capsicum annuum (CAT2) based on morphology, physiology (capsaicin content) and molecular, Random Amplified Polymorphic DNA (RAPD). The results showed that G1/01 had a similar morphology to the varieties of Cakra Hijau, Genie and CAT2 based on habitus, branching, plant height, flower shape and fruit shape, but G1/01 had greater leaf length and width than the Cakra Hijau, Genie and CAT2 and larger fruit size than the Cakra Hijau and Genie. When compared with CAT2, G1/01 has a shorter fruit size even though its diameter was almost the same. The four types of are included in the spicy and very spicy categories. The fruit of G1/01 line showed a change in the level of spiciness from the spicy category when young to the very spicy category when the fruit was ripe red. The RAPD results showed that the Cakra Hijau and Genie varieties were similar to each other while the G1/01 mutant was different from the two. CAT2 shows quite a distance with the other three types. Dendrogram based on morphological, physiological and molecular data shows that G1/01 changes quite large from the Cakra Hijau, but does not similar as CAT2.

Keywords: Capsicum sp., Capsaicin, morphology, RAPD

1. Introduction
Chili pepper is belong to the family Solanaceae originating from Central and South America and then spread to Europe, Africa and Asia [1]. Nowadays, around 30 species have been found, but only 5 species, namely C. annuum, C. frutescens, C. baccatum, C. pubescens and C. chinense are widely cultivated [2]. The most common chili species developed in Indonesia are C. annuum (large chili) and C. frutescens (cabai rawit) [3], but between them, C. frutescens is the most planted species with several cultivar names, such as Sky line, Bara, Cakra Hijau and Cakra Putih [4], and Genie [5].

Based on the colour of the young fruit, chili pepper can be divided into green chili pepper that has dark green colour and white chili pepper that has light green to near white. Cakra Hijau and Genie are two green chili peppers varieties produced by two different seed company that are widely cultivated by chili farmers. Cakra Hijau varieties share some morphological characteristics with Genie varieties, such as the colour and size of the fruit [5, 6]. Genotype 1 (G1) chili that is a descendant of the Cakra Hijau, which was identified as C. frutescens also shows the character of CAT2, which is a purplish-
coloured node and white flowers [7]. The mutation induction of G1 with Ethyl Methanesulfonate (EMS) has produced various green mutants [8]. One of the mutants, G1/01, has tree habitus characteristics, the shape and colour of the leaves, the shape and colour of the fruit and the colour of flowers and nodes similar to the Cakra Hijau and Genie.

A special character of Capsicum genus is to produce capsaicin compounds which cause spicy taste [4]. The pungency level was varies depending on the content of the capsaicin compound it produces. Of the three genotypes of chili pepper (Genie, Cakra Hijau, G1/01) which morphologically have the same character, the level of spiciness and the capsaicin content of the three are unknown. Random Amplified Polymorphic DNA (RAPD) is a molecular marker that can be used to identify the genomic variations of an organism [9]. Based on these facts, this study intends to find out the genetic profile of G1/01 mutants compared to the original type of Cakra Hijau, Genie, and large chili (C. annuum) based on morphological, physiological and molecular characteristics.

2. Methods

2.1 Planting and Maintenance of Plants
The seeds of mutant line G1/01, Cakra Hijau, Genie and large C. annuum (CAT2) were sown in pots and placed in a location with sufficient irradiation. Four weeks after sowing, seedlings were transplanted to new planting media consisted of a mixture of soil and compost (2:1 v/v) in pots with a diameter of 28 cm. Watering was done as needed in the morning or evening. Leaf fertilizer was given once a week in the morning, sprayed on the surface of the leaf. Granular fertilizer was given to plants through the planting media every two weeks.

2.2 Isolation of DNA and Random Amplified Polymorphic DNA (RAPD)
Genomic DNA was isolated from chili pepper young leaf using CTAB methods [10]. RAPD then performed to the DNA template using 5 primers (Table 1) with the program consisted of: 94 °C for 3 minutes denaturation, continued with 45 cycles consisted of denaturation at 94°C for 30 seconds, annealing at 37°C for 30 seconds, extension at 72°C for 1 minute 30 seconds and 1 end extension cycle at 72°C [11]. The PCR mixture (total 10 uL) was consisted of ddH2O 3 uL, PCR mix (Intron) 5 uL, 10 pmol 1 uL primer, DNA 100 ng / mL 1 uL.

| No. | Primer | Sekuen primer(5’-3’) | G+C (%) |
|-----|--------|----------------------|---------|
| 1   | L2     | GGGGTGACGA           | 70%     |
| 2   | L9     | ACCTCGGCAC           | 70%     |
| 3   | L11    | GTCAGTGCGG           | 70%     |
| 4   | L16    | TGCCGAGCTG           | 70%     |
| 5   | L18    | TGCTCTGCCC           | 70%     |

2.3 Morphological Observation
Observations of qualitative and quantitative morphological characters of the chili pepper were conducted in the vegetative and reproductive phases. The morphological analysis refers to Descriptors for Capsicum (Capsicum spp.) by the International Plant Genetic Resources Institute (IPGRI), the Asian Vegetable Research and Development Center (AVRDC) and the Tropical Agricultural Research and Training Center (CATIE).

2.4 Measurement of Capsaicin Content
The level of fruit pungency was measured in two level of fruit maturity, young (green) and ripe (red). The young fruit was taken when the fruit was still green, 35-42 Days After Sowing (DAS), while the mature condition was taken when the fruit was already fully red in colour at the age of 42-65 DAS. As much as 0.5 g of fruit was weighed then added with 5 ml of absolute ethanol and crushed using mortar and pestle. Homogenate was vortexed for 2 minutes and filtered with filter paper. The filtrate was measured using a spectrophotometer with a wavelength of 280 nm. The absorbance value was
substituted in the equation of the capsaicin standard solution curve to determine the concentration of the sample capsaicin [12].

2.5 Data Analysis
Data were analyzed using Paleontological Statistics Software (PAST 3) using Jaccard similarity index Unweighted Pair Group Method with Arithmatic Means (UPGMA) method.

3. Results and Discussion

3.1 Morphological Characters
The four types of chili pepper studied had a shrub habitus. The stems of all genotypes had a round shape. The stem colour was light green to dark green, with purple nodes. The nodes of Genie, Cakra Hijau and CAT2 varieties tend to have a darker purple pigmentation compared to that of the G1/01 line (Figure 1).

![Figure 1. The node and trichomes’s colour of chili peppers. A) Genie, B) Cakra Hijau, C) G1/01, D) CAT2](image)

The four genotypes also exhibit several different morphological characters. The stems of chili pepper varieties of Genie and Cakra Hijau were covered with white trichomes (Figure 1) from the main stem to the leaf stalks. Branches of Genie, Cakra Hijau and G1/01 line were similar, which appear in all parts of the stem from the base to the tip of the stem with dichotomous branching types. Basically, the character of stem was in accordance with the results of research of Arumingtyas et al. (2017). CAT2's stem was different from other genotypes because its branching was centered on the terminal part of the stem. The stem will divide into three or four branches, or become a secondary branch at the stem height of 10 to 40 cm. CAT2’s stem can reach the heights of 50-150 cm [13, 14]. Based on these characteristics, in general the four genotypes are similar. The distinguishing feature of the four genotypes lies in the presence of trichomes on the stem and their branches. Genie and Cakra Hijau varieties have intermediate trichomes on the stem. This shows that Genie and Cakra Hijau represented the characteristic of *C. frutescens*. Another distinctive characteristic observed was the branching type, where the CAT2 has dichotomous branching and focused on the terminal part, meanwhile, G1 / 01 did not have any special characteristics, either trichomes or branching.

The average height of chili plants Genie was 76.5 cm, not significantly different from CAT2 which had an average height of 74.3 cm. Meanwhile, the average plant height of Cakra Hijau and G1/01 line were not significantly different which were 62.7 cm and 58.6 cm, respectively (Figure 2). Generally, cabai rawit (*C. frutescens*) had higher habitus than *C. annum*. This is due to the fact that *C. annum* is an annual plant that after once or at most twice fruiting, the vegetative growth will stopped, in contrast
to *C. frutescens* which is a perennial plant, it will continue to grow and bear fruit many times during its lifetime.

![Figure 2. Average height of Genie, Cakra Hijau, G1/01 and CAT2. Different notation show significant differences](image)

The G1/01 line had an average leaf length and leaf width that was much larger (14.4 cm, 6.5 cm) than the 3 other varieties (Figure 3). The G1/01 line had ovate leaf shape, the other three genotypes have lanceolate leaf shape. The leaves of Genie and Cakra Hijau varieties were covered with white trichomes, such as those on their stems and petioles, while G1/01 and CAT2 did not have trichomes.

Among the four types of chili peppers, the Cakra Hijau variety was the earliest flowering, 84 DAS. Meanwhile, CAT2 flowered one day later. G1/01 flowering time of 86 DAS and Genie was 89 DAS. The chili genotypes had a single flower with white petal colour. The flowers of Genie and Cakra Hijau varieties generally have 5 petals, while the G1/01 and CAT2 were 5 or 6 petals. The four genotypes had one greenish yellow pistil with a white pistil stalk. The number of stamens of the four genotypes was five and situated around the pistil. The pistil of Genie and Cakra Hijau varieties was bluish in colour, while G1/01 and CAT2 were purplish (Figure 4).

![Figure 3. Average leaf length (blue bar) and width (brown bar) of Genie, Cakra Hijau, G1/01 and CAT2. Different notation of the same character indicate significant differences.](image)
The fruit of the four genotypes had a green colour when it was not yet ripe and turns red when ripe. In addition, all four genotypes have smooth fruit surfaces. Each axil consists of one fruit. The fruit of Genie and Cakra Hijau had similarities on term of elongate shape with a pointed tip, and fruit size. G1/01 line had a larger fruit than Genie and Cakra Hijau but shorter than CAT2. Genie, Cakra Hijau and G1/01 line reach maturity at 42-55 days after flowering (DAF) or around 152-165 days after sowing (DAS). Unlike the other three genotypes, CAT2 has a fruit ripening time of around 60-65 DAF or around 170-175 DAS. The largest fresh weight of fruit was showed by CAT2, which was 3.4 g. This variety also had the longest fruit length, which was 9.7 cm. The fruit fresh weight and length of the fruit were different significantly from other genotypes (Figure 5).
Fruit stalk length that exceeds the length of the fruit was a special feature possessed by Genie (Figure 5), even the length of the fruit stalk of this variety was longer than the CAT2 which had the longest fruit length and the highest fruit fresh weight.

3.2 Capsaicin content and fruit spiciness level

The highest level of spiciness of young green fruit was showed by Genie varieties: 56,937 SHU, while in mature conditions was showed by G1/01 line with 73,263 SHU. The level of spiciness based on Scoville heat units (SHU) is divided into several categories, non-spicy (0–700 SHU), mildly spicy (700–3,000 SHU), moderately spicy (3,000–25,000 SHU), highly spicy (25,000–70,000 SHU) and very highly spicy (>80,000 SHU) [15]. Based on this category, the young fruit of the four chili genotypes were categorized as spicy. The level of spiciness of the fruit of four genotypes in ripe conditions did not differ significantly. Genie variety and G1/01 line in ripe conditions are included in the very spicy category (Table 2).

| No. | Genotype     | Level of Spiciness (SHU) | Differences of Spiciness in different level of Maturity (SHU) |
|-----|--------------|--------------------------|--------------------------------------------------------------|
| 1.  | Genie        | 56.937<sup>b</sup>       | 71.147<sup>a</sup>                                          | 14.210                                                      |
| 2.  | Cakra Hijau  | 49.983<sup>ab</sup>      | 69.333<sup>a</sup>                                          | 19.350                                                      |
| 3.  | G1/01        | 36.680<sup>a</sup>       | 73.263<sup>a</sup>                                          | 36.583                                                      |
| 4.  | CAT2         | 34.261<sup>a</sup>       | 65.705<sup>a</sup>                                          | 31.444                                                      |

Note: Different notation letter of the same character indicate significant differences

The level of spiciness can vary, not only at the level of species but also at the level of cultivars. The CAT2 generally has a low level of spiciness. A study of the spiciness level of large chili pepper (C. annum) and cabai rawit (C. frutescens), showed the spiciness of 12,500 SHU for large chili pepper while Cabai Rawit had the spiciness level of more than 82,500 SHU [16]. Based on these results, the large chili pepper was included in the category of moderate spicy and cabai rawit into the category of very spicy.

G1/01 line shows interesting character, i.e. the difference in the level of spiciness in mature and young conditions was much bigger than the other three genotypes. The difference in spicy level in mature and young fruit of G1/01 line was 36,583 SHU. A similar phenomenon was also seen in CAT2, where the difference in spiciness was 31,444 SHU (Table 2). However, the ripe fruit of CAT2 remains at the lowest level of spiciness.

3.3 Molecular Analysis

Based on the results of the RAPD, it seems that the varieties of Genie and Cakra Hijau have many similarities, although there were still some differences between the two observed. The G1/01 line has more in common with Genie and Cakra Hijau varieties but based on molecular character, G1/01 line was more similar to CAT2. These results indicate the characters in the G1/01 line have the characteristic between C. frutescens and C. annum. Meanwhile, CAT2 has more differences compared to the other three chili genotypes. This shows that CAT2 was not only different in morphological characters, but also in molecular characters, indicating a low relationship.
Similarity analysis results based on morphological, physiological and molecular data divided the four chili genotypes into 2 clusters. The first cluster consisted of G1/01 and CAT2 (CAT) with a similarity value of 0.67. The second cluster consisted of chili pepper varieties of Cakra Hijau (CH) and Genie with a similarity value of 0.84 (Figure 7). Based on these results, it was known that the varieties of the Cakra Hijau and Genie have a closer kinship with each other. While G1/01 and CAT2 have lower similarity values, both of these genotypes are in the same cluster showing the relationship between the two was closer to each other compared to the other two genotypes.

The Genie and Cakra Hijau varieties that have been identified as *C. frutescens* showed a close relationship that supports that both varieties are from the same species, however, the observed variation showed that the two was of different varieties. Meanwhile, G1/01 line which has a closer relationship with CAT2 indicated a quite big alteration occurred due to mutation of the G1/01 line caused a shift of characteristic toward *C. annum*. Other possible explanation is the incident of natural crossing between genotypes that can increase heterosis in chili plants [17]. Therefore, special characteristics such as the length of the fruit stalk of Genie, the leaf shape of G1/01 line, and the shape and size of CAT2’s fruit can be distinguishing characteristics, both at the level of species, and varieties.

4. Conclusion
The molecular character of G1/01 line was more similar to that of Genie and Cakra Hijau varieties, however the dendrogram based on morphological, physiological and molecular data showed that G1/01 mutant had closer relationship to CAT2 than to both Cakra Hijau and Genie varieties which
were similar to each other. This indicated that G1/01 changes quite large from the Cakra Hijau, but does not become exactly the same as CAT2. Further research on anatomical and biochemical properties of the genotypes as well as other molecular marker need to be employed for further identifying and profiling genotypes so it can be utilized for parental selection to develop superior quality chili pepper variety.

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